Waterborne Zoonoses

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Waterborne Zoonoses

Identification, Causes, and Control

Edited by
J.A. Cotruvo, A. Dufour, G. Rees, J. Bartram,
R. Carr, D.O. Cliver, G.F. Craun, R. Fayer and
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Contents

Preface

Investigating important emerging issues in water and infectious disease and communicating discoveries create challenges, which are addressed by an initiative being undertaken by the World Health Organization (WHO) Water Sanitation and Health Unit, the US Environmental Protection Agency (US EPA) Office of Research and Development, and other collaborators. The initiative seeks to accelerate the identification of actual and perceived issues, to bring together information and knowledge in critical areas, and to disseminate information to policy-makers and practitioners in a timely fashion. This initiative has resulted in the publication of several cutting-edge documents that critically analyse emerging issues in water and infectious disease and present balanced assessments of how these will impact disease transmission through water with emphasis on management options for preventing and controlling waterborne disease.

Other issues dealt with in the Emerging Issues in Water and Infectious Disease initiative include:

- heterotrophic plate counts and drinking-water safety;
- pathogenic mycobacteria in water;
- the H_2S method for the detection of faecal contamination of drinking-water;
- water recreation and disease;
- respiratory transmission of faecally excreted viruses; and
- toxic cyanobacteria in water.

This publication was developed from the workshop on "Zoonosis and Waterborne Disease," held in Annapolis, Maryland, USA, on 2–4 September 2003. The workshop was sponsored by the WHO units dealing with Water, Sanitation and Health and with Strategy Development and Monitoring of Zoonoses, Foodborne Disease and Kinetoplastidae, working with US EPA's Office of Research and Development and Office of Ground Water and Drinking Water. Twenty-nine experts from 14 countries and diverse disciplines, including sanitary and veterinary microbiology, animal health, agriculture, animal waste management, public health, water epidemiology, medicine, sanitary engineering, food safety, and regulatory policy, attended the workshop. They examined the roles of zoonoses in current and future waterborne disease and prepared the chapters published here.

Participants at the workshop were asked to:

- review current waterborne zoonotic disease threats;
- identify new disease candidates based on disease agent characteristics; and
- evaluate current control strategies to identify agents that might fall outside of the current control envelope.

The workshop participants reviewed information on zoonotic organisms linked to waterborne diseases in humans and focused on the organism characteristics, human activities, and environmental conditions that could lead to future concerns from evolving or emerging organisms. Animal vector factors discussed included feral/wild animals, domestic animals, intensive grazing, feedlots, abattoirs, and other elements. Emergence related to translocation of microorganisms resulting from human and animal movement, food production, irrigation, food handling, distribution from distant areas, climate change, and other appropriate contributing factors was discussed.

This publication was developed from technical inputs to the workshop, workshop deliberations and revisions to the technical materials based on the suggestions of expert technical reviewers.

The goal of this publication is to provide guidance to agencies concerned with human and animal health and water and wastewater service providers worldwide to anticipate potential future waterborne zoonotic disease problems

and to determine whether current practices will be protective or whether new approaches need to be developed or deployed to protect health. This publication presents information on how zoonotic pathogens can be best managed at the source (i.e., through animal management practices, treatment of animal wastes, runoff management); through water treatment (wastewater and drinking-water); or through a combination of multiple barriers.

We hope that this publication provides useful information in describing the significance of zoonotic microorganisms as threats to the quality of ambient water and drinking-water and to public health throughout the world. We hope that this will facilitate the development of cross-sectoral initiatives to manage current health threats and to anticipate and manage health threats from emerging waterborne zoonotic pathogens.

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Jamie Bartram, WHO, Geneva, Switzerland
Carole Bolin, Michigan State University, East Lansing, MI, USA
Peter Braam, WHO, Geneva, Switzerland
Corrie Brown, University of Georgia, Athens, GA, USA
Lynda M. Browning, Scottish Centre for Infection and Environmental Health, Glasgow, Scotland
Rebecca L. Calderon, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA
Richard Carr, WHO, Geneva, Switzerland
John Cicmanec, Office of Research and Development, US Environmental Protection Agency, Cincinnati, OH, USA

Dean O. Cliver, Food Safety Laboratory and WHO Collaborating Centre for Food Virology, School of Veterinary Medicine, University of California, Davis, CA, USA
Joseph Cotruvo, Joseph Cotruvo & Associates, Washington, DC, USA
Gunther Craun, G.F. Craun and Associates, Staunton, VA, USA
Michael F. Craun, G.F. Craun and Associates, Staunton, VA, USA
John Cross, Division of Tropical Public Health, Department of Preventive Medicine and Biometrics, Uniformed Services University, Bethesda, MD, USA
Friederike Dangendorf, Institute of Hygiene and Public Health, University of Bonn, Bonn, Germany
Alfred Dufour, Office of Research and Development, US Environmental Protection Agency, Cincinnati, OH, USA
Andrea Ellis, WHO, Geneva, Switzerland
Takuro Endo, Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan
Ronald Fayer, Agricultural Research Service, US Department of Agriculture, Beltsville, MD, USA
Kate Field, Department of Microbiology, Oregon State University, Corvallis, Oregon, USA
Daniel Y.C. Fung, Department of Animal Science and Industry, Kansas State University, Manhattan, KS, USA
Victor P.J. Gannon, Public Health Branch, Animal Diseases Research Institute, Health Canada, Lethbridge, Alberta, Canada
Frank Humenik, College of Agricultural and Life Sciences, Animal Waste Management Program, North Carolina State University, Raleigh, NC, USA
Sasitorn Kanarat, Hygiene and Microbiology, Ministry of Agriculture, Bangkok, Thailand
Diane Lightfoot, Microbiological Diagnostic Unit, Public Health Laboratory, University of Melbourne, Victoria, Melbourne, Australia
Santiago Mas-Coma, Department of Parasitology, Faculty of Pharmacy, University of Valencia, Burjassot, Valencia, Spain
Graham McBride, National Institute of Water and Atmospheric Research, Hamilton, New Zealand
Francois-Xavier Meslin, WHO, Geneva, Switzerland
Christine Moe, Department of International Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA
Kåre Mølbak, Statens Serum Institut, Copenhagen, Denmark
Yasuyuki Morishima, Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan
Rosa Gabriella Ramírez-Porras, Department of Veterinary Epidemiology and Public Health, Veterinary Faculty, Yucatan, Mexico

Gareth Rees, Askham-Bryan College, Askham-Bryan, York, North Yorkshire, UK
William Reilly, Scottish Centre for Infection and Environmental Health, Glasgow, Scotland
Mark Rice, College of Agricultural and Life Sciences, Animal Waste Management Program, North Carolina State University, Raleigh, NC, USA
Joan Rose, Michigan State University, East Lansing, MI, USA
Stephen Schaub, Office of Water, Office of Science and Technology, US Environmental Protection Agency, Washington, DC, USA
Flemming Scheutz, Statens Serum Institut, Copenhagen, Denmark
Jeevan B. Sherchand, Tribhuvan University Teaching Hospital, Kathmandu, Nepal
Huw V. Smith, Scottish Parasite Diagnostic Laboratory, Glasgow, Scotland
James E. Smith, Jr., Office of Research and Development, US Environmental Protection Agency, Cincinnati, OH, USA
Mark Sobsey, University of North Carolina, Chapel Hill, NC, USA
K. Suresh, Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
Desmond Till, Public Health Microbiologist Consultant, Wellington, New Zealand
Jose Williams, Department of Veterinary Epidemiology and Public Health, Veterinary Faculty, Yucatan, Mexico

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List of acronyms and abbreviations

A/EEC	attaching and effacing *E. coli*
AFLP	amplified fragment length polymorphism
AFO	animal feeding operation
AGI	acute gastrointestinal illness of unknown origin
AIDS	acquired immunodeficiency syndrome
ARCC	average rate of correct classification
ATP	adenosine triphosphate
BFP	bundle-forming pilus
BSE	bovine spongiform encephalopathy
CAFO	concentrated animal feeding operation
CDSC	Communicable Disease Surveillance Centre (England and Wales)
CFU	colony-forming unit
CI	confidence interval
CJD	Creutzfeldt-Jakob disease
CUP	carbon-source utilization
CWD	chronic wasting disease
DAEC	diffuse adherent *E. coli*
DALY	disability-adjusted life year

DBP	disinfection by-product
DEC	diarrhoeagenic *E. coli*
DNA	deoxyribonucleic acid
DT	definitive phage type
EAEC	enteroadherent *E. coli*
EAggEC	enteroaggregative *E. coli*
EHEC	enterohaemorrhagic *E. coli*
EIEC	enteroinvasive *E. coli*
EPA	Environmental Protection Agency (USA)
EPEC	enteropathogenic *E. coli*
epg	eggs per gram of faeces
ESWTR	Enhanced Surface Water Treatment Rule (USA)
ETEC	enterotoxigenic *E. coli*
HACCP	hazard analysis and critical control points
HAV	hepatitis A virus
HBV	hepatitis B virus
HEV	hepatitis E virus
HIV	human immunodeficiency virus
HUS	haemolytic uraemic syndrome
ID	infective dose
ID_{50}	median infective dose
Ig	immunoglobulin
IID	infectious intestinal disease
IPCC	Intergovernmental Panel on Climate Change
LEE	locus of enterocyte effacement
LH-PCR	length heterogeneity polymerase chain reaction
LT	heat-labile enterotoxin
LU	livestock unit
MAP	*Mycobacterium avium* (ssp. *paratuberculosis*)
MAR	multiple antibiotic resistance
MBM	meat and bone meal
MCL	Maximum Contaminant Level (USA)
MCLG	Maximum Contaminant Level Goal (USA)
MOR	matched odds ratio
MPN	most probable number
mRNA	messenger ribonucleic acid
MST	microbiological source tracking
NASBA	nucleic acid sequence-based amplification
NPDES	National Pollutant Discharge Elimination System (USA)
NTU	nephelometric turbidity unit

OIE	Office International des Epizooties (World Organization for Animal Health)
PCR	polymerase chain reaction
PEAS	possible estuary-associated syndrome
PFGE	pulsed-field gel electrophoresis
PT	phage type
QMRA	quantitative microbial risk assessment
QRA	quantitative risk assessment
rDNA	ribosomal deoxyribonucleic acid
REP-PCR	repetitive extragenic palindromic polymerase chain reaction
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
SARS	severe acute respiratory syndrome
SCCWRP	Southern California Coastal Water Research Project (USA)
SMX	sulfamethoxazole
STEC	Shiga toxin-producing *E. coli*
STh	heat-stable enterotoxin (human)
STp	heat-stable enterotoxin (porcine)
TMDL	total maximum daily load
TMP	trimethoprim
T-RFLP	terminal restriction fragment length polymorphism
TSE	transmissible spongiform encephalopathy
UDG	uracil-D-glycosylase
USA	United States of America
US EPA	United States Environmental Protection Agency
UV	ultraviolet
VBNC	viable but non-culturable
vCJD	variant Creutzfeldt-Jakob disease
VTEC	verocytotoxin-producing *E. coli*
WHO	World Health Organization
WSP	water safety plan
YLD	years lived with a disability
YLL	years of life lost to premature death

Section I

Expert consensus

1

Expert consensus

Expert Meeting Group Report

1.1 BACKGROUND

Waterborne disease in both epidemic and endemic forms continues to occur in both developed and less developed countries. Concern for waterborne disease is dominated by pathogens transmitted by the faecal–oral route and by drinking-water. Waterborne transmission also includes diseases transmitted by faecal droplet inhalation (e.g., some adenoviruses) and exposure through contact (e.g., recreational and occupational). It is interconnected with the consumption of shellfish and other harvest fisheries outputs and through indirect exposure to water in foodstuffs when the water is used in irrigation, in food processing, or as an ingredient. Interactions that result in waterborne zoonotic infections are illustrated in Figure 1.1.

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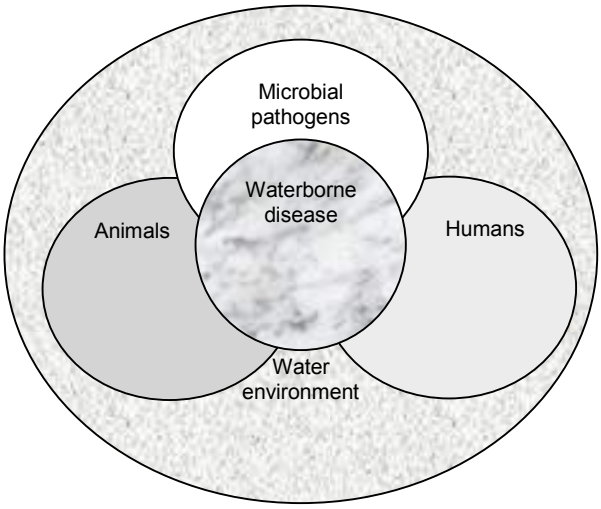

Figure 1.1. Waterborne disease interactions in the water environment.

It is important to place into context the zoonotic component of waterborne disease. Approximately 4 billion cases of diarrhoea occur each year, leading to nearly 2 million deaths. Intestinal worms infect more than a billion people worldwide. The percentage of these illnesses that are caused by zoonotic pathogens is difficult to determine due to a lack of data, but it is thought to be significant. Waterborne zoonotic pathogens cause both gastrointestinal diseases such as diarrhoea and other illnesses such as leptospirosis and hepatitis.

Some waterborne zoonoses are well known, information is limited on others, and it is likely that some, possibly many, remain unrecognized. Several waterborne zoonoses, such as cryptosporidiosis and giardiasis, occur regularly in a variety of countries; others, such as leptospirosis, occur more frequently in tropical countries. High quality information on many aspects of waterborne zoonoses is not available. There is also a need to better integrate the disciplines of human and animal health to anticipate emerging waterborne zoonoses and develop appropriate management responses to prevent them.

The phenomenon of "emergence" and "re-emergence" of infectious diseases in general is now well recognized. Up to 75% of emerging pathogens may be of zoonotic origin. A significant number of emerging and re-emerging waterborne pathogens have been recognized over recent decades. Many of these pathogens have zoonotic sources (e.g., *E. coli* O157:H7), and others have both human and zoonotic sources (e.g., *Cryptosporidium*). There are many pathogens for which there is currently no basis to confirm whether or not the strains implicated in

human infection are from a human or an animal source. There is the added complication of multiple modes of transmission of certain zoonotic pathogens, including through food and secondary transmission.

The long-established World Health Organization (WHO) definition of zoonoses, "those diseases and infections (the agents of) which are naturally transmitted between vertebrate animals and man" (WHO/FAO 1959), was used in this meeting. However, by their nature, (re)emerging pathogens will have incomplete data, and adherence to such a strict definition should not exclude consideration of potential waterborne zoonotic pathogens because some component of their biology or life cycle is currently unknown.

1.2 FUTURE PERSPECTIVE

1.2.1 Driving forces

Available evidence suggests that (re)emerging zoonotic waterborne pathogens will continue to be recognized as being of significant and increasing public health concern due to a range of underlying driving forces. These include:

- changing patterns of water use;
- population factors, including growth, migration, and varying proportions of immunocompromised individuals;
- increasing travel and recreational activities;
- water scarcity, climate change, and severe weather events;
- conflicts and disasters;
- increasing urbanization and colonization of new habitats;
- increasing demand for animal protein and fresh vegetables in the diet;
- increasing use of antibiotics in animals and humans;
- increasingly concentrated animal husbandry practices and increased usage of concentrated feedlots;
- density of domestic pets;
- ecosystem disturbance; and
- international trade patterns in animals, animal products, and other foods.

A number of pathogen characteristics are important in the (re)emergence of zoonotic waterborne pathogens, including their capacity to adapt, mutate, or recombine in response to underlying driving forces such as those listed above and their capacity to acquire resistance to antibiotics.

The consequences of these driving forces strongly indicate that human exposure to zoonotic pathogens through water and the incidence of zoonotic

disease are likely to increase. The impact of these factors will likely differ between and among developed and less developed countries. People without access to improved water supply and sanitation are at higher risk of contracting waterborne zoonoses. Populations with no access to improved water sources or with access to intermediate service will persist for the foreseeable future; for example, just to halve the proportion of people unserved by 2015, 2.2 billion additional people will need access to sanitation and 1.5 billion will need access to water in Africa, Asia, Latin America, and the Caribbean alone (WHO/UNICEF 2000).

Overall human population growth will continue. Urban populations of Africa, Asia, Latin America, and the Caribbean are expected to increase dramatically — Africa more than doubling, Asia nearly doubling, and the urban population of Latin America and the Caribbean predicted to grow by almost 50%. Additionally, populations themselves are changing, characterized by larger proportions of vulnerable subpopulations (e.g., very old or very young, pregnant, infected with human immunodeficiency virus [HIV]). Vulnerable groups may suffer higher rates of infection and more severe illnesses.

Urban agriculture, including the raising of livestock, is a growing phenomenon in many cities and may also increase human contact with animal excreta and abattoir wastes.

Urbanization is likely to lead to increasing populations of urban wildlife such as rats, foxes, skunks, possums, and other opportunistic species and increased human exposure to both feral and domestic animals. The implications of this for human health will depend on the amount of excreta that enters water resources, the nature and reliability of the drinking-water supply, the use of water for recreational or irrigation purposes, and the degree of contamination of water that supports harvest fisheries.

Population growth and (especially) affluence tend to increase demand for meat products. Increasing meat protein demand will likely be satisfied largely through intensified agriculture, particularly in less developed countries. Evidence suggesting that this leads to significant waterborne exposure is now becoming available (e.g., *Campylobacter* in New Zealand). The significance of water in the overall risks of zoonotic infection relates to its role not only in transmission directly to humans or through contamination of food, but also as a vehicle in the transmission to flocks and herds, thereby leading to increased overall environmental contamination and risk of human infection.

Affluence also leads to larger pet populations (including exotic animals). This increases the possibility that diseases carried by pets can be transmitted through water to people (e.g., infections from aquaria, such as *Comamonas* bacteraemia associated with keeping exotic fish or toxoplasmosis from cats).

1.2.2 Control strategies

Opposing the driving forces discussed in section 1.2.1 is the array of public and animal health and other interventions that can control adverse human health impacts. Health risks can be managed through source water protection, water treatment, water distribution, point-of-use treatment, and other interventions, such as vaccination (animals, humans, or both) or risk communication especially to susceptible populations. Source controls and close-to-source controls have particular significance, as they act on a range of known, potential, and unknown pathogens. The implementation of animal waste management systems is likely to lag behind the increase in intensified agricultural production, leading to increasing contamination of water sources and an increase in waterborne zoonoses.

Although (re)emerging pathogens are characterized by uncertainty and inadequate information, few potential zoonotic waterborne diseases present truly new challenges to waterborne disease control per se, and many can be indexed by better characterized agents. Benchmark pathogens that could be applicable to some of these situations include viruses (enterovirus, hepatitis A virus [HAV], and poliovirus), bacteria (*E. coli*), protozoa (*Cryptosporidium* and *Entamoeba*), and helminths (*Ascaris*).

A future challenge will be to better coordinate the efforts of professionals from the variety of sectors involved in human and animal health to anticipate emerging pathogens and reduce water-related transmission via effective control measures. It will be necessary to determine if current management strategies are protective of human and animal health or whether new approaches need to be developed and implemented. In the pursuit of the best use of limited resources for public health benefit, regulators, engineers, and other stakeholders require risk-based targets. The hazard analysis and critical control point/water safety plan concept should be applied to the prevention and control of waterborne zoonoses at all water cycle stages as well as to food products that may be exposed to waterborne zoonotic pathogens. For maximum beneficial impact on public health, management of waterborne zoonoses should be integrated into control of zoonoses through all routes, including food, contact, and water, and control of waterborne disease from diverse sources, as specified in the Stockholm framework (Bartram *et al.* 2001).

1.3 EVALUATION OF ZOONOTIC WATERBORNE PATHOGENS

There are a large number of pathogens potentially transmissible by the waterborne route. Among these are pathogens that are frequent or rare causes of human

disease in any given "setting" and pathogens for which water may be a dominant or minor route of transmission.

The area of (re)emerging pathogens is one in which available information is incomplete. It is normally necessary to make assumptions and/or extrapolations or utilize analogies in order to make informed decisions. The degree of uncertainty makes it important to recognize that lack of information implies neither presence nor absence. In other words, lack of evidence does not mean that the pathogen does not exist in a certain region; instead, it may be the case that no one has looked for it.

There is a need for simple tools or criteria to discriminate among the many potential pathogens and to identify those of particular relevance to waterborne disease and its prevention. It is useful to distinguish between pathogens emerging because of increased recognition and those emerging because of increasing absolute or relative disease occurrence.

1.3.1 Approach

The credibility of any new or emerging zoonotic source of waterborne infectious disease must be established. The criteria for such a pathogen are twofold: first, the credible demonstration of a zoonotic phase, including those that may sporadically traverse species barriers; and second, the credible demonstration of a waterborne transmission route to humans. Several factors can be reviewed in order to determine whether a pathogen arising from an animal reservoir is a potentially significant emerging waterborne pathogen:

- pathogen adaptability;
- introduction into the environment;
- extent and proximity of animal reservoirs;
- persistence of pathogen in the environment and resistance to pollution control measures;
- human behaviour;
- outcome factors (infectivity, severity of adverse health impacts, human immune status); and
- public health factors (potential contribution of waterborne route to overall burden of disease).

These factors may be assessed by comparison with a "group benchmark" pathogen — a pathogen that has been well studied and for which effective control strategies have been developed. Such a benchmark organism must be one that is well documented in all aspects, including its interaction with the water environment. Viruses, bacteria, protozoa, and helminths are the minimum "groups" for which appropriate benchmarks are required (prions may be

considered when more information on their fate and transmission becomes available). A simple system can then be used to compare a potential pathogen with locally relevant benchmarks. It should include a comparison with the benchmark pathogen for a particular factor (e.g., environmental persistence, resistance to chlorine disinfection). While subjective, such an approach could lead to a mechanism for prioritizing risk management decisions.

1.3.2 Pathogen adaptability

Specific factors may encourage rapid/accelerated evolution by pathogens. A well described example concerns the frequent use of antibiotics, leading to increased prevalence of multidrug-resistant strains of bacteria, which may then be introduced to human populations through waterborne and other routes. Viral mutations — such as those leading to the outbreak of severe acute respiratory syndrome (SARS) — and the capacity of some helminths to infect an increasing range of hosts indicate that such evolutionary trends are not restricted to bacteria.

1.3.3 Introduction to the environment

The likelihood that a pathogen will be transmitted through drinking-water, recreational water, or other routes is dependent on numerous factors.

Some of these reflect the characteristics of the pathogen itself, including resistance to environmental "stressors," such as ultraviolet light, desiccation, salinity, temperature, etc.

Along with trends in animal populations, the prevalence of a given pathogen (e.g., *Salmonella*) among animal populations may vary, and the intensity of shedding may be influenced by factors with their own underlying trends, such as the age of animal or seasonality.

There may be increasing introduction of a zoonotic pathogen to the water environment, arising, for example, from changes in control and management practices such as introducing sewage without treatment or through the land application of inadequately treated biosolids produced from agricultural processes. Contamination results from the discharge/runoff of untreated waste or inadequately treated wastewater into water bodies.

In some cases, pathogens have been brought into new geographic areas through the importation of infected animals (both livestock and exotic pets).

1.3.4 Extent and proximity of animal reservoirs

As described in chapters 11, 12, 24, and others, the density of animals (both domestic and wild) in a catchment basin is related to the amount of zoonotic pathogens that enter water sources. A pathogen that can infect a wide range of animals, both domestic and wild, may thus pose more of a challenge than a pathogen that infects only one host rarely present in a catchment basin.

Other factors concern the behaviour of the reservoir animals, the significance of which may vary widely between settings. Thus, pathogen carriage by, for example, urban rats, foxes, and other animals (including pets) may have limited significance where universal reliable piped water supply has been achieved, but it may be more significant where such a supply is absent or unreliable (as is the case for 1.1 billion people at present).

In some areas, it may be possible to manage pathogen reservoirs (both livestock and other animals) by excluding them from catchment basins or by preventing their contact with water (e.g., by installing fences to keep animals from entering rivers). Management of animals in catchments figures significantly in water safety plans implemented in Australia, for example (see WHO 2004 for more information on water safety plans and source protection).

1.3.5 Environmental resistance/persistence

Survival of zoonotic pathogens in water and the environment is a key factor in the transmission of waterborne zoonoses. Many zoonotic pathogens of concern (e.g., *Cryptosporidium*, *Giardia*, *E. coli* O157:H7) can survive for months in the environment under the right conditions. This increases the probability of waterborne transmission. In some cases, pathogens may be able to regrow in the environment (e.g., *Salmonella* has been shown to multiply in the natural environment; see chapter 14).

1.3.6 Human behavioural factors promoting exposure

Human behavioural factors include travel, migration, development of agricultural land, consumption of new food sources, new modes of food preparation, cultural factors, and reuse of wastewater. Migration of humans into undeveloped areas and clearing land for agriculture lead to increasing contact with animals, thereby potentially enhancing waterborne zoonosis risk. The human population is predicted to double between 1990 and 2015 — most of this growth will occur in less developed countries. Rural populations moving into urban areas in search of work, farmers bringing livestock into urban areas, and urban areas expanding into rural areas will all affect potential zoonotic disease foci.

1.3.7 Outcome factors

The primary interest is whether the agent is associated with human disease, as illustrated by the potential for hepatitis E virus (HEV) transmission from porcine reservoirs through water to humans. A range of factors, including age, differential infectivity, dose–response, and genetic differences in susceptibility, come into play when evaluating the outcome of an infection. Immunity in the population (i.e., due to vaccination or previous exposure) varies widely in and between developed and less developed countries. In addition, populations with high or low circulation patterns may interact with the differential levels of immunity to contribute to spread of disease. Malnutrition and the incidence of other diseases will also contribute to human susceptibility to waterborne zoonotic infections. In many areas, an increasingly large percentage of the population is considered to be vulnerable due to age, disease, pregnancy, etc. (see discussion in chapter 4).

It is also necessary to compare pathogens with one another in order to consider their relative public health significance. A pathogen causing self-limiting diarrhoeal disease with a low case fatality rate (e.g., *Cryptosporidium* in developed countries in non-immunocompromised persons) is clearly of lesser priority than one causing more severe disease requiring medical treatment (e.g. *E. coli* O157:H7) or one associated with delayed sequelae (e.g., *Campylobacter*, associated with Guillain-Barré syndrome) or with a high case fatality rate (e.g., *Cryptosporidium* in immunocompromised patients or HEV in pregnant women). In making these comparisons, it is essential to take account of locally specific vulnerability factors. Among these, immunosuppression (especially due to HIV infection and immunosuppressive treatment) is important and increases the importance of the availability of clinical treatments. Other infections may depend on factors such as age of first exposure (some, such as HAV, are relatively minor when acquired young; others, such as *E. coli* O157:H7, present more severity in childhood).

Combined consideration of these factors has been facilitated by the development and increasing application of metrics, especially disability-adjusted life years (DALYs) (WHO 2004). It is possible to estimate a DALY score for an "average" disease course for a given pathogen in specific circumstances and thereby compare it with a chosen benchmark. For simple scoring purposes, more/similar/less severe than the selected benchmark may be useful. Local factors such as access to health care facilities have an important impact on disease control and thereby DALY "scores."

1.3.8 Public health factors

Health risks from waterborne zoonoses should be seen in the context of the overall level of gastrointestinal (or other) disease within a given population. WHO has developed a harmonized framework for the development of guidelines and standards for water-related microbiological hazards (Bartram *et al.* 2001; see chapter 28). This framework involves assessing health risks prior to the setting of health targets, defining basic control approaches, and evaluating the impact of these combined approaches on public health status. Future WHO water-related guidelines are being developed in accordance with this framework.

For a given pathogen, the waterborne route of transmission may be of either limited or overwhelming importance. Thus, for example, where drinking-water is routinely disinfected, salmonellae are unlikely to be significantly distributed by this route; thus, salmonellosis is largely a foodborne infection in well managed water supplies, although waterborne transmission may occur where safeguards break down. It is inappropriate to extrapolate this conclusion to the areas where drinking-water safety is unsure. Local factors must be taken into account in determining whether a pathogen is likely to contribute a minimum of, for example, 5% of total disease transmission from waterborne routes; outbreaks and sporadic cases may need to be considered separately.

1.4 PATHOGEN GROUPS

1.4.1 Viruses and prions

Viruses demonstrate considerable host specificity. The direct human health significance of animal viruses with close human relatives (e.g., HEV in humans and pigs) remains unclear. However, their existence may suggest a historic process of crossover between species. A recent example of crossover is SARS, for which faecal droplet transmission appears to have been likely in some, albeit unusual, settings. Animal viruses with close human relatives, including some caliciviruses, coronaviruses, enteroviruses, picornaviruses, and rotaviruses, and specific viruses, such as HEV, coxsackievirus B5, and Nipah virus, may warrant further research and monitoring for their potential as waterborne zoonotic pathogens.

With respect to prions, bovine spongiform encephalopathy and chronic wasting disease should also merit further investigation as potentially waterborne zoonotic pathogens.

1.4.2 Bacteria

There are several well documented waterborne zoonotic bacterial pathogens, including *Salmonella*, *E. coli* O157:H7, *Campylobacter*, and *Yersinia*. The prevalence of these organisms depends on the nature of the source and the water supply, excreta and other waste disposal processes, and other environmental and climatic factors. Bacteria are a versatile group; their capacity to adapt to the varying conditions that result from the interaction of different driving forces is well recognized. In addition to continued research on the bacteria mentioned above, *Mycobacterium avium* (ssp. *paratuberculosis*) and *Leptospira* may warrant increased investigation and monitoring.

1.4.3 Fungi and microsporidia

Fungi are not normally considered as zoonotic waterborne pathogens. However, there is a significant range of both yeasts and moulds that are zoonotic, including *Trichophyton* spp., *Cryptococcus*, and *Coccidiodes*. Generally transmitted by contact, ingestion, or inhalation, credible waterborne routes may at some stage be demonstrated.

Four species of microsporidia have been found in a wide range of animals as well as in humans. They are commonly found in surface waters, and one suspected waterborne outbreak was reported in Lyon, France. Previously considered to be protozoans, molecular techniques suggest that microsporidia are more likely to be fungi.

1.4.4 Protozoa

Protozoan pathogens originating from animal and human waste have been recorded from water sources throughout the world. A number of well documented waterborne zoonotic protozoa exist, including *Giardia intestinalis*, *Cryptosporidium*, *Toxoplasma gondii*, and *Entamoeba histolytica*. There are other potential candidates, including *Cyclospora*, where waterborne transmission has been demonstrated but a zoonotic route remains to be established.

1.4.5 Helminths

Zoonotic waterborne helminth infections, including those caused by *Ascaris lumbricoides* and *Trichuris trichuria*, account for millions of human cases worldwide. There are also emerging helminthic parasites, some of which may occasionally be transmitted by water from a zoonotic reservoir. In most parasites, only one host is required for completion of the life cycle, and there is usually

strong host specificity. Increasingly, multiple host susceptibility is being recognized, enhancing the likelihood of zoonotic waterborne transmission. In some helminthic parasites, the human host may be only one of several satisfactory hosts. Humans may become only incidentally involved, while animals act as the reservoir for the parasite. Evidence suggests that *Fasciola hepatica* may have significant transmission through drinking-water in some geographic regions. More research and monitoring of this parasite are warranted.

1.5 EXPOSURE/TRANSMISSION ROUTES

Excreted material and other animal waste products are the predominant sources of waterborne zoonotic pathogens. The pathogens use these materials as transport vehicles from the animal reservoir to the particular water environment, where their stability in that environment will influence the infectivity and thereby the risk to humans. The key concentrated sources of the pathogens are therefore:

- wastewater/biosolids from municipal and agricultural processes;
- faeces;
- urine; and
- carcasses and abattoir waste.

Zoonotic pathogens cross the species barrier from vertebrate animals to humans; the waterborne transmission route is one route of waterborne transmission. Water-related disease transmission routes have been described by a number of authors, introducing the concept of waterborne infections, water-washed infections, water-based infections, and infections with water-related insect vectors. The inhalation of contaminated aerosols and ingestion of foods (e.g., shellfish) that have been in contact with contaminated waters are also important. In the following list, the principal vehicles of transmission of waterborne zoonotic pathogens are described:

- drinking-water;
- water contact;
- water used in food preparation, production (including agricultural produce and seafood products), and processing; and
- aerosolized materials.

Identification of the sources and transmission routes of waterborne zoonotic infections is essential for developing and implementing appropriate control mechanisms (see chapters 5, 24, 25, and 26).

1.6 CONCLUSIONS AND RECOMMENDATIONS

The state of the evidence concerning waterborne transmission of zoonotic pathogens is incomplete but suggests that waterborne zoonoses pose a significant threat to public health in many countries. To better control threats to public health from waterborne zoonoses, both now and in the future, the expert group made the following conclusions and recommendations:

- People without access to safe water and sanitation are the most likely to suffer from waterborne zoonoses. Accelerating efforts to increase access to safe water and sanitation would have dramatic beneficial health impacts for a significant portion of the world's population.

- There is a need for a risk-based approach to setting standards for waterborne zoonotic infections. The group recommended that WHO take an active role in the development of evidence-based guidance for treating animal waste. This guidance should promote investigation of cost-effective technologies to meet management targets for different situations.

- Further efforts are needed to develop networks and facilitate regional cooperation that lead towards international harmonization for control of zoonotic pathogens across multiple exposure routes (water, food, contact).

- There is inadequate information on differentiation of human versus animal strains of human pathogens, both in the field (e.g., pathogen typing and microbial source tracking in order to orient control activities to priority concerns) and analytically (e.g., relative infectivity and pathogenicity). Both of these areas are priorities for targeted research.

- Improving understanding is important in harmonizing approaches to waterborne zoonoses. Currently available surveillance data, on both sporadic and outbreak diseases, are of limited use in understanding the importance of zoonotic waterborne infection. Surveillance for waterborne disease in general and waterborne zoonoses in particular has failed to provide a meaningful indication of the associated burden of disease, even in countries with established systems.

- Efforts are needed to devise tools, systems, and management responses to support outbreak investigation and response and to establish effective water-related disease surveillance.

- Anticipatory, "horizon-scanning" perspectives are a key to identifying (re)emerging waterborne zoonoses, as well as identifying new animal diseases, investigating their capacity to be zoonotic, and determining their capacity to be waterborne. Such "horizon scanning" can be successful only if appropriate organizational structures representing both animal and public health sectors are established.

- Molecular biology is a key technology for the future and is currently essential for the characterization of all zoonotic pathogens. However, there is a continuing need for reliance on traditional techniques of identification for many pathogens. This is particularly true for developing countries that are in need of quick, accurate, low-cost analytical tools that can be applied in remote areas. Research on real-time treatment process indicators related to pathogen removal in a range of technological situations is also needed.

- The importance of cross-sector and interdisciplinary collaboration between sanitary microbiologists and engineers, veterinary scientists, and agricultural scientists and engineers in controlling waterborne zoonoses cannot be understated. Networks and regional cooperation continually arise as cross-cutting themes and must be established. Evidence of situations where collaboration could have mitigated the impact of emerging waterborne zoonotic pathogens is amply available in the cases of both cryptosporidiosis and campylobacteriosis. Funding will be more effectively directed should the creation of such coordinating bodies occur, incorporating an appropriate range of different expertise.

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Section II

An introduction to emerging waterborne zoonoses and general control principles

J. Bartram and R. Carr

Infectious diseases cause approximately 26% of all deaths worldwide and 31% of all disability. Water plays a role in the transmission of a significant number of these diseases. In the last 20 years, it has been recognized that many diseases are caused by emerging or re-emerging pathogens, 75% of which are zoonotic. Zoonoses can emerge in several ways — for example, as discrete events that lead to establishment of the pathogen in the human population and subsequent human-to-human transmission (e.g., human immunodeficiency virus) or as "spillover" events where the pathogen resides in animal reservoirs, which occasionally results in the transmission of the disease to humans. Chapter 2

 Waterborne Zoonoses: Identification, Causes and Control. Edited by J.A. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D.O. Cliver, G.F. Craun, R. Fayer, and V.P.J. Gannon. Published by IWA Publishing, London, UK. ISBN: 1 84339 058 2.

further explores some of the ways in which waterborne zoonoses emerge, especially with regard to how changing farming practices influence these events.

Chapter 3 provides a framework for determining what diseases are emerging, what diseases are waterborne, what diseases are zoonoses, or all of the above. The author also discusses pathogen properties that lead to environmental transmission and proposes a modification of the Bradley classification system of water-related diseases to include disease transmission through aerosols and the consumption of contaminated shellfish/seafood. Applying the proposed classification system to determine whether an organism is both waterborne and zoonotic, the author develops three case-studies to test the validity of the criteria.

Chapter 4 explores a variety of factors that influence the distribution and emergence of zoonoses. Many factors relating to the distribution and emergence of zoonoses are related to human activities, including migration, travel, urbanization, land development, changes in dietary practices, and the increasing use of antibiotics. The ability of pathogens to change characteristics, climate change, and the increase in the size of vulnerable human subpopulations also affect the impact and transmission of emerging waterborne zoonoses.

Many of the emerging waterborne zoonoses are difficult to manage. Chapter 5 explores the development of the concept of a control envelope for evaluating emerging pathogens against risks posed by better understood organisms. Effectively managing health risks from emerging waterborne diseases requires a cross-sectoral approach. A variety of risk management tools are available (e.g., quantitative microbial risk assessment, hazard analysis and critical control points, water safety plans, disease surveillance) but must be extended to encompass the entire spectrum of the control envelope to maximize the protection of human and animal health.

2

Emerging zoonotic diseases and water

C. Bolin, C. Brown, and J. Rose

2.1 FACTORS IN DISEASE EMERGENCE

The global burden of infectious disease continues, despite landmark advances in the treatment and prevention of disease. In addition to the well described microbial and parasitic infections of humans and other animals, emerging infectious diseases have received increased attention in recent times. An infectious disease is considered to be emerging if it appears in populations of humans or other animals for the first time or has occurred previously but is increasing in incidence or expanding into new areas.

The emergence of an infectious disease is a complex process involving biological, social, and ecological factors. The Institute of Medicine (1992, 2003) has identified 13 factors key to the emergence of infectious diseases in humans:

 Waterborne Zoonoses: Identification, Causes and Control. Edited by J.A. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D.O. Cliver, G.F. Craun, R. Fayer, and V.P.J. Gannon. Published by IWA Publishing, London, UK. ISBN: 1 84339 058 2.

- microbial adaptation and change;
- human vulnerability;
- climate and weather;
- changing ecosystems;
- economic development and land use;
- human demographics and behaviour;
- technology and industry;
- international travel and commerce;
- breakdown of public health measures;
- poverty and social inequality;
- war and famine;
- lack of political will; and
- intent to harm.

We posit that many of these factors apply to the emergence of infections in domestic and wild animal populations as well.

With the inherent complexity of the biological and social systems involved in disease emergence, it is not possible to accurately predict the infectious agents destined to emerge. However, studies of infections that have emerged (Ewald 1996; Cleaveland *et al.* 2001; Taylor *et al.* 2001) in populations of humans and other animals have revealed the following:

- Viruses, prions, bacteria, and protozoa are more likely than fungi or helminths to be associated with emerging infections.
- Zoonotic pathogens comprise 75% of emerging infectious diseases.
- Pathogens that are subject to relatively frequent mutation or genomic reassortment events (e.g., RNA viruses and viruses with segmented genomes) are more likely to emerge.
- Pathogens that infect multiple hosts or pathogens that infect species that can harbour multiple closely related agents, providing an opportunity for reassortment or recombination (e.g., severe acute respiratory syndrome in cats), are likely to emerge.
- Agents transmissible by more than one route or by indirect contact (e.g., water, food, environmental contamination, vectors) are likely to emerge.

Zoonotic infections clearly play a central role in emerging infectious disease in humans. The exposure of humans to zoonoses follows two major epidemiological patterns. In some cases (e.g., human immunodeficiency virus), there is a point source of exposure of humans to the zoonotic agent, and then the disease is transmitted among, and often maintained in, humans. In other cases (e.g., Ebola

virus), animals serve as the reservoir for the agent, and the infection "spills over" to humans, with little transmission between humans. In this latter situation, the infection will die out in the human population without constant reintroduction from animal reservoirs.

Many emerging zoonotic diseases are transmitted by indirect contact — foods, water, environmental contamination, vectors, etc. — and are not reliant on direct contact between human and wild or domestic animal hosts for transmission. These same features may also be related to increased virulence of the pathogens (Ewald 1996, 1998). Analysis of these factors suggests that the interaction between animals (domesticated and free-ranging) and the water supply may be a rational area of concern for disease emergence. Some of these emerging infections may involve transmission of disease to humans through drinking-water or recreational water activities and the spread of infection to, and within, populations of animals.

2.2 ANIMAL AGRICULTURE AND POTENTIAL ENVIRONMENTAL IMPACT

It is projected that the number of people in the world will be 7.7 billion by 2020 and 9.4 billion by 2050, with the largest increase coming from the developing world. Over the last 25 years, per capita meat consumption in developing countries grew at 3 times the rate in developed countries. If current trends continue, which is predicted, diets will continue to shift from plant-based to increased consumption of meat and dairy products. It is estimated that global livestock production will have to double by 2020 to supply needs. This demand-driven increase in animal agriculture has been termed the "livestock revolution" (Bradford *et al.* 1999; Delgado *et al.* 1999).

Traditional livestock systems are integrated, sustainable, low input, and "closed loop." Historically, animal production in developing countries has been of this type. In traditional livestock rearing, manure is vital fertilizer, and animals return nutrients to the soil in forms that plants can readily use. In addition, in some areas, manure is a vital resource to be used as fuel or building materials. In most traditional systems, manure is effectively utilized and is not a disposal issue (Sherman 2001).

In comparison, modern or industrialized systems are high-input, open-loop systems. These industrialized systems provide significant efficiency in terms of economy of scale, consistency, and value to consumers. Industrialized systems began in the USA over 60 years ago with the poultry industry and have now become the norm for the swine industry also. It is more difficult to industrialize the cattle industries, largely because they are ruminants and benefit from grazing,

but beef feedlots and large dairies are examples of industrialization for this species as well (Sherman 2001). Animal agriculture in the developed world is almost entirely of this industrialized type, so that today, throughout the developed world, there are fewer farms managing larger numbers of animals. Manure produced in industrial systems is usually in excess of agronomic requirements and figures as a major disposal issue in all of these intensive animal production facilities.

Today, there are approximately 1.2 billion cattle, 800 million pigs, and 10 billion chickens in the world. Of the cattle and pigs, three-quarters are resident in the developing world, usually in traditional systems. With respect to chickens, industrialization has occurred more extensively even in the developing world, so that fully half of all the world's chickens are reared under conditions of intensive husbandry (Mason and Crawford 1993). Livestock numbers have been increasing while agricultural land has been decreasing, at a rate of 7% per decade, due to urbanization, commercial forces, and land degradation (Oldeman *et al.* 1991). Livestock densities in both the developed and developing worlds range between 5 and >6000 kg/km^2, with the greatest concentrations in India, China, and Europe (AGA, undated).

The "livestock revolution" predicts that much of the increase in animal production will come from expansion of intensive systems of agriculture in the developing world (Bradford *et al.* 1999; Delgado *et al.* 1999). In fact, globally, the trend is clearly towards industrialization. Intensive systems of animal agriculture are increasing at the rate of 4.3% per year, with much of that increase happening in Asia, South America, and North Africa (Bradford *et al*. 1999; Delgado *et al.* 1999). As traditional systems are replaced by industrial agriculture, livestock density increases.

As mentioned above, manure disposal from industrialized facilities is problematic. Numerous systems have been developed to dispose of or recycle the manure, but many have environmental and health considerations.

Application to fields is the time-honoured manner of manure disposal, but it can cause considerable problems with nitrate leaching if the ground is frozen, there is excess rain, or the soil is very sandy. Runoff of manure into watersheds causes increased microbial proliferation, high biochemical oxygen demand, and altered aquatic microenvironments (Hooda *et al.* 2000). For pigs, 80% of waste is held in liquid storage systems for subsequent decontamination and application to land (De Haan *et al.* 1997). These earthen basins and lagoons work well as long as there are no significant compromises in integrity. Catastrophic events can ensue after natural disasters such as floods or earthquakes, or even because of faulty design. For broilers, virtually all waste goes into stacking pits. After three or four broods are raised in a house, the litter, which contains fibrous material, spilled feed, feathers, and bird excreta, is collected and held in a heat-generating pile for some weeks until pathogenic organisms are destroyed. This material is fed to

cattle, who consume it free-choice in self-feeders as a source of fibre and protein (Rankins *et al.* 2002). Beef and dairy faeces are usually collected as solids and later applied to land. There is an anaerobic slurry storage period, which is designed to decrease microbial content, but it has been shown that certain pathogenic organisms can survive beyond the designated period (Pell 1997; Hooda *et al*. 2000).

Industrialized systems of animal agriculture currently produce 7 billion tonnes of waste per year. As these intensive open-loop systems continue to grow by 4% per year, it is estimated that in 2020, there will be 18 billion tonnes of animal waste for disposal (De Haan 1997).

Some countries have become fairly aggressive in their policies regarding handling of manure. For instance, in the Netherlands in 2000, federal regulations were instituted for any farm housing at least 2.5 livestock units (LU) per hectare. This 2.5 LU is equivalent to the number of animals that excrete 102.5 kg of manure phosphate per year, or 2.5 dairy cattle, 13.9 growing pigs, or 427 broilers. There are 50 000 farms in the Netherlands that fell under this new restriction. Any farm exceeding the allowed production must pay a tax (Jongbloed and Lenis 1998). Over the next decades, industrialized production will increase dramatically in the developing world, where, historically, environmental regulations are much more relaxed.

Great strides have been made in the mapping of food consumption, human dimensions, and livestock-oriented production systems, as well as water use (Gerbens-Leenes *et al*. 2002; Kruska *et al*. 2003). While the tonnage of animal waste has been estimated based on nitrogen in animal excreta (Bouwman and van der Hoek 1997), little effort has been made to address the microbial and zoonotic infectious disease potential due to these changes associated with loading of manure onto land and water.

Faeces and urine from both humans and other animals are likely the largest source of environmental loading of pathogens associated with waterborne disease transmission (Figure 2.1). Loading, prevalence, concentrations, survival, and infectivity need to be compiled for the various pathogens associated with urine and faeces. We must also recognize that more than half the bacteria in the human intestine and more than 99% of environmental bacteria have not been cultured or characterized (Relman 1998; Kroes *et al.* 1999). This is almost certainly also true for the broad array of domestic and wild animals in our environment. Therefore, there is always a risk of the emergence of an as yet unrecognized agent of disease, representing a real challenge to public and animal health officials attempting to anticipate and prevent emerging infectious diseases.

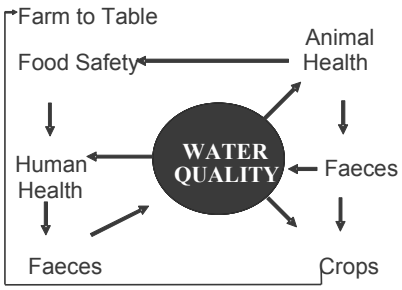

Figure 2.1. Interactions of humans and animals associated with faecal contamination of water.

2.3 RISK ASSESSMENT AND STRATEGIES

An analysis of the known factors that favour disease emergence indicates that zoonotic diseases with the potential to be waterborne are likely to be significant risks for the future. Therefore, assessment of risks and vulnerabilities of modern water treatment processes and also of the various aspects of water treatment processes around the world is essential and must be an ongoing, forward-thinking process. Our definition of "microbiologically safe water" is evolving from the utopian concept of pathogen-free water to a more realistic goal of providing drinking-water for human consumption (European Union Council Directive 98/83/EC and WHO guidelines as quoted by Szewzyk *et al.* 2000) to be "free from microorganisms and parasites … which, in numbers or concentrations, constitute a potential danger to human health." These statements must be interpreted along with a definition of acceptable risk. The US Environmental Protection Agency previously suggested (but has not concluded) that an acceptable risk of infection from potable water might be 1:10 000 per year, i.e., 1 case of the disease in 10 000 exposed persons per year (J. Cotruvo, personal communication, 2003). These types of guidance can be used to conduct mathematical modelling of the risk to the public and animal health of various agents. However, to conduct this risk assessment, key pieces of information are required to be known, or at least predictable, based on mathematical models: 1) the infectious dose–response of the pathogen of interest; 2) the concentration at which the agent can be found in water; and 3) the impact of various water treatment strategies on the reduction in infectivity of the pathogen of interest. While we have reasonable estimates of these factors for some traditional waterborne diseases, there are large gaps in our knowledge of the current and

future risks to the water supply in regards to emerging infectious diseases in general, and zoonotic diseases in particular.

Additional tools and strategies are needed to assess the biological impact of livestock and wildlife populations on the water supply (e.g., source tracking capability), the diversity of organisms in faeces and urine of humans and other animals, and what role on-farm manure handling procedures play in mitigating these risks. This information will help define changes in water treatment required to provide safe, potable water to a growing and increasingly stressed (nutritionally and immunologically) world population in the face of emerging infections and infections that are resistant to current practices. In some cases, this may require additional treatment of water coincident with ingestion by either humans or other animals. As we develop alternative strategies, careful attention must be paid to developing systems and safeguards that work in relatively underdeveloped environments as well as state-of-the-art water treatment facilities.

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3

What are the criteria for determining whether a disease is zoonotic and water related?

C.L. Moe

3.1 INTRODUCTION

A number of new diseases have been recognized in the past quarter century. The term "emerging disease" was used in a seminal Institute of Medicine report in 1992 to describe infectious diseases whose incidence in humans has increased within the past two decades or threaten to increase in the near future (Lederberg *et al.* 1992). Even before the etiological agent in an emerging infectious disease can be identified or characterized, it is critical to understand the source of the agent and the likely and possible transmission routes so that effective prevention and control measures can be established as soon as possible.

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There are a number of newly recognized infectious agents that have been associated with outbreaks of water-related disease or appear to have the potential for waterborne transmission. How did we know that these diseases were water-related? There has been a recent surge in the number of new zoonoses and new forms of existing zoonoses that have been identified (Mahy and Brown 2000). How did we know that these infections were zoonotic? The knowledge that a newly recognized disease is water related and zoonotic is critical for planning public health protection measures. In order to better understand these classifications of "water related" and "zoonotic," it is useful to start with the definitions of these terms and examine the infections that are commonly accepted to fall within these definitions. We can then propose criteria for determining what is a water-related zoonotic disease and test these criteria on newly recognized diseases to see how well they work.

3.2 ZOONOSES

Zoonoses have been defined as "those diseases and infections (the agents of) which are naturally transmitted between vertebrate animals and man" (WHO/FAO 1959). Examples of zoonotic infections have been recognized among all the major groups of infectious agents: prions, viruses, bacteria, protozoa, and helminths. Some of these agents may infect only one type of animal and humans. Others may infect several types of animals as well as humans.

Wilson (2001) explained that there are two types of zoonoses: direct and indirect. In direct zoonoses, an infection is transmitted from animals to humans by direct contact with the animal via a bite, ingestion of animal tissue, or skin contact with an animal (Figure 3.1a). Examples of direct zoonoses are rabies, trichinellosis, and tularaemia. Indirect zoonoses (Figure 3.1b) involve transmission of the infectious agent from animals to humans via a vector or vehicle. Examples of indirect zoonoses include cryptosporidiosis, the plague (*Yersinia pestis*), ehrlichiosis (*Ehrlichia chaffeensis*), West Nile virus, and leptospirosis (*Leptospira interrogans*). Some infections, such as tularaemia, can be transmitted both directly via animal contact and indirectly via water ingestion or inhalation of infectious aerosols. For some zoonoses — avian influenza, Hendra and Nipah viruses, human immunodeficiency viruses HIV-1 and HIV-2, and Ebola virus — it is not clear how the agent was transmitted from animals to humans (Mahy and Brown 2000).

Another key differentiating factor between zoonoses is whether humans are a "dead-end host" (e.g., rabies virus) or whether subsequent human-to-human transmission can occur (e.g., HIV-1 and HIV-2 and cryptosporidiosis). In direct zoonoses, the pathogen is normally transmitted between animals only and is accidentally spread to humans. Because humans are not part of the normal

transmission cycle, we become a "dead-end host" (Wilson 2001). In some cases, transmission may be from the natural animal reservoir to another animal species and then to humans. One indication that the intermediate animal host may not be the true natural reservoir of the infectious agent is if the disease is often fatal in the animal — as in the case of Marburg virus in monkeys or Hendra virus in horses (Mahy and Brown 2000). Some zoonoses emerge when there is a host that is co-infected with human and animal strains of an infectious agent and a new reassortant strain is produced — such as avian influenza viruses that have surface antigens (haemagglutinin subtype H5 or H9) that are new to the human population and to which we have little or no immunity. In this example, pigs can be infected with both human and avian influenza viruses in addition to swine influenza and may serve as the host where reassortant virus strains are created. For some organisms, such as *Cryptosporidium*, it is difficult to differentiate between zoonotic and anthroponotic strains. The origin of some *Cryptosporidium* strains is predicted by molecular typing to be zoonotic, but the epidemiological evidence indicates that the predominant transmission is anthroponotic (Leoni *et al*. 2003).

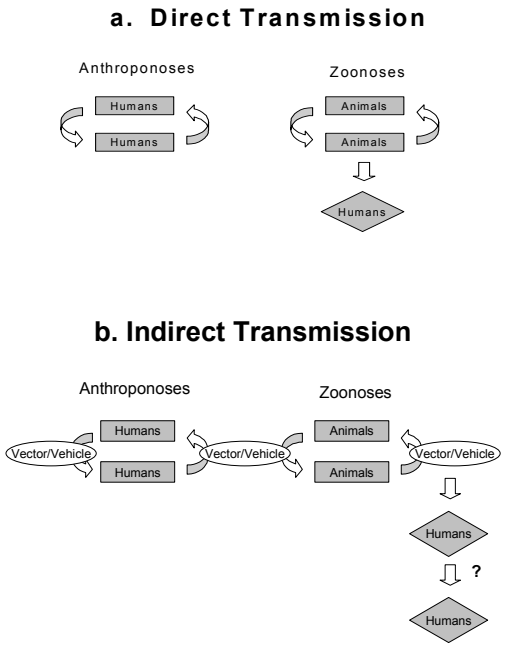

Figure 3.1. Direct (a) and indirect (b) transmission of infectious agents (from Wilson 2001).

3.3 WATER-RELATED DISEASE

3.3.1 Environmental transmission of infectious agents

Many factors affect the ability of an infectious agent to be transmitted through the environment (Figure 3.2) (Feachem *et al*. 1983). First, the infectious agent must enter the environment. For most water-related pathogens, the organism enters the environment via human or animal faeces deposited on land or in water. Some organisms enter the environment via human or animal urine (*Leptospira* and schistosomes). Other organisms may be directly discharged into water, such as guinea worm larvae, which exit via the skin. Some organisms live within mosquitos that must breed near water. It is not always known whether there is transovarial transmission and the organism is actually within the mosquito eggs that are deposited in water. The load of organisms entering the environment depends on the prevalence of the infection in the population of humans or animals, the concentration of the infectious agent in the faeces or urine, and how long an infected individual will shed the organism.

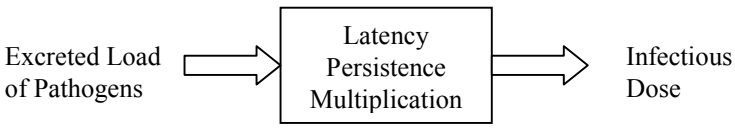

Figure 3.2. Environmental transmission of infectious agents (from Feachem *et al*. 1983).

Once the agent is in the environment, several factors affect the ability of the organism to be transmitted to a human host:

(1) The organism may require time in the environment to undergo further development before it becomes infectious. Latency is the time between the excretion of the pathogen and the time that it is infective to a new host. Most enteric viruses, bacteria, and protozoa have no latent period and are immediately infectious after excretion. Other organisms, such as helminths, typically require time in the environment to develop into an infectious stage and may pass through one or more intermediate hosts (e.g., schistosomiasis).
(2) The ability of the organism to persist in the environment is critical to its transmissibility. The longer an organism can persist, the more likely it is to have the opportunity to come into contact with a

susceptible host. Survival time in water depends on many physical factors (pH, temperature, sunlight) as well as characteristics of the organism. The survival times of water-related pathogens in water range from hours to years. Some pathogens, such as *Salmonella*, *Campylobacter*, and *Vibrio cholerae*, are capable of entering a dormant state described as "viable but non-culturable," which allows them to survive longer under adverse conditions in the aquatic environment and yet maintain their pathogenicity (Hunter 1997). Other pathogens have a stage in their life cycle, such as a spore or oocyst, that is environmentally resistant. *Vibrio cholerae* colonizes the surface of zooplankton, which permits prolonged survival in environmental waters between seasonal epidemics (Tamplin *et al.* 1990). The fate of the organism in wastewater and water treatment processes is also a key element in understanding the risk of waterborne transmission.

(3) The ability of the organism to replicate in the environment is also important. Under favourable conditions, some water-related pathogens can multiply in the aquatic environment. This is not possible for viruses and protozoa that are obligate parasites. However, some enteric and all aquatic bacterial pathogens can multiply in the environment and may reach high concentrations. Some water-related helminth pathogens may be amplified within an intermediate aquatic host.

The final element in this transmission process is the infectious dose or degree of water exposure necessary to transmit the infection. Infectious dose varies widely among environmentally transmitted organisms and is difficult to measure. Limited data on dose–response come from human challenge studies with healthy adult volunteers. The dose that induces infection in 50% of exposed individuals, described as the median infectious dose (ID_{50}), can range from about 10^9 colony-forming units of *Salmonella pullorum* (Teunis *et al.* 1996) to 10–1000 *Cryptosporidium* oocysts (Teunis *et al.* 2002). The ID_{50} can vary by the strain of microorganism and by the host population, depending on age and immune status.

3.3.2 Bradley's classification of water-related diseases

The classification of water-related diseases by Bradley (1977) provides a valuable framework for understanding the relationship between infectious disease transmission and water. This classification system's advantage is that it facilitates planning effective prevention and control measures for a variety

of water-related diseases, depending on the type of agent and transmission route. Bradley (1977) described four main categories of water-related infections: "water-borne infections," "water-washed infections," "water-based infections," and "infections with water-related insect vectors." Some infections may fit into more than one of these categories, and often water is not the only transmission route or even the major transmission route for some of these infections.

The "water-borne infections" are those classically recognized as waterborne disease, such as typhoid and cholera, where an enteric microorganism enters the water source through faecal contamination and transmission occurs by ingestion of contaminated water. Transmission by this route depends on 1) the amount of faecal contamination in the water, the concentration of pathogens in the faecal contamination (determined by the number of infected persons or animals in the environment), and the survival of the pathogenic organism in water; 2) the infectivity of the organism; and 3) individual ingestion of (exposure to) the contaminated water. Control of these infections is generally through improvement of microbiological water quality, either through water treatment or source protection.

"Water-washed infections" are diseases due to poor personal and/or domestic hygiene. These diseases are not due to the presence of infectious agents in water but rather to the lack of readily accessible water. This limits washing of contaminated hands and utensils and thus permits transmission of infectious agents, such as *Shigella* spp. Transmission is again related to the presence of faeces from an infected individual, infectivity of the organism, amount of faecal contamination on the hand or surface, and the persistence of the organism on surfaces. Lack of water for bathing also facilitates the spread of diseases that affect the eyes and skin, such as trachoma, conjunctivitis, and scabies. Control of these diseases is through provision of greater quantities of water, closer, easier access to water, and education to improve personal and domestic hygiene.

"Water-based infections" are worm infections in which the pathogen must spend a part of its life cycle in the aquatic environment. This category is further subdivided into diseases acquired by ingestion of water and diseases acquired by contact with water. The prototype infections in this category are dracunculiasis, due to ingestion of water contaminated with guinea worm (*Dracunculus medinensis*), and schistosomiasis, which is transmitted by contact with water contaminated with species of the trematode genus *Schistosoma*. The original source of the guinea worm is larvae discharged from the female worm that lies in a vesicle, usually on the lower leg or foot of an infected human. The larvae are discharged when the vesicle is immersed in water and are then ingested by a copepod (genus *Cyclops*), where they

develop into the infective stage. Humans become infected when they ingest water containing the copepods (Bradley 1977). Typically, the eggs of schistosome worms enter the aquatic environment from the urine or faeces of an infected human. However, dogs, cats, pigs, cattle, water buffalo, horses, and wild rodents may serve as the reservoir of *Schistosoma japonicum*, which is found in East Asia (Chin 2000). The eggs hatch in the water to produce miracidia, which infect snails, develop into the infective stage, and are shed by snails into the water over a period of months. Humans become infected when the free-swimming infective larvae penetrate the skin during water contact (Bradley 1977). Control of dracunculiasis and schistosomiasis is through protection of the water source and the user by limiting skin contact with water and by eradication of intermediate hosts.

The types of water contact diseases most frequently encountered in industrialized countries are those associated with recreational exposure to contaminated marine water, freshwater lakes, ponds, creeks, or rivers, and occasionally treated water in swimming pools, wave pools, hot tubs, and whirlpools. In developing countries, risk of water contact diseases may be from bathing or doing laundry in contaminated surface waters. Water contact diseases are also often associated with occupational exposure to waters. While many water contact diseases are associated with enteric organisms and ingestion of water with faecal contamination, there are some diseases of the ear, eye, and skin that are associated with actual water contact and systemic illnesses associated with penetration of a pathogen through an open wound or abrasion. Reported recreational water outbreaks have involved *Giardia*, *Cryptosporidium*, *Shigella sonnei*, and *Escherichia coli* O157:H7 that presumably entered the gastrointestinal tract via ingestion. Other recreational water outbreaks have involved ingestion of, contact with, or inhalation of indigenous aquatic organisms such as *Naegleria*, *Pseudomonas*, *Legionella* (Lee *et al.* 2002), several *Vibrio* species, and several *Mycobacterium* species (Dufour 1986). Epidemiological/microbiological studies indicate that *Staphylococcus aureus* skin and ear infections are often associated with recreational use of water, and the source of these organisms may be other bathers or the water (Charoenca and Fujioka 1995). *Vibrio vulnificus* can cause serious wound infections when a skin injury occurs in marine water or from contact of pre-existing wounds with marine water (Klontz *et al.* 1988). Cyanobacterial toxins have been associated with contact irritation after bathing in marine or fresh waters (Codd *et al.* 1989). An additional cause of water contact infections is the *Leptospira* species that are neither enteric organisms nor aquatic organisms, but enter water via the urine of infected domestic and wild animals (Dufour 1986). Leptospirosis is probably the most widespread zoonosis worldwide and occurs in urban and rural areas of both

developed and developing countries. Because of the non-specific symptoms of leptospirosis, it is believed to often be misdiagnosed and under-reported (WHO 2003c).

"Infections with water-related insect vectors" are those transmitted by insects that breed in water, such as mosquito vectors of malaria, or insects that bite near water, like the tsetse flies that transmit sleeping sickness. Control of these infections is through the application of pesticides, destruction of breeding grounds, and construction of piped water supplies.

3.3.3 Other water-related transmission routes

Two additional water-related modes of transmission of infectious agents are transmission by inhalation of water aerosols and transmission by the consumption of raw or undercooked shellfish or contaminated fish. The major pathogens associated with aerosol transmission are *Legionella* spp., especially *L. pneumophila*, the etiologic agent of Legionnaire's disease and Pontiac fever. *Legionella* are ubiquitous in water and soil and are capable of prolonged survival and reproduction in the aquatic environment. Growth within free-living amoebae appears to enhance survival and provide protection from routine disinfection (Winn 1995). Outbreaks of legionellosis have been associated with aerosols from cooling towers and evaporative condensers of large buildings or with hot and cold water systems in hospitals, hotels, and other institutions. *Legionella* can proliferate in hot water tanks maintained at 30–54 °C (Wadowsky *et al.* 1982), and exposure to aerosols from showerheads can occur. These infections are controlled through minimizing exposure to contaminated aerosols and routine cleaning and disinfection of water systems with adequate doses of chlorine, chloramines, or ozone (Muraca *et al.* 1990; Winn 1995). Water quality guidelines generally include limits on *Legionella*.

The potential for aerosol transmission of *Mycobacterium avium* and other non-tuberculous mycobacteria and the risk to the immunocompromised population continue to be a concern. Like *Legionella*, these organisms are frequently isolated in environmental and treated water systems and are able to colonize and propagate within water distribution systems (Jenkins 1991). Wendt *et al.* (1980) reported the isolation of non-tuberculous mycobacteria from aerosol samples near the James River in Virginia, USA. The isolates (mostly *M. intracellulare*) were biochemically similar to those recovered from human clinical specimens, suggesting that airborne mycobacteria derived from fresh water might be a significant source of infection. Non-tuberculous mycobacteria have also been isolated from hot water systems, and, like *Legionella*, they can survive in co-culture with protozoa (Ford 1999).

Bivalve molluscan shellfish serve as vehicles of enteric disease transmission because of their ability to concentrate enteric organisms from faecally contaminated water in their tissues. Numerous outbreaks have been attributed to the consumption of raw or undercooked oysters, clams, and mussels (Morse *et al.* 1986). Many pathogens, including hepatitis A and E viruses, human caliciviruses (Norwalk-like viruses), pathogenic *E. coli*, *Salmonella typhi*, and species of *Shigella*, *Vibrio*, *Plesiomonas*, and *Aeromonas*, have been implicated in shellfish-borne disease (Hackney and Potter 1994a, 1994b). Shellfish and some species of fish may also serve as vehicles for algal toxins. Toxic species of *Gonyaulax* and *Gymnodinium* are concentrated by filter-feeding molluscs and can cause paralytic shellfish poisoning among shellfish consumers (Carmichael *et al.* 1985). Reef-feeding fish can concentrate toxic dinoflagellates of the genus *Gambierdiscus*, which cause ciguatera seafood poisoning among consumers (Carmichael *et al.* 1985). Paragonimiasis is a trematode disease involving the lungs (Chin 2000). Infection is from consumption of raw, pickled, or partially cooked freshwater crabs or crayfish containing infective larvae. In humans, the larvae excyst, move through the intestinal tract to the lungs, and develop into egg-producing adult worms. Eggs expectorated into the sputum are swallowed and excreted in faeces. Faecal contamination of fresh water allows the larvae to hatch, penetrate snails and undergo a cycle of development, and emerge and penetrate crabs and crayfish.

3.4 CRITERIA FOR ZOONOTIC WATER-RELATED DISEASE

Given these characteristics of zoonotic diseases and water-related diseases, what criteria are useful for determining whether a disease is both zoonotic and water related? Do we limit these criteria only to "infections," or should they also apply to intoxications and other adverse health effects associated with zoonotic organisms? How do these criteria apply to organisms with complex life cycles that involve multiple stages, such as schistosomiasis? The following draft criteria seem to be a reasonable starting point:

(1) *The pathogen must spend part of its life cycle within one or more animal species.* It should be able to replicate or undergo development within an animal host and within a human host. However, the organism may not always cause symptomatic disease in either the animal or human host. The stringency of this criterion rests on the definition of "animal." Should

organisms that infect aquatic animals or fish or those that invade copepods or snails be included?

(2) *Within the life cycle of the pathogen, it is probable or conceivable that some life stage will enter water* — via faeces, urine, or tissue of an infected animal or human, because it is originally an aquatic organism, or possibly within the eggs of an insect vector. The organism must be able to persist in water for at least a few hours or days in order to be transmitted by exposure to water. Replication in water is not necessary. It is recognized that the predominant life stage in water may be different from the predominant life stage in the animal or human host. A stringent form of this criterion would be that there must be evidence that the organism can be detected in water.

(3) *Transmission of the pathogen (or toxin produced by the organism) from animal source to human must be through a water-related route:*
- water ingestion;
- water contact;
- inhalation of water or wastewater aerosols;
- consumption of shellfish or other seafood harvested from waters impacted by animals or animal waste; and
- consumption of seafood infected with a pathogenic organism.

These initial criteria are quite broad and encompass organisms with very different life cycles and transmission pathways, especially if one includes vector-borne water-related infections. It is arguable whether water-washed infections associated with poor hygiene and lack of adequate water and infections associated with water-based or water-related vectors should be included in these criteria, because one could also classify these infections as person-to-person transmission or vector-borne transmission. However, the case can be made that water management decisions will affect the transmission of these diseases, so they could be considered water related. For specific areas of interest, such as faecal–oral water-related zoonotic disease, the criteria can be narrowed to include only those organisms that 1) infect the enteric tract of animals and humans, 2) are shed in faeces, and 3) are transmitted via water ingestion or consumption of seafood from harvest waters contaminated with animal faeces.

Using these criteria, one can apply a modification of the Bradley classification system to water-related zoonotic diseases, as illustrated in Table 3.1.

3.5 APPLICATION TO SELECTED EXAMPLES

The purpose of these criteria is to help identify emerging water-related zoonotic diseases and provide some insight into how to control and prevent these diseases

using public health experience with other diseases. In order to test their usefulness, we will apply them to three examples of emerging diseases.

Table 3.1. Classification of water-related zoonotic diseases

Category	Zoonotic examples	Relevant control strategies
Waterborne via drinking-water	Salmonellosis, *E. coli* O157:H7, cryptosporidiosis, giardiasis, campylobacteriosis, microsporidiosis, toxoplasmosis, balantidiasis, yersiniosis, tularaemia, cysticercosis	Improve microbiological water quality through water treatment; protect drinking-water sources from contamination by animal faeces
Waterborne via recreational water contact	Leptospirosis, cryptosporidiosis, giardiasis	Protect water source from animal contamination
Water-washed	Cryptosporidiosis, giardiasis, balantidiasis, hepatitis E virus?	Increase water quantity to improve hygiene; promote hand washing
Water-based	Schistosomiasis (*Schistosoma japonicum*)	Protect user, control aquatic hosts, surface water management
Water-related insect vectors	West Nile virus, Rift Valley fever virus, yellow fever virus, sleeping sickness (African trypanosomiasis)	Protect user, control vector, surface water management
Inhalation of water/wastewater aerosols	Mycobacteria	Protect individuals who have occupational exposure; limit human exposure to geographic areas impacted by aerosols
Aquatic food	Paragonimiasis	Avoid ingestion of raw or undercooked crustaceans; prevent faecal contamination of freshwater crab and crayfish habitats, control snails by molluscicides

3.5.1 Severe acute respiratory syndrome (SARS)

Severe acute respiratory syndrome (SARS) is an example of an emerging disease where many questions about zoonotic reservoirs and water-related transmission are still unanswered. In the Guangdong Province of China, cases of an unknown respiratory illness with high fever, dry cough, dyspnoea, myalgia, and sometimes diarrhoea began to appear in November 2002 (Wu 2003). These cases were not reported to the World Health Organization (WHO) and did not receive much official attention until an outbreak occurred in Hong Kong in

February 2003. The disease quickly spread to more than 30 countries, including Vietnam, Singapore, Canada, Ireland, the USA, and Taiwan (Wu 2003). WHO issued a global alert about SARS on 12 March 2003. Understanding the transmission, identifying the etiological agent, and enacting effective control measures have occupied much of the public health community since that alert. In retrospect, it is impressive how quickly the causative agent (SARS coronavirus) was identified, cloned, and sequenced. Although there were more than 8400 estimated cases and 900 estimated deaths attributed to SARS by early August 2003 (WHO 2003a), the epidemic seems to have slowed, and control efforts seem successful.

Some epidemiological observations raised questions about the origins of the SARS virus and a possible animal reservoir early in the investigation of the epidemic. A WHO team reviewing the histories of the early cases in Guangdong noticed that a relatively high proportion of the cases had some connection with the food industry and that people who lived near markets were also more likely to be cases. Also, animal market workers, but not the general population, were found to have antibodies to the SARS virus (Normile and Enserink 2003). There appeared to be no link between SARS and eating specific foods. Once the sequence of the SARS virus was known, it was evident that it was different from the coronaviruses commonly found in domestic animals (pigs, cattle, chickens) and may have evolved in an isolated reservoir for some time before jumping to humans.

A study from the University of Hong Kong and the Shenzhen Center for Disease Control focused on exotic animals available in markets in Guangdong and reported the detection of a SARS-like coronavirus from six masked palm civets and also from a racoon dog (Normile and Enserink 2003). However, another research team from the China Agriculture University in Beijing was not able to detect SARS virus in any samples from civets or dozens of other species that were sampled. Neither research group felt that civets were the primary reservoir for the SARS virus, but rather that the civets may have become infected from contact with another animal reservoir in the wild or in the market holding facilities (Normile and Enserink 2003). Four international research teams are starting further studies of domestic and wild animals to seek animal reservoirs for the SARS virus. Other laboratory studies are attempting to infect different animal species with the SARS virus. The ban on civets and 53 other wild animal species from the markets of Guandong was lifted in August 2003 (Normile and Yimin 2003).

Evidence that the SARS virus could be spread by wastewater or faecally contaminated water is mixed. Transmission of the virus by aerosolized wastewater was suspected during the investigation of the SARS outbreak at Amoy Gardens in Hong Kong (Hong Kong Department of Health 2003). This is

a large housing estate built in 1981 with approximately 20 000 residents. An index SARS case visited a relative in Block E building in March 2003 and experienced diarrhoea during his visit. By mid-April, there were 321 SARS cases in Amoy Gardens, and 41% of the cases were concentrated in Block E. The surrounding buildings (B, C, and D) also had much higher SARS incidence rates than the other 11 buildings. Cases appeared earlier in Block E than in the other blocks, and the timing of these cases suggested a point source exposure. Survey data indicated that 66% of these cases had diarrhoea. Laboratory studies have shown that many SARS cases excrete coronavirus in their stools.

Environmental investigations (Hong Kong Department of Health 2003) indicated that the U-trap in the bathroom floor drains were usually dry and that there was a cracked sewer vent pipe in Block E. Tests with oil droplets suggested that aerosols travelled upwards in the light wells (utility channels) between each floor and that droplets could enter a bathroom through the floor drain because of negative pressure generated by exhaust fans when the bathroom door was closed. The investigators hypothesized that a virus in the faeces of the index SARS case was transmitted to a few other residents in Block E. These cases then caused greater contamination of the sewage system in Block E and transmission to other residents. Residents in nearby buildings became infected via person-to-person contact with their neighbours and perhaps from other environmental contamination. However, environmental samples of air and water in the Amoy Gardens were negative for the SARS virus.

Given that the SARS virus is excreted in faeces and these results, it seems possible that SARS could have been transmitted through wastewater droplets. However, one would have expected to have seen more examples of SARS transmission via exposure to wastewater in the various other settings where the epidemic spread. Possibly, this transmission route did contribute to SARS in other countries but was not recognized due to inadequate investigation; it is also possible that the Amoy Gardens buildings provided a unique environment that allowed the virus to accumulate to unusually high levels and expose residents to a high dose.

To date, the SARS coronavirus has been detected in animals and humans, is likely to enter water via faeces of infected persons, and *may* be transmitted via wastewater aerosols. It is not clear that SARS coronavirus causes disease in animals, and it has not yet been detected in water or wastewater.

3.5.2 *Pfiesteria* species

Pfiesteria, a genus of dinoflagellates, was first described in laboratory studies of fish mortality (Noga *et al.* 1993) and in association with major fish kills in North Carolina, USA (Burkholder *et al.* 1992). *Pfiesteria* spp. have been

detected in waters with fish kills from the mid-Atlantic to the Gulf Coast. Laboratory studies indicate that these organisms have a complex life cycle, including a toxic stage (Burkholder 1999). Ulcerative lesions, narcosis, erratic behaviour, and death have been observed in several fish species exposed to active cultures of *Pfiesteria* and *Pfiesteria*-like organisms (Burkholder and Glasgow 1997).

Public concern about possible human health hazards posed by *Pfiesteria* spp. began in 1995, when adverse health effects were reported among investigators working with this organism in the laboratory (Glasgow *et al.* 1995). Glasgow *et al.* (1995) described three laboratory workers with exposure to *Pfiesteria* cultures via direct contact with hands and arms and potential inhalation of aerosols from open aquaria. The exposed persons experienced various combinations of symptoms, which included numbness and tingling in hands and feet, skin lesions, respiratory and eye irritation, headaches, abdominal cramps, difficulties with mental concentration and memory, and personality changes. Most of the prominent symptoms subsided after cessation of exposure, although resolution of some symptoms took several months.

In August 1997, a fish kill occurred on the Pocomoke River in Maryland, USA, which triggered a study of 24 individuals with varying degrees of exposure to the Pocomoke waters and other estuaries in the Chesapeake Bay and 8 unexposed waterworkers (Grattan *et al.* 1998). Subjects were asked about water exposure and health symptoms, examined by a medical team, and tested with a neuropsychological screening battery. Individuals who reported high exposure (6–8 h per day in affected waterways with extensive skin contact and exposure to aerosolized spray) were significantly more likely than occupationally matched controls to complain of neuropsychological symptoms, headache, skin lesions, or a burning sensation of skin on contact with water. Nineteen study subjects exposed to affected waters had significantly reduced scores compared with 19 non-exposed subjects (matched on age, gender, education, and occupation) on three neuropsychological tests that indicated difficulty with learning and higher cognitive function. The investigators reported a dose–response effect and that study subjects who reported the greatest exposure to the affected waters had the lowest scores on selected neuropsychological tests. No consistent abnormalities were detected in the physical examinations or laboratory assessments of the study subjects. As with the reported cases among laboratory workers, the major neurocognitive problems appeared to be transitory, and the test scores for all study subjects returned to within normal ranges by 3–6 months after cessation of exposure. However, pre-exposure cognitive test performance for these individuals is not known, and some study subjects complained of persistent symptoms for a longer period of time. This study is the first systematic investigation of human health

effects after exposure to a fish kill associated with *Pfiesteria*. However, the study was limited by the small number of subjects and the facts that they were largely self-selected, exposure status was self-reported and may have been affected by recall bias, and the medical team was not blinded to the exposure status of the subjects during the medical and neuropsychological evaluations.

In response to concerns about *Pfiesteria* and human health and the many different types of health problems that were claimed to be associated with *Pfiesteria* exposure, the US Centers for Disease Control and Prevention and a panel of experts drafted criteria for "possible estuary-associated syndrome" (PEAS) to describe the adverse consequences of exposure to *Pfiesteria* and related organisms (Centers for Disease Control and Prevention 1999). The key criteria included in the definition were 1) exposure to estuarine water with a fish kill or fish with lesions consistent with *Pfiesteria* or *Pfiesteria*-like organisms, 2) symptoms of memory loss and confusion, 3) three or more other symptoms from a list of clinical features that have been reported in previous incidents of *Pfiesteria* exposure, 4) symptoms developing within 2 weeks of exposure to estuarine water and persisting for 2 weeks or longer, and 5) inability of the health care provider to identify another cause for the symptoms. Using these criteria, three prospective cohort studies of fishers and other waterworkers were launched in North Carolina, Virginia, and Maryland to explore the health risks of estuary exposure (Moe *et al.* 2001).

There is still controversy about whether *Pfiesteria* spp. are a primary causative agent of fish mortality or only a contributing factor, as well as about the presence and characteristics of the toxin and the health risks to persons who are exposed to waters with *Pfiesteria* spp. in the natural environment. Based on current knowledge, *Pfiesteria* spp. have been detected in the tissues of diseased fish (but not in humans), have been detected in water, and *may* cause adverse human health effects via exposure through water contact or water aerosols to a toxin. Whether *Pfiesteria* spp. fit the proposed criteria for a water-related zoonotic disease depends on whether 1) fish are considered to be "animals," 2) there is sufficient evidence that there is a toxic form of the organism that causes adverse health effects in fish and humans, and 3) there is sufficient evidence that exposure to the toxin occurs via either water contact or inhalation of water aerosols. If PEAS is a water-related zoonotic disease, the risks of adverse health outcomes associated with *Pfiesteria* spp. may be significant only at intense exposures similar to those experienced by the laboratory workers.

3.5.3 Bovine spongiform encephalopathy (BSE)

Bovine spongiform encephalopathy (BSE) is a transmissible, neurodegenerative, fatal brain disease of cattle. It has a long incubation period of 4–5 years; once

symptoms appear, however, the duration of the disease is weeks to months. Between November 1986 and November 2002, 181 376 confirmed cases were reported in the United Kingdom. There have been an additional 3286 cases reported in other European countries since 1989 (WHO 2003b). The etiologic agent is believed to be a prion — an infectious, self-replicating, mutated protein. Although the exact nature of the etiologic agent is still being debated, it is clear that the agent is highly stable and resists freezing, drying, and high temperatures. The disease appears to be transmitted between cattle by animal feed (meat and bone meal) that contains tissue from BSE-infected cows or scrapie-infected sheep.

Variant Creutzfeldt-Jakob disease (vCJD) is a rare, fatal degenerative brain disease of humans. It is important to note that vCJD is different from the classic form of CJD that is endemic throughout the world and affects older adults. The first vCJD cases were reported in 1996; through November 2002, 139 cases have been reported (WHO 2003b). The majority of these cases (129) were in the United Kingdom, and the average age at death was 29 years (WHO 2003b). The similarities in the human disease and cattle disease, long incubation period, similar symptoms, and similar morphology of infected brain tissue suggest that these diseases are both caused by the same agent. Transmission from cattle to humans is believed to occur through ingestion of beef and beef products from BSE-infected cattle. Human susceptibility to the disease is also likely to involve host genetic factors (McCormack *et al.* 2002).

When evaluating BSE as a candidate for a water-related zoonotic disease, several unknowns exist. The BSE agent clearly causes disease in cattle, and strong evidence suggests that the same agent is also responsible for a similar disease in humans. It is conceivable that the BSE agent could enter water if tissue from infected cattle (slaughterhouse waste or landfill leachate) enters water. There have been several incidents of public concern about the possibility of BSE-contaminated water (WaterTech Online 2000). However, Gale (1998) suggested that the likely concentration of BSE prions in the environment would be too low to pose a significant risk, even after a lifetime of exposure to contaminated water. The survival of the agent in water is unknown, although the agent is believed to be very stable in general. Currently, there are no reports of the detection of BSE agent in water, but it is likely that no methods exist to test water samples for this agent. There has been concern about the possible threat to groundwater supplies in the United Kingdom from buried carcasses of infected cattle.

3.6 SUMMARY

There has been a recent surge in the number of newly recognized zoonotic diseases, some of which have water-related transmission routes. There is striking variety in the species and life cycles of water-related zoonotic organisms and how these organisms can move from animals to humans. A set of broad criteria has been proposed to help determine whether a disease is both zoonotic and water related. The application of these criteria to different emerging diseases identifies the critical gaps in our understanding of these diseases. The control of the diseases that meet these criteria involves water management strategies. However, many of these organisms can be transmitted to humans by more than one route, and water may not be the dominant transmission route. Therefore, control of water-related transmission may be necessary but not always sufficient to prevent these diseases. The stringency of the criteria proposed here can be adjusted to examine specific types of diseases and specific types of water-related transmission, depending on the application.

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4

Impacts of anthropogenic and environmental factors on the distribution of zoonoses

F. Dangendorf

4.1 INTRODUCTION

The geographical distribution of diseases has shown major changes over human history. With the improvement of urban sanitation, the development of antibiotics, and the development of vaccines, as well as improved individual hygiene behaviour and a higher standard of living, there has been a considerable decrease in infectious diseases since the end of the 19th century in developed countries that made the transition to modern industrial societies.

Since the 1980s, an increase in epidemics has occurred worldwide — for example, the return of diphtheria and tuberculosis in Russia (Müller 2000),

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bovine spongiform encephalopathy in Great Britain (Kurth 1997), cholera in South America (Chin 2000), and Ebola in South Africa (Kurth 1997). Infectious diseases remain among the most frequent causes of death globally.

The question arises as to the cause of the increase in infectious diseases, including water-transmittable diseases, and whether further increases would be expected. Numerous risk factors that can influence the occurrence and spreading of infectious diseases are discussed in the technical literature. These include geographical aspects, environmental changes, socioeconomic factors, and pathogen-specific factors, as well as individual behaviour. Internal and external migration, population growth, lack of living space, impoverishment, military actions, uncontrolled and rapid urban growth, environmental changes as a result of changes in land use, air pollution and climate change, construction of irrigation systems, dams, de- and reforestation, the development of mass tourism, mass production in the food industry, as well as an increase in international trade are only some of the risk factors to be mentioned (Haggett 1994; Kurth 1997; Kovats *et al.* 1999; Githeko *et al*. 2000; Patz *et al*. 2000; WHO 2002a; Woolhouse 2002). Some risk factors contributing to the increased occurrence of zoonoses in the Mediterranean region are, according to Mantovani and Prosperi (1995), the changing patterns of migration of people and animals, the densities of human populations, domestic animals, and wild animals, types of farming, pig-rearing, the presence of stray dogs, slaughtering methods, food and living patterns, and trade.

Human activities have profound effects on the environment. Table 4.1 summarizes the impact of some anthropogenic factors on the distribution of zoonotic diseases.

4.2 PATHOGEN RESERVOIRS AND MOBILITY

Human migration has been the main source of epidemics throughout recorded history. Trade caravans, religious pilgrimages, and military conflicts facilitated the spread of many diseases, including plagues and smallpox (Wilson 1995).

The mobility of people continues to increase due to social, economic, and political factors. Worldwide, about 125 million people are migrant workers, immigrants, or refugees searching for education, employment, or safety, making them vulnerable to infectious diseases that are not endemic in their home countries (Theron and Cloete 2002). Furthermore, natural hazards such as flooding, earthquakes, and hurricanes often force people to search for new settlement areas. The increasing number of military conflicts brings soldiers into new environments. Large numbers of troops from mid-latitude countries operate in tropical and subtropical climates (Haggett 1994).

The consequences of mobility are that people carry pathogens, insect vectors, immunity due to past infections, vulnerability, genetic material, cultural preferences, behavioural patterns, and technology to new environments, which influence their risk for infection and their capacity to introduce diseases in the new region (Wilson 1995).

Table 4.1. Impact of human activities

Cause	Effect
Mobility due to population growth, urbanization, social inequality, stress	Migration movements (e.g., into slums of large cities)
	Spatial proximity to waste disposal, sewage, and contaminated rodents or other roaming animals
	Collapse of the public health infrastructure
	Invasion of a non-immune population by an infectious agent
Mobility due to national and international conflicts	Exposure of soldiers and refugees to a range of diseases not encountered in their home region or country
	Lack of medical care, hygiene, safe drinking-water
Mobility due to travel	Increased potential for disease spread due to air travel
	Decreasing travel times
	Increasing size of aircraft
Trade, transboundary animal transport, import of exotic animals	Deficient surveillance for infected animals
Agricultural practices	Land use, manure deposits, manure application, livestock, dairy farming, drained areas, mass production of food and animals
Water control, irrigation projects	Change of ecology of large regions
	Surface water serves as breeding site
	Increased soil salinity
Human habits	Changing consumption patterns
	Eating raw or undercooked fish or meat
	Pet-keeping
	Close contact with (infected) animals
	Colonization of new environments
	Lack of knowledge of self-protective lifestyles
Infrastructure changes	Development of technical systems
	Water supply distribution network
	Cooling towers
	Air conditioning
Demographic factors	Increase of risk groups
	Aged population
	Immunocompromised patients

Cause	Effect
Pathogen-specific factors	Decrease or increase of pathogenicity
	Antibiotic-resistant pathogens
Climate change	Increasing warm winters benefit the proliferation of infectious agents
	Changes in the geographical range of pathogens, vectors, and reservoirs
	Increased seasonality in rainfall
	Storm frequency increases
	Floods, prolonged droughts

4.2.1 Population growth and urbanization

The world population will grow from the current 6.3 billion to an estimated 8.9 billion people by the year 2050. Nearly all of the 2.6 billion increase will be in the developing countries of Africa, Asia, and Latin America (UNFPA 2003). Due to social, economic, and political factors, there is an increasing shift of populations to urban areas. Nearly half of the world population now lives in urban settlements. The number of large cities increased sharply during the last several decades (Moore *et al*. 2003). Current data indicate 18 cities with populations greater than 10 million (UNPD 2003).

Urbanization is often associated with rapid and unplanned growth, poverty, and environmental degradation. Substandard housing, crowding, air pollution, water pollution, overusage of water sources, and inadequate sanitation facilities and services are mostly related to rapid urban growth (Moore *et al*. 2003).

Negative effects on human health evolve from a higher risk of disease contacts through crowding and pollution (Haggett 1994). Crompton and Savioli (1993) showed that in developing countries, high rates of intestinal parasitic infections occur where rural/urban migration results in poor periurban settlements. Furthermore, the aggregation of human populations into high-density urban regions has important effects in providing host reservoirs for maintaining infection chains (Haggett 1994). Disease vectors, such as rodents and insects, find new habitats within the changing urban landscape and may come into close contact with people.

The poor population in urban areas often does not have access to the municipal water supply. The people often depend on common pumps or surface water sources, which are often faecally contaminated due to inadequate solid waste collection (Moore *et al*. 2003). Municipal supplies are also often unreliable.

In industrialized countries, heavy faecal contamination of surface waters can occur as a result of the introduction of treated or untreated sewage.

Characteristic sources are municipal or private sewage discharges, industrial effluents, and recreational activities. Enteric viruses, for example, are predominantly found in rivers or seawater polluted by discharges from densely populated and industrial areas (Johl *et al*. 1991; Van Olphen *et al*. 1991; Payment and Franco 1993).

The increasing growth of populations with the extension of settlement areas, as well as the extension of agricultural areas into new environments and up to catchment areas of drinking-water resources, must be seen as risky environmental changes.

4.2.2 International and national conflicts

Increasing numbers of cross-boundary military conflicts bring soldiers into new environments. Large numbers of troops from mid-latitude countries operate in tropical and subtropical climates, where they come into contact with a different spectrum of infectious diseases (Haggett 1994).

Thousands of people abandon their villages and cities as a consequence of military actions. Severe water-associated outbreaks occur due to the lack of medical care, hygiene, drinking-water, and healthful behaviour among refugees.

4.2.3 Travel

Travel patterns of tourists have changed over several decades. Bradley (1988) showed that over the last four generations, the spatial range of travel has increased 10-fold. In particular, air travel has increased the potential for spread of disease, not only through decreasing travel times, but also due to the increasing size of aircraft (Haggett 1994). Important aspects of these problems include the transmission of foodborne and waterborne diseases, the translocation of insect vectors, the rapid transport of people with subclinical infections as well as direct transmission while in the aircraft, and the transmission of zoonoses through animal transport (Royal and McCoubrey 1989).

With the rising popularity of international travel to exotic locations, travellers are often exposed to pathogenic agents that are not endemic in their countries. This could result in an invasion of a non-immune population by the infectious agents, which often leads to a more severe manifestation of diseases (Wilson 1995).

Problems of water supply in many developing countries arise from insufficient technology with regard to well heads, water treatment, supply systems, and hygienic behaviour. Water resources have been polluted over the years due to inadequate protection of surface reservoirs and groundwater from faecal and toxic wastes. Travellers from countries with high standards of water

supply have higher risks of acquiring waterborne diseases than the indigenous population, because the residents have developed immunity to many of the local waterborne pathogens.

Shigellosis (bacillary dysentery), typhus, and paratyphus are typical diseases of travellers, which are of minimal importance as endemic diseases in developed countries (RKI 2000). Hepatitis A also often occurs in travellers. For residents of countries with low sero-prevalence levels, such as Sweden, vaccination is recommended before travel to countries where the disease is endemic (Bottiger and Christenson 1998).

4.3 TRADE

A huge volume of plants, animals, and other materials is transported all over the world. The globalization of markets brings fresh fruits and vegetables over long distances to places where they are not grown. A consequence of shipping the goods is the unintended transfer of microorganisms from and to different ecosystems and populations (Wilson 1995).

Millions of calves and cattle are transported across, from, and to Europe every year. Most of these animals are going to slaughterhouses in the respective countries or in another community state or coming or going abroad. These transports can cause severe stress in animals, entailing poor welfare. Furthermore, they increase risks of spreading infectious diseases over large distances. According to Hartung *et al*. (2003), "Existing legislation does not provide enough protection to transported animals especially over long distances largely because considerable parts of the regulations are not sufficiently based on scientific evidence."

Worldwide, over 50 species of helminth parasites from fish, crabs, crayfish, snails, and bivalves are known to cause human infections. Some of the zoonotic diseases are more prevalent than others and can pose serious health hazards. The majority of seafood zoonoses occur along coastal regions where seafood products are commonly consumed. However, improvements in transportation technology and food handling allow fresh seafood to be distributed throughout the world. Thus, the potential to acquire parasitic infections from seafood is not limited to coastal populations.

Global food trade is expected to increase due to increases in global income levels, improved transportation networks, including improved refrigeration, and the fact that the growing population requires larger quantities of nutrition and safe food (Buzby 2001). There has been a remarkable increase in the consumption of animal products in countries such as Brazil and China, although the levels are still below the levels of consumption in North America and most other industrialized countries (WHO 2002a).

4.4 AGRICULTURAL AND HUSBANDRY PRACTICES

The contamination of the environment with enteric pathogens from agriculture depends upon the number of infected non-human hosts, the number of transmissive stages excreted, host behaviour, and agricultural practices. Enteric pathogens may enter the environment via storage and spread of farmyard manure, on-farm discharge of faecally polluted water to land or to watercourses, runoff from pastureland into water bodies, or the disposal of faecally contaminated waste from abattoirs (Slifko *et al*. 2000).

Several studies have shown that the most frequent association for *Cryptosporidia* and *Giardia* is between surface water sources and the presence of a high density of domestic and wild animals. *Cryptosporidia* occurrence has been frequently correlated with dairy farming and density of fallow deer in the catchment area, whereas *Giardia* cysts were predominately associated with the presence of urban sewage (LeChevallier *et al*. 1997; Atherholt *et al*. 1998; Hsu *et al*. 1999; Payment *et al*. 2000; Kistemann *et al*. 2002). Adenoviruses and enteroviruses were found in lakes and rivers whose catchment areas were influenced by poultry and sheep farming (Till *et al*. 2000).

In many countries — for example, in Southeast Asian countries — untreated night soil is used as a fertilizer in agriculture or as food in aquaculture (Bo *et al*. 1993). Contamination with pathogens and eggs of parasites affects soil, groundwater and surface water, vegetables, and fish. This poses health risks to workers and farmers and to consumers eating raw fish taken from ponds fertilized with night soil. In a province in south China where the cultural preference is to consume raw fish, the infection rate with *Clonorchis sinensis* reached 100% in some areas (Bo *et al*. 1993).

Modern animal husbandry has tended to increase the number of animals raised per unit area. Feeding and watering of animals are automated, and the use of antibiotics supports animal growth rates. These changes have introduced some new risks. In particular, the increased concentration of animal excreta can be hazardous to human health and the environment (Cole *et al*. 1999).

Numerous wastes are produced by intensive swine production. They are associated not only with potentially transferable antimicrobial resistance patterns, but also with several infectious agents that can be pathogenic to humans (Cole *et al*. 2000). Hepatitis E, for example, is thought to be transmissible through swine (Worm *et al*. 2002).

Bacterial enteric diseases due to *Salmonella enteritis* and *Escherichia coli* O157:H7 (enterohaemorrhagic *E. coli* [EHEC]) are examples of diseases associated with changing farming practices and consumer habits (Meslin 1992). Cattle are asymptomatic natural reservoirs of *E. coli* O157:H7. It has been reported that about 30%, and as high as 80%, of all cattle are carriers of this

pathogen. High-producing dairy cattle are fed large grain rations in order to increase feed efficiency. This is thought to be correlated to higher shedding of EHEC than using a forage diet (Callaway *et al*. 2003). Other reservoirs of EHEC are sheep, goats, red deer, horses, dogs, birds, and flies. The bacteria can survive in liquid manure, non-liquid manure, and drinking troughs. Foods that are irrigated, washed, or prepared with polluted water are also a common cause of infection (Doyle 1990).

Campylobacter spp. are common in poultry, particular chickens. In production systems, poultry flocks can be infected through the environment, overcrowded conditions, and their low immune status (Sahin *et al*. 2002). Tully and Shane (1996) indicated that newly introduced birds (ostriches, emus, rheas) may contaminate soil and water with pathogens such as *Mycobacterium* spp. and *Salmonella* spp. Mass production of animals, development of large meat factories, and international trade of meat products and animals are believed to be the reasons for the increased prevalence of yersiniosis in humans (Neubauer *et al*. 2001).

4.5 WATER CONTROL AND IRRIGATION

Large water projects have caused social and environmental disasters. Malaria, bilharzia, and other tropical diseases have been caused in numerous cases by dam construction and development of new irrigation systems. Such projects can expand habitats for mosquitos, aquatic snails, and flies, which spread disease among resettled agricultural populations. Excess irrigation and poor drainage can cause increased salinization of soils. High salt concentrations can adversely affect vegetation, wildlife, and cultivated crops (Jobin 1999).

World population growth means that more dams and irrigation systems will be needed for survival in Africa and other dry areas. Efficient use of water resources and sustainable designs and proper operation must be implemented more widely (Jobin 1999).

The use of inadequately treated wastewater in irrigation and faecal sludge in soil amendment and fertilization is often associated with an elevated prevalence of intestinal helminth infections and diarrhoeal diseases in both workers and food consumers (Mara and Cairncross 1989).

4.6 CHANGING HUMAN BEHAVIOUR

The preparation and consumption of meat or fish are associated with specific cultural preferences. In recent years, globalization of the food supply has changed consumer habits. As a consequence, some foodborne diseases have shifted to new areas.

Previously, the majority of seafood zoonoses occurred along coastal regions where seafood products are commonly consumed. Through the improvement of transportation facilities, seafood is now available worldwide. Changing consumption habits — such as eating raw seafood dishes (e.g., sushi and sashimi) and the tendency to reduce cooking times when preparing seafood products — have increased the risk of fish-borne parasitic zoonoses in areas where the products were previously not common (Deardorff 1991).

Foodborne zoonoses may also occur due to inadequate preparation of meat, vegetables, and fruits. People now more frequently eat raw, undercooked, smoked, salted, pickled, or air-dried meat, which may have a higher risk of infection (Slifko *et al*. 2000).

The highest biodiversity of all terrestrial ecosystems is to be found in tropical forests. The invasion into formerly untouched areas by hunters, villagers, tourists, and others has led to intensified contacts with free-living animals. Increased contacts between people and animals in tropical forests and high population densities of humans and domestic animals pose a high risk for the emergence of new diseases (Ludwig *et al*. 2003). Construction of roads in previously forested areas provides access for non-immune, non-protected populations (road workers, tourists, miners, etc.) into new environments (Patz *et al.* 2000).

Deforestation is mostly connected to conversion of the area into grazing land for cattle and agricultural or settlement areas. With the introduction of new animal or plant species, new habitats for parasites or vectors can be created. The replacement of former forests by agricultural land use attracts new settlers and migrants who frequently have a lack of immunity to the endemic zoonoses and also lack of knowledge of protective hygienic habits (Patz *et al*. 2000).

4.7 INFRASTRUCTURE CHANGES

Legionnaires' disease is a classic example of the fact that an ecological milieu for pathogens that does not constitute a danger for human beings in their natural environment can be created by infrastructure changes. The development of new technologies is usually associated with improvements in the quality of life, but it also can create new problems. With the extended use of water in large constructed systems, like drinking-water distribution systems, warm-water plumbing systems, air conditioning systems, cooling towers, etc., the risk of Legionnaires' disease has increased in the last decades (McDade 2002).

Only since the 1980s has it been recognized that proliferation of microorganisms in treated drinking-water without particular nutrients is possible (Christian and Pipes 1983). This regrowth of heterotrophic organisms usually does not involve bacteria from animal or human faeces but instead involves

organisms that occur naturally in the aquatic environment and the soil (Botzenhart and Hahn 1989). These organisms reach the drinking-water via the raw intake water or during pipe construction and repair. They have the capability to multiply in water systems in which they are both nourished and protected by biofilms from physical removal, disinfectants, and temporarily high temperatures. Examples of hygienically relevant microorganisms are *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Aeromonas*, *Mycobacterium*, and *Acinetobacter* (Szewzyk *et al*. 2000). Some microorganisms of faecal origin have the ability to survive and be transported in the drinking-water supply network. These include *E. coli*, *Cryptosporidium*, *Giardia*, and enteroviruses (Flemming 1998).

Most of the heterotrophic organisms that proliferate in water systems are not pathogenic, but some pathogens can be harboured in biofilms. Biofilms form from a matrix of extracellular polymer substances. The biofilms serve as habitat for the microorganisms. Depending on the nutrient supply, they can multiply and can access the water. The formation of biofilms in the pipe system is encouraged or limited by certain factors. The colonization is dependent on the pipe material used, the age of the pipe system, corrosion phenomena, stagnation conditions in the system, temperature, pressure, disinfectant residuals, nutrients, and pH. Furthermore, the tolerance of biofilms to disinfection agents is of importance to drinking-water supply systems (Flemming 1998).

Infrastructures in water supply are subject to a generally long-term planning perspective, and correspondingly the capacities of the pipe systems are only slowly adapted to new trends. Pump stations, conduits, and storage systems are closely connected. Problems in the pipe system can be caused by overhead traffic, underground displacement, corrosion, frost, and construction work. Leakage and breaks lead to losses of water as well as to impairments in the drinking-water quality.

Recontamination in primarily clean drinking-water can be associated with home plumbing that has not been installed and maintained appropriately or that is out of date. Sites at particularly high risk are hospitals, nursing homes, day care centres, and other public institutions where high-risk populations may reside.

4.8 DEMOGRAPHIC CHANGES

Particular health risks arise in populations whose resistance against a pathogenic microorganism has been reduced. These groups of people are an environment that can support proliferation of some pathogens. For example, the course of gastrointestinal infections is often more severe in young people, especially

infants, and in the aged, rather than in healthy adults. Severe health complications can also arise as a result of dehydration (Chin 2000).

In the USA, the portion of high-risk groups in the total population is approximately 20% (Gerba *et al*. 1996). High standards of living and progress in medical science have led to an increasing life expectancy and a higher portion of older people, who are more vulnerable to infections. The fatality risk from bacterial gastroenteritis outbreaks is up to 10 times higher in nursing homes than in the general population. Intestinal diseases can have grave complications in immunocompromised patients. As a result of chemotherapies or after organ transplantation, patients are very vulnerable to viral infections of the gastrointestinal tract. Cryptosporidiosis often appears in patients with acquired immunodeficiency syndrome (AIDS) (Gerba *et al*. 1996), and clinical treatment is generally not effective.

An additional at-risk group is the increasing number of patients in home nursing, which can be seen in Europe, due to attempts to reduce costs in health care. The occurrence of at-risk groups in several European countries is shown in Table 4.2. It can be assumed that the ratio of the population at risk (i.e., with an increased risk of infection) to the normal European population is 1:6 (Exner and Kistemann 2003).

Table 4.2. Occurrence of "at-risk" populations in different European countries (adapted from Exner and Kistemann 2003)

	UK	Germany	The Netherlands
Total population	60 million	82 million	16 million
Over 65 years old	9 million	13 million	2 million
Under 1 year old	600 000	800 000	100 000
People living with cancer	1 million	–[a]	160 000
Discharged from hospital within previous 2 weeks	200 000	–	60 000
Hospital outpatients at home	–	1 270 000	–
AIDS cases	15 000	–	91
Total at-risk persons	>1 in 6	>1 in 5.6	>1 in 6.3

[a] No data.

Worldwide, nearly half of all people are under the age of 25 (UNFPA 2003), out of which about 617 million are children under the age of 5 (UNPD 2003). More than 10 million children die each year, most from preventable causes, and almost all in developing countries. Key issues include malnutrition and deaths due to infectious diseases and poor general sanitation (Black *et al*. 2003).

Gastrointestinal infections are one of the principal causes of morbidity and mortality among children. For children under 5 years of age in developing

countries, a median of 3.2 episodes of diarrhoea per child-year was calculated. Estimates of mortality revealed that 4.9 children per 1000 per year in these areas die as a result of diarrhoeal illnesses in the first 5 years of life (Kosek *et al*. 2003).

About 41% of child deaths occur in sub-Saharan Africa, and another 34% in south Asia. Most of the children live in rural areas, but poor periurban populations also have high mortality rates. Unhygienic and unsafe environments, including ingestion of unsafe water, inadequate availability of hygiene, and lack of access to sanitation, cause 88% of child deaths from diarrhoea (Black *et al*. 2003).

4.9 PATHOGEN CHARACTERISTICS

4.9.1 Genetic mutations

The ability of an infectious agent to cross the species barrier is a complex multifactorial process. New types of pathogens or new forms of adaptation of microorganisms can be explained by mutations for the most part. The mechanisms of the microbial evolution can either decrease or increase the pathogenicity or virulence of a pathogen. In addition, microorganisms can leave their natural host and become endemic in a new species (Kurth 1997).

RNA viruses — for example, human immunodeficiency virus (HIV) and influenza viruses — show extremely high mutation rates. As a result, RNA virus populations consist of a dynamic swarm of mutants, even within a single host individual. Maintaining such a genetically highly diverse population allows rapid adaptation to a new environment and host (Ludwig *et al*. 2003).

The recent outbreak of severe acute respiratory syndrome (SARS) demonstrated the capacity of viruses to change their hosts. The SARS agent was identified as belonging to the family of coronaviruses. It is believed that within an animal host, the coronaviruses developed unique genome sequences that have crossed over to people in rural areas of a southern Chinese province (Pearson *et al*. 2003). Even though the outbreak was stopped, the infectious disease may return. As with the influenza viruses, development of mutations may be favoured by immunity in people and the use of antiviral drugs (Pearson *et al*. 2003).

Another prominent example is the formation of pathogenic *E. coli* strains that may have taken up virulence by genetic diversification. In contrast to the harmless *E. coli*, five variations of the species are of human pathogenic importance: EHEC, EIEC (enteroinvasive *E. coli*), ETEC (enterotoxigenic *E. coli*), EPEC (enteropathogenic *E. coli*), and EAEC (enteroadherent *E. coli*) (Eisenstein and Zaleznik 2000). EHEC is currently one of the most important

strains of waterborne pathogens, even though it is easily eliminated by basic water treatment. In 2000, in the town of Walkerton, Ontario, Canada, an estimated 2300 people became ill and 7 died from exposure to EHEC-contaminated drinking-water (Hrudey 2003), which was not adequately disinfected.

4.9.2 Drug resistance

Antibiotics inhibit bacterial growth by interfering with vital cell functions such as protein synthesis. Every time an antibiotic is used, there is selective pressure to allow the growth of resistant organisms, because the antibiotics selectively kill or reduce the viability of only the sensitive bacteria. This leaves the resistant ones with the opportunity to proliferate more in the host or environment. Through the process of natural selection, the resistant bacteria very rapidly become the dominant variants in the population (Levy 1998). In addition, pathogens can acquire new antibiotic-resistant genes from other species in the environment (Woolhouse 2002).

The selection of antibiotic-resistant and drug-resistant pathogens has become more frequent through massive and increasing use of antimicrobial drugs in humans, food animals, and fruits and vegetables. Drug-resistant infections in humans are often associated with prolonged hospital stays, and they increase the risk of complications and death.

According to McGeer (1998), nearly half of the antimicrobial drug use in North America is in agriculture, out of which the majority is given to animals to promote growth rather than to treat any existing infections. The main risk to humans from agricultural antibiotics is that the drugs used in animals are similar to those used in humans. As a result, resistance mechanisms developed in agricultural settings may be effective against both human and animal antibiotics (McGeer 1998).

In the last decade, for example, there has been a worldwide increase in the *Salmonella typhimurium* phage type DT104. It is common in cows and pigs and is also often isolated in humans with *Salmonella* infections. The phage type DT104 seems to have increased virulence, and it is additionally associated with multiple resistance to antibiotics (FVO 2001).

4.10 THE IMPACT OF CLIMATE CHANGE

In recent years, there is stronger scientific evidence that climate is changing on a wide range of temporal and spatial scales due to both natural variability and external (e.g., anthropogenic) factors.

The 20th century has experienced an increase in precipitation of 0.5–1% over most mid- and high latitudes of the continents in the northern hemisphere, accompanied by an increase in the frequency of heavy precipitation events. It is likely that the global average water vapour concentration and precipitation will increase further during the 21st century. In areas where an increase in mean precipitation is anticipated, larger year-to-year variations in precipitation are very likely to occur (IPCC 2001).

The regional effects of climatic changes have, in fact, not been sufficiently analysed. An increasing occurrence of extreme weather conditions has also been registered in temperate zones — for example, the flooding events of the Rhine, Meuse, Oder, Danube, and Elbe in the 1990s and early 21st century (WHO 2002b). In general, it is likely that for many mid- and high-latitude areas, primarily in the northern hemisphere, statistically significant increases have occurred in the proportion of total annual precipitation derived from heavy and extreme precipitation events. It is likely that there has been a 2–4% increase in the frequency of heavy precipitation events over the second half of the 20th century (IPCC 2001).

By the year 2100, the mean sea level is projected to rise by 9–88 cm on a global scale. More than half of the world's population now lives within 60 km of the sea (WHO 2001). According to the WHO (2001), some of the most vulnerable regions are the "Nile delta in Egypt, the Ganges-Brahmaputra delta in Bangladesh, and many small islands including the Marshall Islands and the Maldives."

The links between climate change and human health are still very poorly understood. However, climate change, together with other ecological and demographic changes, can have adverse effects on human health. This hypothesis may be supported by the concurrence of ongoing changes in patterns of human disease and the advent of climate changes (Kovats *et al.* 1999).

4.10.1 Seasonality

Seasonality in some disease incidence is closely connected to weather patterns. Vector-borne diseases in particular show a strong correlation to seasonal weather changes. For example, the death rate from malaria is highest at the end of the rainy season (Patz and Lindsay 1999).

Climatic cycling also seems to have a strong influence on the pattern of the emergence of diarrhoeal diseases. Several studies show a direct correlation between seasonal precipitation patterns and the occurrence of gastrointestinal diseases (Atherholt *et al*. 1998; Curriero *et al*. 2001). In some areas, weather patterns are changing also, with more prolonged droughts. Falling water levels and reduced river runoff increase the risk of cyanobacterial blooms and also

make water treatment more difficult, as the levels of sediments and organic material increase (Leder *et al*. 2002).

Apart from natural seasonal variability, climate is also influenced by other factors that may have an influence on weather events in several parts of the world at the same time. The El Niño Southern Oscillation is a complex climate phenomenon that can be detected every 2–7 years. The El Niño changes the oceanic currents and thereby the weather in many parts of the world. The regions where the phenomenon has a strong effect on climate are southern Africa, East Africa, Peru, and Southeast Asia (WHO 2001).

The El Niño effect forces warm equatorial Pacific water to flow from the western to the eastern Pacific, bringing heavy rainfall to some regions, while others suffer from drought (Patz and Lindsay 1999). Correlations could be found between the occurrence of the El Niño phenomenon and cumulative outbreaks of malaria, rodent-borne hantavirus, or Rift Valley fever in certain countries, especially during the strong El Niño years 1997 and 1998 (Patz and Lindsay 1999).

4.10.2 Heavy rainfall and floods

High rainfall and storms change the drainage conditions of flowing waters. The straightened courses of rivers and creeks, which result in improvement of land for cultivation by means of irrigation, drainage, or soil conservation, can also contribute to extreme flood situations. Microorganisms become mobile through dispersion of sediments. The avulsion of the surface of the soil and high discharge of settlement sewage increase the microbial burden, which can be transported over long distances as a result of the high flow rate of the water. Curriero *et al*. (2001) proved that outbreaks due to surface water contamination showed the statistically strongest association with extreme precipitation during the months of disease outbreaks. It is estimated that about 50% of waterborne outbreaks occur as a consequence of heavy rainfall events (Curriero *et al*. 2001). In a study on microbial loads of flowing waters during heavy rainfall, an increase of the concentrations of parasites was especially found in water samples that were taken parallel to the flood wave at 2-h intervals. It was determined that between 0.31% and 0.77% of the mean expected annual discharge can enter the waters during heavy rainfall events (Kistemann *et al*. 2002).

Indirect effects of floods on human health include damage of water supply systems, disruption of pipes, damage to sewage disposal systems, and insufficient supply of safe drinking-water. Standing water and migration of rodents or other host animals may expand the range of vector habitats, which

may lead to an increase in zoonotic and waterborne infectious diseases (WHO 2002b).

4.10.3 Temperature

The accumulation of greenhouse gases in the lower atmosphere due to human activities has led to a reduction of the efficiency with which the Earth's surface radiates heat to space. As a consequence, the global average surface temperature (the average of near surface air temperature over land and sea surface temperature) has increased since 1861, and scientists of the Intergovernmental Panel on Climate Change (IPCC) forecast an increase in average global temperature of 1–3.5 °C by the year 2100 (IPCC 2001).

During the 20th century, the average surface temperature experienced an increase of 0.6 ± 0.2 °C. The decade from 1990 to 2000 was the warmest decade and 1998 the warmest year in the instrumental record since 1861. This increase in temperature is likely to have been the largest of any century during the past 1000 years (IPCC 2001).

The global rise in the mean annual temperature will facilitate spreading of tropical and subtropical diseases, such as malaria and leishmaniasis, to moderate latitudes (Haggett 1994). The extent of the rise in temperature and its spatial manifestation are, however, still speculative.

The 10 °C annual isotherms, which are regarded as the approximate northern boundary for the occurrence of leishmaniasis and its vectors in the Mediterranean area (Piekarski 1952), are moving farther north. These tendencies are already observable today, as sandflies have been identified in northern France and southern Switzerland (Ashford and Bettini 1987) and, in 1999, for the first time in Germany (Naucke and Pesson 2000).

4.11 SUMMARY AND CONCLUSION

Anthropogenic and ecological factors influence the transmission of zoonoses. In this chapter, old and new risk factors for the emergence of these diseases were discussed in the framework of environmental and climate changes and human factors. Although it is still difficult to forecast the future impact of geographical and ecological effects on the distribution of infectious diseases, there is a greater awareness of this potential problem among policymakers, researchers, and others.

Increased risks for the transmission of waterborne and zoonotic diseases can be related to the mobility of populations, high density and close proximity of domestic and feral animal and human populations, agricultural practices, heavy rainfall events, and demographic factors. Improved surveillance and monitoring

and greater commitments to sanitation and water management are needed to detect global and ecological changes and to ensure protection from associated re-emerging and emerging infectious diseases.

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5

The control envelope and risk management

R. Carr and J. Bartram

5.1 INTRODUCTION

As discussed in chapter 1, emerging waterborne zoonoses will continue to pose new challenges to public health. As time passes, the spectrum of pathogens changes in response to pathogen evolution, environmental change, progressive technological developments, increasing scientific capacities, and changes in human behaviour and the vulnerability of populations (WHO 2003a). The foodborne and waterborne pathogens that currently pose the greatest threat to public health are also frequently the most recently identified (at least in developed countries) (Tauxe 2002). As the routes of transmission for an emerging/(re)emerging pathogen become known, strategies for controlling the pathogen are developed, and the threat from the pathogen is significantly reduced, bringing other pathogens to higher visibility (Tauxe 2002).

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This chapter explores different factors that are associated with the transmission of waterborne zoonotic diseases and evaluates the ability of current disease management strategies to effectively manage the risks they pose. It introduces the concept of a "control envelope," which provides a framework for evaluating the threats from different pathogens against threats from well known benchmark organisms. Using the control envelope, emerging waterborne zoonotic pathogens can be assessed as being (a) firmly within the control envelope and thus do not pose unique management challenges; (b) on the edge of the control envelope, i.e., of concern but should be manageable with the right strategies; or (c) outside of the envelope and will require new approaches for managing associated health risks. The control envelope concept provides a useful starting point for developing risk management strategies, such as hazard analysis and critical control points (HACCP) and water safety plans (WSPs).

5.2 CONTROL ENVELOPE

Most emerging pathogens will have some similarities to existing pathogens and thus may be adequately controlled by current management strategies, technologies, and/or infrastructure. This package of interventions, henceforth referred to as the control envelope, includes the severity of health outcomes and the technological and organizational responses that may be used to control the disease. The control envelope offers a starting point for screening emerging waterborne pathogens against known properties of other pathogens (e.g., disease severity) and the effectiveness of current disease control measures. The concept of the control envelope provides a useful tool for predicting under which conditions certain pathogens will pose the greatest threats (see Table 5.1). A pathogen that is likely to exceed any one of the control envelope parameters could be flagged as a potential threat and management interventions introduced accordingly.

For example, in cases where inadequate water treatment or short-term under-performance leads to pathogen breakthrough, it is of much greater concern if the organism causes a severe illness (e.g., *Escherichia coli* O157:H7) than if the pathogen causes a mild self-limiting illness (e.g., norovirus) (see Table 5.1). Of course, if the pathogen causes a severe illness in a special subpopulation, such as individuals infected with human immunodeficiency virus (HIV), then the consequences can also be grave. *Cryptosporidium* causes a mild self-limiting illness in most individuals; in immunocompromised individuals, however, the illness may persist indefinitely (this is exacerbated by the fact that, until recently, there was no effective chemotherapy). In the 1993 *Cryptosporidium* outbreak in

Milwaukee, USA, of the 54 associated deaths, 85% of the victims were infected with HIV (Hoxie *et al*. 1997).

Table 5.1. Examples of known waterborne zoonotic pathogens and exceedances of control envelope parameters (from Mas-Coma *et al.* 1999; Hermon-Taylor and El-Zaatari 2004; WHO 2004)

Pathogen	Control envelope parameter of concern
Cryptosporidium parvum	Widespread domestic and wild animal reservoirs
	Environmentally persistent
	Resistant to treatment processes
	Low infectious dose
	Limited immune response
	Vaccine development unlikely
	Severe outcomes in immunocompromised populations
	No effective treatment
Toxoplasma gondii	Widespread domestic and wild animal reservoirs
	Severe outcomes in immunocompromised and fetuses
	Environmentally persistent
	Resistant to treatment processes
Campylobacter jejuni	Widespread domestic and wild animal reservoirs
	Antibiotic-resistant strains
	Severe outcomes in immunocompromised and delayed sequelae
	Low infectious dose
Escherichia coli O157:H7	Widespread in domestic animal reservoirs
	Severe outcomes in children
	Low infectious dose
	Persistent in certain environments
	Resistant to antibiotics
Mycobacterium avium ssp. *paratuberculosis*	Causes severe illnesses in susceptible individuals
	Infects a wide range of animal hosts, both domestic and wild, and is widespread in various regions
	Difficult to isolate, culture, and identify
	Evades the immune system by surviving in macrophages
	Can be carried asymptomatically for years
	Environmentally persistent
	Resistant to heat and chlorination and can survive in biofilms and distribution systems
	Has multiple transmission routes, including drinking-water, recreational water contact, and the inhalation of aerosols
	Resistant to a number of antibiotics and may reappear after treatment is completed

Pathogen	Control envelope parameter of concern
Hepatitis E virus (HEV)	Geographic spread and frequency of infection of animal reservoirs unknown
	Severe outcomes in pregnant women
	Low infectious dose
	Detection and cultivation difficult
	Thought to be environmentally persistent
	Thought to be resistant to treatment processes
	No medical treatment available
Fasciola hepatica	Widespread in domestic and wild animals in certain regions
	Disease of moderate to high severity
	Low infectious dose
	Limited immune response
	Environmentally persistent
	Resistant to treatment processes
	Can infect a variety of snail intermediate hosts in a wide range of habitats
	Can develop drug resistance
	Human variant has novel properties that facilitate the transmission through drinking-water

The control envelope will differ significantly from country to country and will depend on many different factors, including climate, level of technological development, access to water supply and sanitation, farming practices, etc. For example, waterborne zoonotic pathogens such as certain strains of *E. coli* (non-enterotoxigenic) or *Salmonella* will pose little threat to people with access to well managed water supplies where disinfection is routinely practised, but could contribute to a significant burden of waterborne disease in situations where drinking-water is not adequately disinfected.

Each of the following subsections looks at key areas for the control of emerging waterborne diseases, including livestock and animal management; source water protection; water storage, treatment, and distribution; and point of use/household. For each key area, pathogen–human factors, technological/management interventions, and examples of factors that might lead to a breach in the control envelope are discussed. In addition, some relevant pathogen properties and human characteristics are briefly considered at the end of the section.

5.2.1 Livestock and animal management

Livestock and animal management is a key component of the control envelope because it involves prevention of pollution at the source. Table 5.2 gives an overview of some important disease management considerations. Chapters 24 and 25 provide more details on livestock and animal management strategies to reduce the transmission of waterborne zoonoses.

Table 5.2. Control envelope for livestock/animal management

Pathogen–human factors	Technological/management interventions	Examples of factors that might lead to a breach in the control envelope
Wide range of zoonotic reservoirs (domestic/wild) Geographic range of zoonotic reservoirs Proximity to human populations Exposure to antibiotics	Vaccination of domestic animals Hygienic rearing conditions for animals Adopting water quality and food quality standards for livestock Treatment of animal wastes at source Exclusion of animals from catchment basins Chemotherapy for livestock (only as necessary) Stop use of antibiotics as growth promoters Develop probiotics to exclude pathogens or reduce shedding from the intestinal tracts of domestic livestock Controlling the international trade of livestock and exotic pets	Use of antibiotics as growth promoters has led to the creation of multiple drug-resistant pathogens and in some cases may have led to the transfer of virulence genes to other pathogens *C. jejuni* rapidly spread to other chickens in a flock through non-disinfected water Infected livestock introduced into a country due to improper quarantine procedures Pregnant women contracting toxoplasmosis from handling cat litter boxes

5.2.1.1 Pathogen–human factors

Important control envelope considerations for livestock and animal management include the host specificity of the pathogen, i.e., can it infect a wide range of both domestic and wild animals or just a few. Likewise, the geographic spread of animal reservoirs in the catchment basin is a key factor. The exposure of domestic animals to antibiotics can select for antibiotic-resistant pathogen strains and in some cases may promote the transfer of

virulence genes from one bacterial species or strain to another (Tauxe 2002). The proximity of humans to animal reservoirs will increase the potential for human exposure to emerging waterborne (and other) zoonoses. Other examples of important considerations are presented in Table 5.2.

5.2.1.2 Technology/management interventions

For livestock/animal management, a number of technologies and interventions to control disease transmission are available. The interventions fall roughly into two categories: 1) preventing or treating infections in animals (e.g., vaccination, adopting water and food quality standards for livestock, hygienic rearing conditions, chemotherapy of infected animals); and 2) preventing pathogens from entering water sources (e.g., waste treatment at source, exclusion of animals from catchment basins, establishing vegetative buffer zones, regulating the international trade in livestock and exotic pets). For example, in the 1980s, *Salmonella* Enteritidis swept through chickens worldwide, but Australia and New Zealand managed to avoid this, at least in part through strict quarantine procedures (Crump *et al*. 2001).

5.2.1.3 Examples of factors that might lead to a breach in the control envelope

Antibiotic-resistant strains of *Campylobacter jejuni* infecting humans in the USA have been directly linked back to poultry (CDC 2002). *Campylobacter jejuni* has been observed to be rapidly transmitted through a flock of chickens by contaminated, non-disinfected water, and *E. coli* O157:H7 may be transmitted to other cattle through contaminated water troughs (Tauxe 2002). *Mycobacterium avium* (ssp. *paratuberculosis*) (MAP), thought to be a cause of Crohn's disease in humans, was introduced into sheep, cattle, and possibly humans in Iceland by the importation of infected sheep (see Box 5.1) (Hermon-Taylor and El-Zaatari 2004).

5.2.2 Source water protection

Table 5.3 describes some elements associated with the control envelope for source water protection. Chapter 26 includes more information on catchment protection from livestock and other animals.

5.2.2.1 Pathogen–human factors

Some of the factors that influence disease transmission in animals/livestock are also relevant to source water protection — e.g., the prevalence and geographic spread of infected animals in the catchment basin. For example, in

a study in the United Kingdom, Chapman (2000) found that 16% of the cattle in herds were infected with *E. coli* O157:H7. Other studies have indicated that from 1 to 10% of cattle in North America and Europe are infected with *E. coli* O157:H7. *Escherichia coli* O157:H7 has been found to infect cattle, sheep, deer, and chicks (Armstrong *et al.* 1996). A significant percentage of livestock herds in Europe and North America test positive for MAP, and MAP has been found to infect a wide range of both domestic and wild animals (Hermon-Taylor and El-Zaatari 2004). Pathogens that are environmentally persistent and resistant to treatment processes will generally pose management challenges (e.g., *Cryptosporidium*, *Giardia*, *Fasciola hepatica*).

Box 5.1. Introduction of MAP-infected sheep and association with Crohn's disease in Iceland

In the 1930s, 20 sheep were imported into Iceland from Germany. Although the sheep appeared healthy, at least some of them were asymptomatically infected with *Mycobacterium avium* ssp. *paratuberculosis* (MAP). The sheep were distributed to 14 farms. In 1938, at five of the original farms, sheep were diagnosed with Johne's disease (a disease in livestock that has symptoms similar to those of Crohn's disease in humans and is caused by MAP). The disease eventually spread to cattle on the same farms and slowly spread throughout all of Iceland's sheep population (Hermon-Taylor and El-Zaatari 2004).

MAP has been reported as a probable cause of Crohn's disease in humans and is frequently identified in patients with Crohn's disease (Hermon-Taylor *et al*. 1998; Sechi *et al*. 2001). Prior to the 1930s, Crohn's disease had never been recognized in Iceland; subsequently, the increase in the disease in human populations has mirrored the spread of MAP throughout livestock on the island (Hermon-Taylor and El-Zaatari 2004). In Iceland, Crohn's disease increased from 0.4 cases per 10^5 population in the 1950s to 5.6 cases per 10^5 population in the 1990s — a 1300% increase (Bjornsson 1989; Bjornsson *et al*. 1998; Bjornsson and Johannsson 2000; Hermon-Taylor and El-Zaatari 2004).

MAP is a pathogen that would not easily be managed within the current control envelope (see Table 5.1). For more information concerning MAP and its association with Crohn's disease, see Hermon-Taylor and El-Zaatari (2004).

5.2.2.2 Technological/management factors

Strategies for source water protection incorporate both management interventions and technological solutions. Management can include animal exclusion from catchments and establishing vegetative buffer zones. Technological solutions include installing wastewater treatment facilities (or

upgrading old ones), repairing leaking sanitation systems, and covering water storage reservoirs.

Table 5.3. Control envelope for source water protection

Pathogen–human factors	Technological/management interventions	Examples of factors that might lead to a breach in the control envelope
Proximity of zoonotic reservoirs to water sources Ubiquity of animal reservoirs in catchment basin Human and animal waste treatment Soil runoff potential Integrity of sanitation systems Use of human/animal wastes as fertilizers Environmental persistence Resistance to treatment processes	Exclusion of animals from vulnerable areas (fencing) Human/animal waste treatment at source Wastewater treatment Buffer zones Protection of wells/springs, groundwater Reduction of soil erosion Repair of leaking sanitation systems Covering drinking-water reservoirs Treatment of animal/human wastes prior to use as fertilizers	Heavy rainfall events often precede disease outbreaks Failure of animal waste storage lagoons during severe storm events Failure to site or protect wells adequately Lack of vegetative buffer zones Animals allowed access to riparian zones

5.2.2.3 Examples of factors that might lead to a breach in the control envelope

Heavy rainfall events wash large amounts of contaminants, including animal faeces, into watersheds. These severe weather events often precede waterborne disease outbreaks. Curriero *et al*. (2001) analysed disease outbreak data in the USA and compared the data with rainfall events from the period 1948–1994. Sixty-eight per cent of the disease outbreaks were significantly associated with heavy rainfall (above the 80th percentile for severity of rainfall). Outbreaks associated with groundwater sources occurred 2 months after severe rainfall events. Among the contributing factors in the 2000 outbreak of *E. coli* O157:H7 in Walkerton, Canada, was a failure to protect groundwater from animal runoff. Additionally, the well was shallow (5–8 m in depth) and located an insufficient distance (less than 100 m) from sources of animal manure (Hrudey 2003). A 1996 US Environmental Protection Agency (EPA) survey of rural wells found 40% contaminated with *E. coli* (not necessarily O157:H7) (Tenenbaum 2002).

5.2.3 Water storage, treatment, and distribution

Water storage, treatment, and distribution form the core components of the traditional water supply industry and have always been considered as key elements for protecting public health. Examples of important control envelope considerations for these processes are presented in Table 5.4. Treatment technologies for both animal and human wastes are discussed in more detail in chapters 25 and 26.

Table 5.4. Control envelope for water storage, treatment, and distribution

Pathogen–human factors	Technological/management interventions	Examples of factors that might lead to a breach in the control envelope
Environmental persistence Resistance to treatment processes Ability to grow in environment	Sanitary surveys to identify sources of contaminants Cover water storage reservoirs or store in confined aquifers Change treatment technologies Change disinfectants Optimize treatment processes Process monitoring to complement indicator organism monitoring Repair distribution systems Manage water distribution systems to reduce development of biofilms Repair leaking sewers	Severe storm events that overload or incapacitate treatment facilities Animal access to storage reservoirs (e.g., birds) Normal fluctuations in treatment processes Over-reliance on microbial indicators Ingress of contaminants into distribution system Loss of pressure in distribution system

5.2.3.1 Pathogen–human factors

Environmental persistence and resistance to treatment processes are important pathogen characteristics in water storage, treatment, and distribution. *Cryptosporidium* and *Giardia* are both persistent environmentally and extremely resistant to chlorine. *Escherichia coli* has been found to survive in biofilms in distribution systems even when high residual chloramine concentrations are present (Williams and Braun-Howland 2003). *Mycobacterium avium* complex (MAC) is also resistant to chlorine and can grow in biofilms (AWWA 1999). Pathogens that have more severe outcomes are important because short-term under-performance can lead to pathogen breakthrough.

5.2.3.2 Technological/management interventions

Prevention of disease transmission through water storage, treatment, and distribution is to some extent a function of system design. The supply of safe water requires a multi-barrier approach that compensates for short-term treatment performance fluctuations. Conducting sanitary surveys is an important procedure for identifying potential sources of contamination. Optimizing water treatment performance through process and indicator organism monitoring and selecting appropriate treatment processes are also necessary. Covering reservoirs or using confined aquifers for water storage can reduce contaminant ingress. Water distribution systems should be adequately maintained and kept under positive pressure. Distribution systems should be managed to control biofilm formation.

5.2.3.3 Examples of factors that might lead to a breach in the control envelope

Even in well managed water treatment systems and settings where health care infrastructure is adequate, risk management strategies will occasionally fail. For example, the efficiency of water treatment processes varies over time and spatially through the treatment system (Gale and Stanfield 2000). Events such as heavy rainfall, system overloading, short-circuiting, membrane failures, or inadvertent recycling of filter backwash can cause pathogen breakthroughs. The May 2000 *E. coli* O157:H7 outbreak in Walkerton, Canada, that affected over 2000 people was a result of the failure to protect drinking-water supplies from farm runoff, a failure in the water disinfection processes, and a failure to communicate laboratory results to local public health officials (Kondro 2000; WHO 2004).

Moreover, monitoring for pathogens or indicator organisms is not conducted on a real-time basis, and thus contamination is already through the system before it has been detected. Even where routine monitoring is in place, contamination slugs can be missed. Gale and Stanfield (2000) modelled *Cryptosporidium* breakthrough events and demonstrated that a significant proportion (33%) of random 100-litre spot samples would detect zero oocysts, but that a very small percentage of samples would contain hundreds or even thousands of oocysts. The high-concentration samples could easily be missed by the monitoring process. This was the case for the 1993 *Cryptosporidium* outbreak in Milwaukee, USA, that affected 403 000 people (MacKenzie *et al*. 1994). Similarly, *Cryptosporidium* was detected in 26% of filtered drinking-water samples taken from 82 different treatment plants in one survey (Aboytes and LeChevallier 2003).

Friedman *et al*. (2003) demonstrated that significant volumes of water could enter the distribution system through small leaks when pressure in the system was not maintained. Also, Kirmeyer *et al*. (2001) showed that soil and water in close proximity to distribution systems often contained evidence of faecal contamination, including the presence of enteric viruses, and thus posed a potential contamination risk during low-pressure events.

5.2.4 Point of use/household

The point of water use and the household represent another element of the control envelope. Poor conditions of water, sanitation, and hygiene (personal, domestic, and food) can affect the transmission of waterborne zoonoses at the point of water use (including in hospitals and other facilities). Table 5.5 presents examples of important point-of-use considerations in the transmission of waterborne zoonoses.

Table 5.5. Control envelope for point of use/household

Pathogen–human factors	Technological/management interventions	Examples of factors that might lead to a breach in the control envelope
Transmission through poor personal, domestic, or food hygiene High probability of secondary or person-to-person transmission Reservoirs include domestic animals or exotic animals kept as pets Vaccination Immune status	Point-of-use water treatment Improve personal, domestic, and food hygiene Access to adequate household sanitation Access to improved water supply Prevent cross-connections Regulate the keeping of exotic pets Vaccination/chemotherapy of household pets Protect household water/ food supply from animals Risk communication to sensitive subpopulations	Poorly designed sanitation systems or cross-connections Pregnant women handling litter boxes Unsanitary conditions that lead to exposure to diseases transmitted by rats (e.g., leptospirosis, HEV) Poor personal, domestic, and food hygiene

5.2.4.1 Pathogen–human factors

Pathogens that infect household pets (e.g., toxoplasmosis in cats) or other animals that might be found in living spaces (e.g., *Leptospira* or HEV, which can infect rats) pose a threat. Diseases that have high secondary infection rates

through person-to-person spread (e.g., *E. coli* O157:H7) are also of concern. People that do not have access to adequate water supply and sanitation and/or practise poor hygiene habits will be at increased risk. A number of human factors will also be important at this point, including immune status (normal or immunocompromised), vaccination, and a number of other factors that will be further discussed in section 5.2.6.

5.2.4.2 Technological/management interventions

Hygiene education and increasing access to improved water supply and sanitation will impact the household transmission of waterborne zoonoses (and other waterborne diseases as well). Designing sanitation systems and identification and removal of household cross-connections are also important interventions. Risk communication targeted to vulnerable populations (e.g., pregnant women, immunocompromised individuals) has been effective for helping to control health risks in some cases. For example, pregnant women could be advised about the dangers of handling cat litter boxes. People suffering from chronic liver diseases could be discouraged from eating raw oysters.

5.2.4.3 Examples of factors that might lead to a breach in the control envelope

Many cats are infected with *Toxoplasmosa gondii* and can transmit this disease to humans, mainly through contact with litter boxes, but possibly also through contaminated water in contact with food (e.g., in the garden) or by being consumed directly (e.g., if a household well is poorly protected). Pregnant women and HIV-infected individuals are at increased risk from toxoplasmosis because the consequences are much more severe (CDC 2003). In the 2003 severe acute respiratory syndrome (SARS) outbreak, a proportion of victims in one apartment block were thought to have been exposed to the SARS virus by inhalation of faecal droplets. People were exposed to faecal droplets containing the SARS virus through a combination of a poorly designed sanitation system and a ventilation system that pulled the faecal droplets into their apartments (SHWF 2003).

5.2.5 Pathogen properties that influence the control envelope

Pathogen properties will have a great impact on various parameters of the control envelope. Table 5.6 describes some pathogen factors and how they influence the control envelope. Characteristics of specific bacteria, viruses, protozoa, fungi, and helminths are described in more detail in Section V.

A number of pathogen properties impact the way in which they can be managed within the current control envelope. Pathogen virulence and infectivity can differ by orders of magnitude between different strains. Many of the recently emerged pathogens have very low infectious doses (ID) (Tauxe 2002). For example, *E. coli* O157:H7 may have an ID of 50 or less according to the study of an outbreak associated with the consumption of dry cured salami in the USA (CDC 1995). Other pathogenic strains of *E. coli* have much higher IDs, in the order of 100 000–1 000 000 organisms (Teunis *et al*. 1996). Part of this difference may be attributed to the acquisition of a new characteristic — for example, the transfer of a virulence gene from another organism or the ability to survive in low-pH environments. *Escherichia coli* O157:H7 can survive under certain circumstances at pHs as low as 2 (Conner and Kotrola 1995) and can thus be transmitted in acidic foods, such as apple cider (Miller and Kaspar 1994).

Table 5.6. Pathogen properties and how they impact the control envelope

Properties	Examples of potential control strategies
Virulence/infectivity Severity of outcome Epidemic potential Environmental survival/growth Resistance to water treatment processes Lack of host specificity Ability to develop new characteristics (e.g., antibiotic resistance) Susceptibility to host immune response Susceptibility to chemotherapeutic agents	Surveillance of immunocompromised populations Surveillance of human and animal populations for infection and disease Development of isolation, identification, and culture techniques/technologies Research on pathogen virulence/transmission factors Vaccine/chemotherapy development Elimination of non-essential use of antibiotics Research on treatment trains and pathogen removal

The ability of an organism to change is an important characteristic. Some pathogens can rapidly develop new characteristics, such as new surface proteins, to evade host immune responses (e.g., *Plasmodium falciparum* or HIV) or develop antibiotic resistance. For example, one study demonstrated that *C. jejuni* in chickens were converted from 100% sarafloxacin-susceptible strains to 100% sarafloxacin-resistant strains within days of first being exposed to the antibiotic (McDermott *et al*. 2001). There is also some evidence to suggest that sublethal doses of antibiotics increase the transfer of virulence genes between different strains of bacteria, potentially leading to the development of new serotypes — possibly even *E. coli* O157:H7 (Zhang *et al*. 2000; Tauxe 2002).

Interventions to control the disease risks from emerging waterborne zoonoses will often need to be tailored to the specific organism and may include:

- the development of rapid and simple isolation, identification, and/or culture techniques/technologies;
- improved human and animal surveillance to identify emerging pathogens of possible concern;
- reducing the use of antibiotics for growth promoters in livestock;
- research on vaccines and medicine for both humans and animals; and
- research on effective treatment trains and pathogen inactivation.

5.2.6 Human characteristics and their impact on the control envelope

Human characteristics, including genetic, socioeconomic, and behavioural factors, are all likely to have a profound impact on the transmission of waterborne zoonoses. Table 5.7 gives examples of some human characteristics and how they affect the control envelope.

Table 5.7. Human characteristics and their impact on the control envelope

Human characteristics	Technological/management interventions
Immune status	Education of public, especially high-risk populations
Age	Identifying high-risk populations
Pregnancy	Improvement of hygiene practices
Medications taken	Vaccination
Presence of other diseases	Research into new vaccines/ chemotherapeutic agents
High-circulation vs. low-circulation environments	Increasing access to improved water supply and sanitation
Genetic susceptibility	Increasing the distance between animals and humans
Malnutrition	Behaviour modification (e.g., cooking food thoroughly)
Access to improved water supply and sanitation	Point-of-use water treatment
Personal, domestic, and food hygiene behaviour	
Proximity to animal reservoirs	

Pathogen properties and human characteristics interact to determine disease transmission. Human immune status is a key factor and can be altered in several ways, depending on the environment. In conditions where there is high circulation of pathogens (i.e., sanitary standards are low), early exposure to a pathogen such as hepatitis A virus (HAV) may lead to a mild self-limiting

illness and lifelong immunity. The same virus can cause a relatively serious disease in people that are infected as adults (i.e., in low pathogen circulation environments). Other diseases can cause acute illnesses (e.g., *E. coli* O157:H7) or chronic infections in children in the same environments, potentially leading to serious health consequences (e.g., hepatitis B virus [HBV]).

Human immune status can be affected by diseases (HIV, cancer), age, drugs, pregnancy, malnutrition, genetics, etc. According to Exner and Kistemann (2003), approximately one out of six people in the United Kingdom, Germany, and the Netherlands fits the definition of immunocompromised. The simple act of taking an antacid tablet for indigestion can make an individual more susceptible to infection with a pathogen that would not normally survive in the acidic environment of the stomach. Behaviour also plays a very important role with regard to disease susceptibility. For example, people that eat inadequately cooked meat or seafood — especially shellfish — increase their risks of contracting an infection.

Important interventions to address human dimensions of the control envelope largely focus on improving access to water supply and sanitation, risk communication, vaccine/medicine development, and behaviour modification. These interventions have been discussed elsewhere in this chapter in more detail.

5.3 RISK MANAGEMENT

Risk management strategies include HACCP-like approaches that identify control points and use data from epidemiological studies (where they exist), microbiological evaluations of water or food products, and quantitative microbial risk assessment (QMRA) to develop WSPs that are based on the multi-barrier principle.

The control envelope not only covers interventions from farm to fork but goes beyond, because it can also include altering human and animal immunity through vaccination and other processes. Effective management strategies will require a vast range of expertise, including veterinarians, range management experts, farmers, water resource managers, hydrogeologists, civil engineers, health professionals, and others. Prevention of pollution at the source is the most effective solution, and thus management strategies that focus on reducing the pathogens in animal reservoirs or treating waste that contains the pathogens before it reaches water sources are likely to have the greatest impact on health protection.

5.3.1 HACCP

HACCP is a systematic approach to the identification, assessment, and control of hazards (Codex Alimentarius Commission 1997) during production, processing, manufacturing, preparation, and use of food, water, or other substances to ensure that the food, water, or other substances are safe when consumed or used. The HACCP system incorporates safety control into the design of the whole process rather than relying solely on end-product testing. The HACCP approach provides a preventive and thus a cost-effective method for ensuring product safety. Initially created for the food processing industry, HACCP has subsequently been applied to a number of different processes, including drinking-water treatment, aquaculture production, and the use of sewage sludge in agriculture (Havelaar 1994; Garrett *et al.* 1997; Godfree 2000).

Application of HACCP systems in many different manufacturing or treatment processes has led to more efficient prevention of adverse health effects associated with the consumption or use of the products. For example, the implementation of an industry-wide HACCP programme for seafood processors in the USA is thought to have averted 20–60% of the normal number of seafood-borne illnesses (Birley and Lock 1998). A similar programme for the prevention of foodborne listeriosis in the USA reduced the incidence and mortality of this disease by 44% and 49%, respectively, over a period of 4 years (Billy 1997).

Limited evidence suggests that implementation of HACCP-like processes into a water quality assurance framework (WSP) will also reduce water-related disease incidence and outbreaks. For example, in the USA, disease outbreaks associated with drinking-water supplied from surface water decreased from 31.8% of all drinking-water outbreaks in 1995–1996 to 11.8% in 1997–1998 (Barwick *et al*. 2000). Much of this reduction was attributed to a partnership between the US EPA and drinking-water agencies to implement HACCP-like preventive procedures designed to optimize water treatment at their facilities (Barwick *et al*. 2000; Godfree 2000). The US EPA Interim Enhanced Surface Water Treatment Rule (US EPA 1998) also includes HACCP-like strategies for reducing drinking-water-related disease (Godfree 2000). In north-west England, the implementation of a HACCP-like process to protect the watershed has at least partially contributed to a lack of *Cryptosporidium* outbreaks in the catchment basin since 2000 and a general decline in the background incidence rate from 1400 cases per year to approximately 400 per year in each of 2001 and 2002 (Godfree 2003). It is likely that further use of these programmes in water treatment, water supply, and water reuse processes will have similar results.

Deere *et al*. (2001) outlined the development of a HACCP system for drinking-water and presented some case-studies for identifying critical control points.

5.3.2 Epidemiological evidence and QMRA

Epidemiological studies are needed to determine the actual disease transmission due to a specific exposure. Data from epidemiological studies are crucial for determining actual health risks, but studies must be large enough to capture significant differences in levels of disease due to a specific exposure. Additionally, epidemiological studies can be expensive.

QMRA (further discussed in chapter 29) provides an alternative or supplementary framework to epidemiology for identifying potential excess risk for defined pathways of particular pathogens from source to recipient. QMRA translates the environmental occurrence of pathogens into the probability of infection (microbial risk) following the paradigm used for chemical risk assessment and has the potential to provide much greater sensitivity in identifying risk. The usefulness of QMRA, however, is dependent upon the quality and appropriate use of available data for describing the occurrence, persistence, and human dose–response of pathogens in the environment (Petterson and Ashbolt 2003).

For emerging pathogens, data on their environmental occurrence may be very limited. Before QMRA can be effectively used as a tool to manage risk, techniques and technologies for identifying, quantifying, and assessing the viability of pathogens need to be developed (see Section VI for further information). Similarly, research on identifying appropriate indicator organisms for emerging waterborne zoonoses or other key parameters for monitoring (e.g., particle counts, turbidity) needs to be conducted.

Given the vagaries in data on immune status and variability, QMRA can only predict potential excess risks for specific pathogens and pathways. Further, limited dose–response data are available, especially for potential animal reservoirs, and in some cases these data vary by more than 1000-fold for different strains of the same pathogen. For the time being, therefore, QMRA should not be seen as directly comparable to epidemiological data, but rather as a tool to assess the sensitivity of changes in performance of (treatment) elements and to identify major risk groups and pathways (Petterson and Ashbolt 2003).

5.3.3 Water safety plans

WSPs are used to develop a systematic programme for protecting water quality from catchment to tap. In the case of waterborne zoonoses, important elements of the plan will include managing animal disease reservoirs outside what

normally might be considered the responsibilities of a water supplier. This aspect of pollution prevention is critical, as large percentages of animals may be carriers of pathogens. Similarly, infected animals may be widespread within the catchment basin. For example, *Cryptosporidium*, MAP, *C. jejuni*, and *F. hepatica* can infect a wide range of both domestic and wild animals and may infect animals throughout the catchment basin. WSPs could be expanded to include the delivery of appropriate quality water to livestock.

Both HACCP and WSPs should include analysis of the health impacts that might be associated with infrequent catastrophic system failures (e.g., flooding of a treatment facility or sabotage) and develop management strategies for such events to control health impacts. For example, WSPs should include procedures for informing health authorities in emergencies and alerting the public in the case of a boil water notice.

For water supply services, water quality management is critical. The detection of waterborne zoonotic pathogens in both raw water and water delivered to consumers is often slow, complex, and costly, which limits early warning capability and affordability. Reliance on water quality determination alone is insufficient to protect public health (UNESCO-WWAP 2003).

The most effective and protective means of consistently ensuring a supply of acceptably safe water is the application of a preventive "quality assurance" framework. A preventive framework, developed to manage water quality, works in an iterative cycle, encompassing assessment of public health concerns, risk assessment, establishing health-based water quality targets, and risk management. Feeding into this cycle are the determination of environmental exposure levels for both humans and animals and the estimation of what constitutes a tolerable risk (see chapter 28) (UNESCO-WWAP 2003; WHO 2004).

According to UNESCO-WWAP (2003), water quality management may be through a combination of protection of water sources, control of treatment processes, and management of the distribution and handling of water. It has five key components:

(1) water quality targets based on critical evaluation of health concerns (both human and animal health);
(2) system assessment to determine whether the water supply chain (up to the point of consumption/use) as a whole can deliver water of a quality that meets the above targets;
(3) monitoring of the control points in the supply chain, which are of particular importance in securing water safety;

(4) management plans documenting the system assessment and monitoring and describing actions to be taken under normal and incident conditions (this includes documentation and communication); and
(5) a system of independent surveillance that verifies that the above are operating properly.

It is important that water quality targets, defined by the relevant national health authority, are realistic under local operating conditions and are set to protect and improve public health. Formal water supply agencies have a basic responsibility to provide safe water and would be expected to develop and implement management plans to address points 2–4 above (UNESCO-WWAP 2003).

The management plans, or WSPs, developed by water suppliers should address all aspects of the water supply and focus on the control of water production, treatment, and delivery of drinking-water. The control of the microbial quality of drinking-water requires the development of WSPs that, when implemented, provide the basis for process control to ensure that pathogen loads are acceptable. Implicit within this process is that a tolerable disease burden has been defined at national and local levels and that water quality targets, which have been established to improve public health, are achievable (UNESCO-WWAP 2003).

5.3.4 Disease surveillance and targeted studies

Surveillance of public health status and trends is a very important tool for risk management. As the disease transmission characteristics of pathogens become better known, then methods for their control can be developed. Similarly, surveillance facilitates the prioritization of risk management by putting disease risks into the context of the overall burden of waterborne disease within a society (see chapter 28).

Surveillance can be used to answer a number of questions. First, is the disease (or severe outcome) present in the population (immunocompetent or immunocompromised populations)? If the disease or severe outcome exists in the population, is there evidence of transmission through water? Another important consideration is the ability of the surveillance system to detect emerging diseases, and at what level.

Routine surveillance of currently known waterborne zoonoses, including *Cryptosporidium*, *E. coli* O157:H7, etc., is important. However, to identify emerging threats, Tauxe (2002) suggests that increased surveillance efforts be directed towards pathogens that cause severe disease in immunocompromised

individuals and further investigation of organisms that cause asymptomatic infections in various animals.

In some ways, immunocompromised individuals are the sentinels of emerging zoonotic infections. Evidence that humans are being exposed to emerging or re-emerging pathogens will first appear in these individuals. Severe illnesses will be more common in these subpopulations, increasing the probability that the pathogen will be identified/isolated from blood or other normally sterile tissues rather than in food or faeces, where there are large numbers of other organisms (Tauxe 2002). For example, *C. jejuni* was first recognized as a cause of bacteraemia in children with leukaemia (King 1962). It was only after selective isolation procedures were developed that the organism could be identified in the faeces of children with diarrhoea (Tauxe 2002).

According to Tauxe (2002), many of the emerging zoonoses identified in recent years share similar features, including the following:

- cause asymptomatic infections in their animal hosts;
- animal hosts act as long-term carriers of the organism;
- cause infections in humans in relatively low doses; and
- are transmitted through products, such as water or foods, that are not cooked before consumption.

Therefore, closer surveillance of asymptomatic infections in animals may yield important clues for managing emerging zoonotic pathogens.

Targeted studies of populations looking for serological evidence of infection can also be useful. For example, cryptosporidiosis was often thought to be largely a developed country problem. However, when studies started to look at serological evidence of *Cryptosporidium* infection in various developing countries, they found it at very high levels. In Anhui, China, over half of the children by age 5 demonstrated antibodies to *Cryptosporidium*. In a poor area of Brazil, over 90% of the children developed antibodies to *Cryptosporidium* by the age of 1 (Ungar *et al*. 1988; Zu *et al*. 1994).

5.3.5 International networks

The international trade in food products and the general growth in international travel make it increasingly important to develop international networks to monitor the spread of both known and emerging pathogens. Products grown with contaminated water may cause health effects at both the local and international levels, and international trade in agricultural products across regions is growing. Exports of contaminated fresh produce from different geographical regions can facilitate the spread of both known pathogens and

strains with new virulence characteristics into areas where the pathogens are not normally found or have been absent for many years (Beuchat 1998). Infected livestock (see Box 5.1) and exotic pets may also introduce emerging pathogens into new regions.

Modern processing methods for meat products also facilitate the spread of pathogens across regions. Large packing/processing plants process meat from various regions. In a 1993 *E. coli* O157:H7 outbreak in the USA, the source trace-back showed that beef used in the product had come from several suppliers and three different countries. Armstrong *et al.* (1996) estimated that one infected cow used to produce ground beef could contaminate up to 8 tonnes of meat. Moreover, pathogens may be introduced into areas where natural immunity is absent, resulting in more severe health consequences.

SARS also provides an example of how fast a disease can cross the globe in the era of modern travel. All of this illustrates the need for international surveillance networks that can facilitate cooperation to control the transport of waterborne and other emerging zoonoses into new regions. For example, the World Health Organization and an international network of national governments have established a network of 148 laboratories to monitor polio eradication efforts. Reported cases of polio are evaluated quickly, and necessary actions can be taken to prevent outbreaks from growing. Because of these efforts, wild polio strains currently exist in only a handful of countries, and the disease is scheduled to be completely eradicated by 2005 (WHO 2003b). Similar cooperative efforts will be needed in the future to prevent the dissemination of emerging waterborne zoonoses.

5.4 CONCLUSION

Many of the emerging waterborne zoonoses are difficult to manage. Developing the concept of a control envelope provides a framework for evaluating emerging pathogens against risks posed by better understood organisms. The control envelope is defined broadly and may require professionals to consider risk management strategies outside of their areas of expertise (e.g., water supply and distribution). Effective management of emerging waterborne zoonoses thus requires cooperation across a broad range of disciplines. A variety of risk management tools are available (e.g., QMRA, HACCP, WSP, disease surveillance) but must be extended to encompass the entire spectrum of the control envelope to effectively manage many of the newly emerging waterborne zoonoses.

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Section III

Water-related zoonosis disease impacts — geographical prevalence

A. Dufour

Surveillance of waterborne infectious disease has become an important aspect of public health practice. Although not all countries do so, there is a growing recognition of the value of detecting and quantifying the occurrence of cases and outbreaks of disease. A formal surveillance programme combined with aggressive epidemiological investigations will produce data leading to the identification of emerging pathogens, sources of etiological agents, and susceptible populations. The Legionnaire's disease pneumonia in 1976 is a classic example of identifying an emerging pathogen and the follow-up tracking of the disease. Surveillance of subsequent outbreaks identified various exposure scenarios that resulted in legionellosis. Identification of sources and risks associated with this microbe has led to better risk assessments and control measures for *Legionella*. A similar history is found for the zoonotic protozoan

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pathogen, *Cryptosporidium*. This microbe was well known to veterinarians as an intestinal disease in cattle, especially calves. Epidemiological evidence collected during outbreaks clearly established the linkage between the water environment and cryptosporidiosis in humans. These emerging and re-emerging diseases, such as cholera, might not have received broad attention without active surveillance systems in place. The globalization of food markets and the potential rapid dissemination due to the ease of international travel are yet other reasons to maintain active surveillance of infectious disease.

On a local or regional basis, surveillance of infectious disease will frequently alert authorities to a breakdown in water treatment processes or to the poor design of waste management facilities. The increase in zoonotic disease outbreaks should serve to strengthen efforts to maintain a watch for infectious disease outbreaks and should encourage a closer working relationship between medical and veterinary epidemiologists to improve surveillance systems.

The first chapter in this section describes emerging and re-emerging disease in Africa, Asia, and South America. Most of the cases and outbreaks described are associated with bacteria, perhaps giving a false impression that viruses and protozoa may not be prevalent on these continents, which is well known not to be true. The authors do point out that this imbalance may be a function of the types of literature reviewed. The difficulties of addressing the collection of health data are discussed, and solutions to some of these problems are listed. The most important of these was the need to intervene with education and training programmes to enable countries to take appropriate steps to reduce waterborne disease.

Chapter 7 discusses water shortages in Mexico, Central America, and the Caribbean and the effect this has had on the health of individuals in this region. The chapter closes with a suggestion that an international network should be created, to share information between countries and to use the information to make decisions about the control of water-related zoonotic diseases.

In chapter 8, zoonotic disease outbreaks associated with drinking-water are described, as well as the deficiencies related to inadequate or interrupted treatment of surface waters. Outbreaks associated with bacteria were associated with untreated groundwater, inadequately disinfected groundwater, and distribution system contamination. It is suggested that better surveillance and outbreak investigation will lead to the reduction of waterborne risks.

The final chapter provides descriptions of zoonotic diseases, their etiological agents, and treatment.

6

Tropical organisms in Asia/Africa/South America

K. Suresh and H.V. Smith

6.1 INTRODUCTION

Zoonotic infections, many transmitted by water, pose a serious threat to global health and the economy. One-third of the world's population lives in countries with some level of water stress, a number that could increase to two-thirds by the year 2025. Twenty per cent of the world's population in 30 countries faces water shortage (Tema 2001). Water use is expected to increase by 40% over the next two decades, and it is estimated that 3 billion people will face water shortages by then. The impact of waterborne and foodborne (Slifco *et al*. 2000) zoonotic pathogens on human health is expected to be significant.

In Africa, Asia, and Latin America, at least 600 million urban dwellers live in unhealthy homes or neighbourhoods. Over a billion people lack access to safe

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drinking-water, increasing their vulnerability to diarrhoeal and parasitic diseases (WHO 1999). Parasitic diseases in infancy can lead to systemic immune system imbalances, increasing stunting and reducing cognizance (Berkman *et al*. 2002). Worm infestations also diminish the efficacy of certain vaccines (e.g., tuberculosis, human immunodeficiency virus [HIV], and malaria; Markus 2002). In a series of 1377 refugees and asylum seekers entering Sweden, intestinal parasites were more frequently recovered in refugees from south-east Asia, Africa, and Latin America (infection rates of 48%, 43%, and 42%, respectively) than in those from Eastern Europe (22%) and the Middle East (32%) (Benzeguir *et al*. 1999). Lack of resources can lead to a resurgence of disease in disease-free areas. According to WHO (1999), success can breed complacency, particularly when diseases have low visibility and limited impact. For waterborne zoonoses, key determinants of human health can lie outside the direct control of the health sector, being rooted in inadequate sanitation, unwholesome water supply, poorly regulated livestock and agricultural practices, environmental and climate change, education, etc. In order to execute effective surveillance, prevention, and control of waterborne zoonoses, useful background information on occurrence must be available.

This chapter highlights organisms (parasites, bacteria, and viruses) found in Asia, Africa, and South America that either are zoonotic or possess the potential to be zoonotic.

6.2 PARASITES

6.2.1 *Ascaris*

The ova of many genera of zoonotic parasites can be found in surface water and groundwater. In properly operated conventional water treatment plants, however, they are excluded from drinking-water by their size (>30 µm). Water is not regarded as a major route of transmission for *Ascaris*, although *Ascaris* ova can be found in the air and in dust and can be transferred to uncovered drinking-water sources. *Ascaris* ova are sticky and can adhere to items such as utensils, furniture, fruit, vegetables, money, door handles, and fingers (Kagei 1983). *Ascaris* infections are widespread throughout many parts of Asia, Africa, and South America. The risk of transferring other geohelminth ova, including *Trichuris* and *Taenia* spp., to uncovered drinking-water sources must also be recognized.

6.2.2 *Cryptosporidium* and *Giardia*

Giardia duodenalis cysts, *Cryptosporidium* spp. oocysts, and spores of microsporidia have also been detected in aquatic environments; however, their smaller size (range 1–17 µm) allows them to penetrate water treatment systems and cause epidemic outbreaks of waterborne disease following the consumption of treated drinking-water (Table 6.1; Smith 1998). These protozoan parasites parasitize the enterocytes, causing an imbalance in food and fluid absorption, which can lead to diarrhoea.

Table 6.1. Some protozoan parasites and the waterborne route of transmission

Organism	Disease/symptoms	Geographic distribution	Transmissive stage (size range) and route of infection
Giardia duodenalis	diarrhoea, malabsorption	widespread	cyst (8–12 µm), ingestion
Cryptosporidium parvum	diarrhoea	widespread	oocyst (4.5–5.5 µm), ingestion
Cyclospora cayetanensis	diarrhoea	widespread	oocyst (8–10 µm), ingestion
Entamoeba histolytica	dysentery, liver abscess	widespread	cyst (9–14.5 µm), ingestion
Microsporidia	diarrhoea, hepatitis, peritonitis, kerato-conjunctivitis, etc.	widespread	spore (1.8–5.0 µm), ingestion/contact with eye
Toxoplasma gondii	lymphadenopathy, fever, congenital infections	widespread	oocyst (10–12 µm), ingestion
Blastocystis hominis; *Blastocystis* sp.	diarrhoea	widespread	cyst (4–6 µm), ingestion
Free-living amoebae (e.g., *Naegleria fowleri*, *Acanthamoeba* spp.)	primary amoebic meningoencephalitis, granulomatous amoebic encephalitis, keratitis	widespread	cyst (7–18 µm for *Naegleria*; 15–28 µm for *Acanthamoeba*), inhalation, contact with conjunctiva

Giardia and *Cryptosporidium* have become significant waterborne pathogens in the developed world for three reasons. First, giardiasis and cryptosporidiosis are indigenous infections with a low infectious dose; second, densities of environmental contamination with infective cysts and oocysts [(oo)cysts] are sufficient to pollute the aquatic environment; and third, (oo)cysts are small enough to penetrate water treatment processes and are less sensitive to the disinfectants commonly used in water treatment. Undoubtedly, waterborne

outbreaks of protozoan parasitic infections following contamination from sewage, wastewater effluent, muck spreading, slurry spraying, etc., leading to the contamination of potable water, pose significant problems for both the developed and developing countries of the world. For example, two waterborne cryptosporidiosis outbreaks occurred in Japan (Smith and Rose 1998).

Potential sources of oocysts and cysts detected in raw water (adapted from Smith *et al*. 1995 with permission) are as follows:

- contribution from infected animals;
- pasturing of infected livestock;
- infected wild animals, including "on-farm" rodents;
- watering of infected animals;
- infected domestic/companion animals;
- carriage by water-roosting or aquatic birds;
- contribution from human activities;
- disposal of contaminated faeces and non-controlled effluents from farms;
- accidental spillages from poorly constructed slurry stores and middens;
- slurry spraying and muck spreading;
- intensive husbandry of livestock;
- disposal of faeces from infected animals at abattoirs;
- disposal of sewage sludge to land; and
- disposal of contaminated backwash sludge.

Surveys of occurrence in a variety of countries indicate that *Cryptosporidium* oocysts and *Giardia* cysts can occur commonly in the aquatic environment (Smith *et al*. 1995; Smith and Rose 1998; Gold and Smith 2002) (Tables 6.2 and 6.3). (Oo)cysts have been detected in wastewater, surface waters, groundwater, springs, and drinking-water samples, including those treated by disinfection alone, filtration, direct filtration, and conventional methods. Data collated by Smith and Rose (1998) and Gold and Smith (2002) indicate that *Cryptosporidium* oocysts and *Giardia* cysts occur at similar densities in various countries, with the highest oocyst densities being found in surface waters, and that (oo)cysts can be found in drinking-water in the absence of increased levels of disease among consumers.

Standardized methods, equipment, and commercial kits are available for detecting *Cryptosporidium* oocysts and *Giardia* cysts, but not for microsporidian spores. Equipment and commercial kits are expensive, particularly for developing countries; however, as for bacteriological and viral methods, standardization is paramount in order to determine the significance of occurrence and the threat to public health of waterborne (oo)cysts.

Table 6.2. Occurrence and density of *Cryptosporidium* oocysts and *Giardia* cysts in surface waters (adapted from Smith and Rose 1998; Gold and Smith 2002)

Country	Number of samples	Occurrence of *Crypto-sporidium* oocysts (% samples positive)	Density of *Crypto-sporidium* oocysts (oocysts/ litre)	Occurrence of *Giardia* cysts (% samples positive)	Density of *Giardia* cysts (cysts/ litre)	Year
USA	11	100	2–112	–	–	1987
USA	222	–	–	43	0.5–1	1989
USA	101	24	0.005–252.7	–	–	1990
UK (Scotland)	262	40.5	0.006–2.3	–	–	1990
USA	35	97.1	0.18–63.5	–	–	1991
Germany	9	78	–	–	–	1991
UK	691	52.2	0.04–3	–	–	1992
UK	375	4.4	0.07–2.75	–	–	1992
UK (Scotland)	53	–	–	33	0.01–1.05	1993
Canada	22	0	–	32	–	1993
Spain	8	50	<0.01–0.31	63	<0.01–0.21	1993
Canada	249	–	0.005–0.34	100	0.005–0.34	1996
Canada	1760	6.1	–	21	–	1996
Honduras	–	–	0.58–2.6	–	3.8–21	1998
Taiwan	31	72.2	–	77.8	–	1999
Czech Republic	–	–	0–74	–	0–4.85	2000

A particular feature of protozoan parasites is that their transmissive stages cannot be amplified readily *in vitro*, and standard methods reflect this problem. Detecting small but significant numbers of organisms in water concentrates limits the range of tests that can be performed on a defined number of organisms, particularly when attempting to extract sufficient DNA from a sample for molecular typing. Currently, the genus *Cryptosporidium* (and, to a lesser extent, *Giardia*) is being revised, which leads to much confusion. Recent genetic analyses reveal that more than one species of *Cryptosporidium* can infect susceptible immunocompromised (*C. meleagridis*, *C. felis*, and *C. muris*; Morgan *et al*. 2000; Guyot *et al*. 2001; Pedraza-Diaz *et al*. 2001a, 2001b; Cacciò *et al*. 2002; Gatei *et al*. 2002) and immunocompetent (*C. meleagridis* and *C. felis*; Katsumata *et al*. 2000; Pedraza-Diaz *et al*. 2001a, 2001b; Xiao *et*

al. 2001) human hosts, but *C. parvum* and *C. hominis* remain the most common species infecting humans. Additionally, the transmissive stages of differing human-infectious species possess similar morphologies and morphometries and cannot be distinguished using the standardized methods for detecting oocysts in water concentrates.

Table 6.3. Occurrence and density of *Cryptosporidium* oocysts and *Giardia* cysts in treated waters[a] (adapted from Smith and Rose 1998; Gold and Smith 2002)

Country	Number of samples	Occurrence of *Crypto-sporidium* oocysts (% samples positive)	Density of *Crypto-sporidium* oocysts (oocysts/ litre)	Occurrence of *Giardia* cysts (% samples positive)	Density of *Giardia* cysts (cysts/ litre)	Year
USA	36	17	0.005–0.017	0	–	1991
USA	82	26.8	–	16.9	–	1991
UK (Scotland)	15	7	0.006	–	–	1995
UK (Scotland)	106	–	–	19	0.01–1.67	1993
Spain	9	33	<0.01–0.02	22	<0.01–0.03	1993
Brazil	18	22.2	–	–	–	1993
Canada	42	5	–	17	–	1993
Canada	249	–	–	98.5	0.045–1.72	1996
Canada	1760	3.5	–	18.2	–	1996
Germany	12	66.7	0.008–1.09	83.3	0.02–1.03	1996
UK	209	37	0.007–1.36	–	–	1998
Taiwan	31	38.5	–	77	–	1999

[a] Waters for potable supply receive different treatments in different areas of the world; whereas some of the waters in this table received a number of treatments before being considered usable for potable supply, others may have received minimal treatment.

Clinical parasitological studies of protozoan parasites have been scarce and sporadic in many parts of Asia, Africa, and South America, and few generalized conclusions regarding transmission can be drawn from them. In Guinea-Bissau, *Cryptosporidium* was the most important risk factor for developing childhood diarrhoea (Sodemann *et al*. 1999). *Cryptosporidium* was the most common

pathogen (17%) identified in stools analysed from 75 consecutive HIV-seropositive patients with chronic diarrhoea admitted to a Nairobi hospital (Mwachari *et al.* 1998). A survey of intestinal parasites among the HIV-positive asymptomatic injecting drug users in north-east India revealed that *Cryptosporidium* sp. (94.4%) and *Isospora* sp. (10.7%) were most commonly seen (Anand *et al.* 1998). In a hospital-based study in India, 7.2% (151/2095) of stool samples were positive for *Cryptosporidium* (Nath *et al*. 1999). In Malaysia, 2% of the 237 stool specimens from children receiving cancer chemotherapy were found to be positive for *C. parvum* (Menon *et al*. 1999). Of 31 water samples collected from nine potable water treatment plants in Taiwan, 77.8% were positive for *Giardia* cysts and 72.2% for *Cryptosporidium* oocysts (Hsu *et al*. 1999).

A study in Japan showed that *Cryptosporidium* oocysts were detected in 35% (9/26) of filtered water samples (geometric mean concentration 1.2 oocysts/1000 litres) and *Giardia* cysts in 12% (3/26; geometric mean concentration 0.8 cyst/1000 litres). The estimated $\log_{10}$ removal efficiency of *Cryptosporidium* oocysts and *Giardia* cysts by rapid sand filtration was 2.47 and 2.53, respectively (Hashimoto *et al.* 2002). The 1996 cryptosporidiosis outbreak in Ogose, Japan, forced wastewater treatment authorities to rethink the relevance of *Cryptosporidium* contamination levels in wastewater and watersheds and to develop countermeasures in wastewater treatment plants. A nationwide survey of *Cryptosporidium* occurrence in raw and treated wastewater identified relatively low densities (Suwa and Suzuki 2001).

Genetic characterization of 15 *G. duodenalis* isolates (8 from Anhui Province, China, and 7 from Seoul, Korea) revealed the same two major lineages, Assemblages A and B, described by Thompson *et al*. (2000). All Korean isolates fell into Assemblage A, whereas four Chinese isolates were in Assemblage A and four in Assemblage B. Two *G. microti* isolates and two dog-derived *Giardia* isolates fell into Assemblage B, but *G. ardeae* and *G. muris* were unique (Yong *et al*. 2000).

6.2.3 Toxoplasma

A Central American outbreak of toxoplasmosis, associated with the consumption of oocyst-contaminated water, has been documented. In 1979, 32 US Army soldiers demonstrated evidence of symptomatic infection with *Toxoplasma* after their return from manoeuvres in Panama. Epidemiological evidence indicated that the most likely vehicle for transmission was the ingestion of creek water contaminated with oocysts excreted by jungle cats, consumed during manoeuvres in the jungle (Benenson *et al*. 1982). Interestingly, most of the affected individuals claimed to have treated their drinking-water with iodine tablets. Primary chlorination is thought to be

unlikely to either prevent *Toxoplasma* oocysts from sporulating or kill sporulated oocysts. As for most zoonoses in Asia, Africa, and South America, this is likely to be an underestimate, because of the lack of tools for oocyst detection in the environment.

6.2.4 *Blastocystis*

Although *Blastocystis* has often been incriminated as a pathogen, much debate surrounds this classification. Widely distributed, it is found in numerous animal hosts. Prevalence in animal workers is higher (44%) than in a normal population (17%) (Suresh *et al*. 2001), suggesting that close proximity with animals may facilitate transmission (Rajah Salim *et al.* 1999). It has been shown that the *in vitro* induced cysts from cultured vacuolar forms of human isolates of *Blastocystis* (Suresh *et al.* 1993) and cysts from *Blastocystis*-infected patients' stools (Moe *et al.* 1997) can cause experimental infections in laboratory-bred rats and mice, respectively, but the zoonotic potential for *B. hominis* remains undecided. Phylogenetic studies (Yoshikawa *et al*. 2003) indicate that isolates tested from humans and animals appear to be *B. hominis.* Sequence and phylogenetic analysis of partial ssu rDNA of *Blastocystis* from a human, a pig, and a horse, sharing a common subgroup, indicated that the pig and horse isolates were monophyletic and closely related (92–94% identity) to *B. hominis*, suggesting the possibility of *B. hominis* being a zoonosis (Thathaisong *et al*. 2003). Twelve *Blastocystis* isolates from primates, when analysed genetically by polymerase chain reaction (PCR) using diagnostic primers and PCR-restriction fragment length polymorphism (RFLP) of SSUrDNA, showed for the first time genetic similarity between the isolates from primates and the genotypes of *B. hominis*. However, it was unclear whether the isolates examined were zoonotic (Abe *et al.* 2003). Viable cysts have been demonstrated in sewage effluent in Pakistan (Zaman *et al*. 1994) and in Malaysian sewage and rivers (K. Suresh, T.C. Tan, and H.V. Smith, unpublished data).

6.2.5 *Strongyloides*

The free-living (heterogenic) life cycle stages of *Strongyloides stercoralis* can be found in warm, moist soil and in sand filter beds of wastewater treatment works, particularly in warm climates. They have to be differentiated from the plethora of nematodes present in biofilms in these environments. While *Strongyloides* is not normally thought of as a waterborne zoonotic agent, transmission via water may be more frequent than suspected in the warmer climates of Asia, Africa, and South America.

6.3 BACTERIA

Many bacteria can be transmitted to humans through water. However, a number of important waterborne bacteria that cause human disease — e.g., *Vibrio cholerae*, *Salmonella typhi*, *Shigella dysenteriae*, and others — arise primarily from human wastes and thus are not considered to be zoonoses. This section presents information on waterborne zoonotic bacteria only.

6.3.1 *Salmonella* and *Shigella*

Between 1990 and 1991, of 3222 *Salmonella* strains identified at the National *Salmonella* and *Escherichia* Centre in Kasauli, India, 2894 were from humans, 226 from poultry, 84 from animals, and 18 from reptiles, birds, and other sources. Fifty-three serotypes were identified, including four serotypes reported for the first time in India (*S. kedogou*, *S. VP. bornheim*, *S. kisarawe*, and *S. madras*) (Mahajan *et al*. 1998).

An Indonesian surveillance study, conducted over a 2-year period among 6760 patients with debilitating diarrhoeal diseases, revealed that 587 (9%) of stools were positive for the following bacteria: *Shigella flexneri* (39%), *Salmonella* spp. (26%), *Vibrio* spp. (17%), *Shigella sonnei* (7%), *Campylobacter jejuni* (4.4%), *Salmonella typhi* (3%), and *Shigella dysenteriae* (2.3%).

From January 1983 to December 1992 in Malaysia, 20 874 *Salmonella* isolates were typed into 97 serotypes belonging to 22 Kauffmann-White groups (Yasin *et al.* 1996). The submissions represented a 100% increase over the previous 10-year period.

Studies have consistently shown that non-typhoidal *Salmonella* bacteraemia is more common during the rainy season in tropical Africa; sources and modes of transmission remain unknown, but clustering of cases in the rainy season suggests a waterborne/water-associated route. Gracey *et al.* (1979) found a high rate of carriage among healthy adults and children in Jakarta, Indonesia, where 48% of specimens of river water used for drinking contained *Salmonella* (Graham *et al*. 2000).

Of 62 faecal specimens collected from mountain gorillas (*Gorilla gorilla beringei*) in the Bwindi and Mgahinga National Parks, Uganda, in January 1999, 19% had *Campylobacter* spp., 13% *Salmonella* spp., and 6% *Shigella* spp. The prevalence of positive specimens was not related to the year of habituation of a gorilla group to humans. *Campylobacter*, *Salmonella*, and *Shigella* infections were distributed unevenly among gorilla age classes: 80% of enteropathogens and all *Shigella* (*S. sonnei*, *S. boydii*, and *S. flexneri*) were isolated from subadult and adult gorillas (age range 6.0–11.9 years). The prevalence of *Campylobacter* and *Salmonella* infections among human-habituated gorillas doubled during the last 4 years, and isolation of *Shigella* for the first time from

mountain gorillas may indicate increased anthropozoonotic transmission of these enteropathogens (Nizeyi *et al.* 2001).

6.3.2 *Campylobacter*

Campylobacter jejuni is a major cause of paediatric diarrhoea in developing countries, where free-range chickens are presumed to be a common source. Peruvian strains, from monthly surveillance and diarrhoeal cases, were compared by RFLP, rapid amplified polymorphic DNA, and Lior serotyping. RFLP analysis of 156 human and 682 avian strains demonstrated identical strains in chickens and humans in 70.7% (29/41) of families, and 35–39% of human isolates from diarrhoeal and non-diarrhoeal cases were identical to a household chicken isolate (Oberhelman *et al*. 2003).

Of a total of 620 samples collected from healthy animals and animal handlers in Calcutta, India, 128 (20.6%) were positive for *Campylobacter* spp. (*C. jejuni* 14.5%, *C. coli* 4%, and *C. lari* 2%). The isolation rate was highest in chickens and ducks (39.3% positive). The isolation rates in diarrhoeic cattle, sheep, and dogs were 22.2%, 33.3%, and 16.6%, respectively; in healthy animals, the rates were 5.3%, 1.4%, and 8.3%. In healthy pigs, the carriage rate of *Campylobacter* spp. was high (37.1%). Of the 140 human faecal samples processed, 10 (7.1%) were positive for *Campylobacter* spp., of which 9 isolates were *C. jejuni* and 1 was *C. coli* (Chattopadhyay *et al.* 2001). Macrorestriction (pulsed-field gel electrophoresis) profiles of human and porcine isolates suggest that *C. hyointestinalis* transmission from pig to human is possible (Gorkiewicz *et al.* 2002).

Campylobacter and *Salmonella* accounted for 40% and 24%, respectively, of 1707 diarrhoeal specimens collected over a 7-year period (1989–1996) from Oita district, Japan. Initially, *Campylobacter* was most prevalent. It then decreased, while *Salmonella* continued to increase; at the end of the study period, *Salmonella* was more prevalent than *Campylobacter*. Increases in *Salmonella* were due to the appearance of *S.* Enteritidis (Narimatsu *et al.* 1997).

6.3.3 *Escherichia coli*

During October 1992, a large outbreak of bloody diarrhoea affecting thousands of individuals, some of whom died, occurred in South Africa and Swaziland (Isaacson *et al.* 1993). *Escherichia coli* O157 was isolated from 22.5% of 89 stool samples, and epidemiological investigations implicated waterborne spread. In some areas, cases were mainly men who drank surface water from fields, while women and children who drank borehole water were spared. *Escherichia coli* O157 was isolated from 14.3% of 42 samples of cattle dung and 18.4% of

76 randomly collected water samples. The underlying problem seems to have been cattle carcasses and dung washed into rivers and dams by heavy rains after a period of drought.

In a semiurban slum of Varnasi, India, 53.7% of milk samples from supplementary milk feeds of 149 children were bacterially contaminated (*E. coli* [13.4%], *Klebsiella* spp. [5.4%], *Enterobacter* spp. [5.4%], *Pseudomonas aeruginosa* [4.7%], *Shigella* spp. [2.7%], and others [22.1%]), of which 16.1% were potentially enteropathogenic. The rate of contamination was significantly higher ($P < 0.001$) in lower income groups (73.4%), lower castes (69.6%), and illiterate mothers (69.3%) (Ray *et al*. 2000).

6.3.4 *Brucella*

From 1992 to 1997, 3532 patients with pyrexia of unknown origin were tested for brucellosis, and 28 (0.8%) were seropositive. Males outnumbered females by 3:1. Seroprevalence was age-related among males, but not among females. The highest number (43%) of positive males belonged to the age group 21–30 years. The majority of patients had titres of 1:160 or 1:256; high titres of 1:512 and 1:1024 were found in 21.4% of patients (Kadri *et al.* 2000).

6.4 VIRUSES

Recently, many zoonotic viruses have emerged in Australia and south-east Asian countries. Increased tourism, greater influx of migrant workers, deforestation, and current intensive farming and husbandry practices in many parts of these regions prove ideal for zoonotic transmission. Below are discussed some emerging zoonotic viral diseases that may have the potential for waterborne transmission.

6.4.1 Menangle virus and Tioman virus

The new Menangle virus (Family Paramyxoviridae) causes stillbirth, mummification, occasional abortions, and deformities in pigs. The virus was isolated from lung, brain, and heart tissues of infected piglets, and serum from two workers contained high-titre, convalescent-phase neutralizing antibodies to the virus (Philbey *et al.* 1998). Tioman virus was isolated from urine of flying fox (*Pteropus* sp.) found on Tioman Island off the eastern coast of peninsular Malaysia (Chua *et al.* 2001).

6.4.2 Hendra virus

Recognized in 1994 after an explosive outbreak of severe, fatal respiratory disease, which killed 13 of 20 infected racehorses (Murray *et al*. 1995), Hendra

virus has also been responsible for a human fatality (Rogers *et al*. 1996). Black fruit bats (*Pteropus alecto*), infected subcutaneously, intranasally, or orally, contract subclinical infections. Hendra virus is widely distributed in Australian pteropid bats, with serological evidence of infection in an average of 42% of wild-caught bats, the number of seropositives varying with species (53% of 229 *P. alecto*, 47% of 195 *P. poliocephalus*, 12% of 115 *P. scapulatus*, and 41% of 99 *P. conspicillatus*) and age, but not geographic distribution (H. Field, unpublished data). Based on morphology and preliminary sequencing data of the M and F genes, Hendra virus is a member of the Paramyxoviridae (Hyatt and Selleck 1996).

6.4.3 Australian bat lyssavirus

Closely related to rabies virus, this virus was first identified in Australia in a fixed brain specimen from a young black flying fox (*Pteropus alecto*) with unusual neurological symptoms. To date, two human infections have been attributed to Australian bat lyssavirus (Hooper *et al*. 1996).

6.4.4 Highly pathogenic avian influenza

Highly pathogenic avian influenza (fowl plague) is highly infectious and contagious. Waterfowl are reservoirs and are the main source of introduction of the disease into domestic poultry flocks. The recent emergence of a new strain (H5N1) of influenza A virus, the avian flu, in Hong Kong accentuates the importance of south-east Asia as a melting pot for emerging microbial agents. H9N2 viruses were isolated from 4.7% of chickens and a smaller percentage of ducks, geese, domestic and wild pigeons, and quail and from environmental swabs in a market (Shortridge 1999).

6.4.5 Nipah virus

An outbreak of Nipah virus in Malaysia and Singapore in October 1998 highlights the epidemiological significance of changing environments and movements of animals from their natural habitats. Nipah virus takes its name from a village in peninsular Malaysia where the virus was first isolated from a human victim (Hendra virus is a close relative; Chua *et al*. 2000). The virus persists in low numbers in the island flying fox (*Pteropus hypomelanus*) (Chua *et al*. 2002), a fruit bat, and the Malayan flying fox (*P. vampyrus*). The virus replicates explosively in pigs, causing respiratory and/or neurological syndromes followed by death (Mohd. Nor *et al*. 2000), and causes severe encephalitis in humans (Mohd. Taha 1999). Of the 269 human cases of viral

encephalitis associated with Nipah virus infection reported in Malaysia in 1999, 108 were fatal (Malaysian Ministry of Health 2001). Several possible routes of transmission between farms in farming communities were suggested, including sharing boar semen and transmission by dogs and cats. It is suspected that dogs and cats were infected with urine and excreta from lorries carrying affected pigs and subsequently introduced the virus to uninfected farms.

Of great concern is the changing geographic distribution of fruit bats, which spread Nipah virus and other, as yet unknown, diseases to susceptible communities. In common with most countries in south-east Asia, peninsular Malaysia has a great diversity of bat species. At least 13 species of fruit bat (Suborder Megachiroptera), including two flying fox species and at least 60 species of insectivorous bats (Suborder Microchiroptera), have been described (Aziz *et al*. 2002).

6.4.6 Haemorrhagic fevers and hantavirus

Hantaviruses (Bunyaviridae) constitute a genus of antigenically, genetically, and epidemiologically related viruses. Laboratory outbreaks due to a causative virus called Seoul virus have been reported in Japan, the Republic of Korea, the People's Republic of China, Russia, France, Belgium, the Netherlands, and the United Kingdom (Lee 1996). Infection with Puumala or PUU virus was also found in the grey-sided vole (*Clethrionomys fufocanus*) in Hokkaido, Japan, but was not associated with human disease (Kariwa *et al.* 1995). Human infection from infected rodents is thought to occur through inhalation of excreta. In 1996, person-to-person transmission was documented in an outbreak of hantavirus pulmonary syndrome (HPS) in southern Argentina caused by the Andes virus. Following the 1993 US outbreak, many South American countries reported either isolated cases or outbreaks of HPS. In 1993, Brazil identified a cluster of cases in a rural area near São Paulo caused by a new virus (Juquitiba virus; Vasconcelos *et al*. 1997). In 1995, Paraguay reported an outbreak, and a new hantavirus (Laguna Negra virus) was isolated from the putative rodent reservoir, *Calomys laucha* (Johnson *et al*. 1997). In South America, HPS affects all ages and both sexes but has a higher prevalence among adult males from rural settings. Reported human seroprevalences vary greatly from region to region, from <2% to 40% among central Paraguayans. Chile and Argentina appear to have more paediatric cases (Pini *et al*. 1998).

6.4.7 Severe acute respiratory syndrome (SARS)

This coronavirus was reported as a new emerging disease in China in November 2002, subsequently spreading around the world to Canada within 5 months. The World Health Organization has recorded more than 83 380 cases and 770 deaths from SARS. Transmission is primarily through large droplets and aerosols, but

contact with contaminated surfaces has also been suggested. The virus can also be excreted in stools. This raises the question as to whether the SARS virus can survive wastewater and water treatment and whether it can be spread by the waterborne route.

6.5 CONCLUSIONS

It is evident from this brief review that, while awareness of zoonotic waterborne diseases exists in Asia, Africa, and South America, different countries have different human health priorities. Bacterial diseases seem to take precedence over viral and protozoal diseases. It is important to remember that these data are based on peer-reviewed scientific publications, which underestimate the amount of work done in the field; much is being done, but its publication is often not a priority.

Water use is expected to increase by 40% over the next two decades, and it is estimated that 3 billion people will face water shortages by then. We may know little about whether the organisms listed are transmitted by the waterborne route or their occurrence in water in Asia, Africa, and South America, but their impact on health, sanitation, and, particularly, drinking-water is potentially great. Of importance is that it is these countries that are likely to suffer greatly from potable water shortages; therefore, we must consider (and control) routes that might contaminate drinking-water with these organisms.

There have been some major diagnostic and epidemiological inroads into investigating zoonotic waterborne bacterial infections, depending on their regional importance; better detection and surveillance systems have reduced the spread of zoonotic waterborne bacterial infections. Our literature searches suggested that while there are more publications on clinical and epidemiological investigations for viral and bacterial diseases in Africa, Asia, and South America, there appear to be as many publications on environmental occurrence of the zoonotic parasitic protozoa as there are on disease occurrence.

Often environmental detection and epidemiological investigations are beyond the scope of government health departments. It is our experience that environment and health ministries do not always work together, and recognition of the potential for waterborne disease is not always conducive to increased income generation from newer industries, such as tourism. For other, less well appreciated, zoonotic waterborne diseases, only sparse, basic, clinical occurrence data are available. Hard-pressed diagnostic and epidemiological centres do not require further remits. One issue that has not been addressed in this review, but should be seriously considered, is the role of water in food production.

There is a definitive need for standardizing both diagnostic and epidemiological tools. Molecular tools are recent additions to our armoury, and, with the exception of research projects, it is impracticable to suggest that all can be adopted into health care systems in developing countries to serve infectious disease diagnosis and micro- and macro-epidemiology. The fact that clinical and epidemiological investigations are ongoing in such countries must be seen as a bonus.

The potential for reducing the economic and human cost of disease depends not only upon the identification and successful treatment of susceptible hosts, but also upon the implementation of control measures that break the transmission cycle. As for the prokaryotic zoonoses, typing and subtyping systems are available for *Cryptosporidium* and *Giardia* (Strong *et al*. 2000; Thompson *et al*. 2000; Homan and Mank 2001; Mallon *et al*. 2003). Particularly for disinfection-insensitive organisms such as *Cryptosporidium* and *Giardia*, there is a distinct need to adopt molecular tools for environmental detection and disease tracking, as microscopy is unable to determine whether those organisms detected in the environment are viable or infectious to humans. Utilizing standard methods may still lead to an underestimation of *Cryptosporidium* and *Giardia* contamination, as will confusion from detecting (oo)cysts that are not infectious to humans and that have no significance to public health. Technologies including PCR, sequencing, and microarrays have application in the environment, especially where waterborne transmission is known to occur. Development of standardized molecular biological tools for diagnostic and epidemiological purposes must be encouraged.

More effective diagnoses carried out in clinics and laboratories in the developing world will enhance the targeting of treatment and lead to a reduction in morbidity. Increased drug resistance is a continuing theme, particularly in countries where treatment programmes are deemed too expensive. People often stop taking the treatment once the symptoms disappear, increasing the risk of developing drug-resistant forms of disease. Also, self-medication can be commonplace. Even for the drug-sensitive intestinal parasitic protozoa, metronidazole resistance is on the increase.

Providing that accurate statistics are collected, the view of the importance of zoonotic waterborne diseases held by governments and international agencies will be more realistic and lead to better targeting of aid and research funds. It is evident that the prevention and control of zoonotic waterborne diseases are now more feasible than ever before.

Probably the most important intervention is to motivate, educate, and train the populations affected by these diseases to enable them to take the necessary interventions themselves to prevent the transmission of these pathogens. In addition to public information programmes and school-based health education, training courses on important zoonotic waterborne diseases should be

encouraged for primary health and day care workers, workers in diagnostic laboratories, and water treatment plant operators (Warhurst and Smith 1992; WHO 1992; Smith and Ahmad 1996).

Experience in east Africa and south-east Asia suggests that empowerment of local communities works well, following agreement with local, district, and national governments. Based on experience, we suggest the following to motivate and empower such communities:

(1) Form smart partnerships with local nongovernmental organizations to deliver similar projects in disparate areas.
(2) Develop multidisciplinary projects to address complete agendas (empowerment, health, environment, and education) in specified areas.
(3) Set up similar projects in different countries with the same/similar module.
(4) Instigate quality diagnostic testing systems for all parameters (clinical and environmental samples).
(5) Liaise with policymakers and water providers to ensure that recommendations are translated into meaningful projects.
(6) Identify high levels of commitment among all partners.

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7

Incidence of the major zoonotic diseases transmitted by water in Mexico, Central America, and the Caribbean

R. Ramírez-Porras and J. Williams

7.1 INTRODUCTION

Adequate water resources, both surface water and groundwater, are increasingly difficult to find due to increasing human population, per capita consumption, and impacts of human activities on the environment. The quantity and quality of water resources throughout the world have been affected. Improved and expanded use of wastewater collection systems has

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decreased public health risks in urban areas; however, not all wastewaters receive adequate treatment before being discharged. The variety and densities of human pathogens present in the wastewater in a region are related to the population from which they originate, the wastewater collection and treatment system, the current diseases in the human population, and the management of wastewater by agriculture, animal production, and industry (Aguiar Prieto *et al*. 1998; Sánchez-Pérez *et al.* 2000). The need to provide universal clean water and basic environmental sanitation is the environmental health priority for Latin American countries due to the high rates of diarrhoeal and other waterborne diseases. Diarrhoea accounts for a significant burden of disease in Latin American countries and is responsible for many deaths annually. Children under 5 years of age account for 85% of all deaths attributed to diarrhoea. The majority of this disease burden falls upon poor periurban and rural households (HEMA 2002).

Almost half of the population in developing countries is at high risk of exposure to waterborne diseases, including gastroenteric diseases such as dysentery, giardiasis, hepatitis A, rotavirus, typhoid fever, and cholera. Diarrhoeal diseases are a significant cause of mortality and morbidity in developing countries (OPS 2000).

In recent years, there has been increasing concern about zoonotic diseases that can be transmitted by water. In some European countries and the USA, there have been increasing numbers of cases of diseases related to emerging pathogens, such as the protozoan *Cryptosporidium*, the bacteria *Escherichia coli* O157:H7, *Campylobacter*, and *Salmonella* Enteritidis, and hepatitis E virus (Benenson 1997; Binder *et al*. 1999; Hubálek 2003).

This chapter describes the current incidence of the most important zoonotic diseases transmitted by water in Mexico, Central America, and the Caribbean, with emphasis on emerging pathogens.

There are many differences between countries with respect to the health status of their populations. The quality of the health services, the condition and efficiency of the surveillance systems, and the national economic conditions are some of the factors that will determine the incidence of gastrointestinal diseases in the human population.

In general, each country has some form of epidemiological surveillance system that receives information from all of the different health sectors; however, many factors determine the efficacy of these surveillance systems (OPS 2000, 2002).

7.2 COUNTRY-SPECIFIC INFORMATION

7.2.1 Mexico

Children under 1 year of age continue to be those most affected by intestinal diseases, with cumulative incidence of intestinal diseases higher than 28 000 per 100 000. Mortality from these diseases in children under 5 years of age was 25 per 100 000 in 1999. The average annual incidence of bacterial infections was 34 per 100 000 in 1997–2000. Approximately 200 reported deaths per year were due to food poisoning between 1997 and 1999 (OPS 2002). In 2002, 19 305 cases of bacterial food poisoning, 6 323 520 cases of digestive infectious diseases, and 4 878 503 cases of intestinal infectious diseases caused by viruses and other organisms, including those not defined, were reported (SUVE 2002). Gutiérrez-Cogco *et al.* (2000) reported that 199 different serotypes were identified in 24 394 *Salmonella* specimens collected from 1972 to 1999 in public and private health laboratories and analysed with the Kauffmann-White method. The most frequent serotype was *S.* Typhimurium (20.4%), followed by *S.* Enteritidis (18.3%). In the past few years, the frequency of *S.* Enteritidis has been increasing, surpassing that of *S.* Typhimurium since 1991, so that *S.* Enteritidis is currently the most frequently isolated serotype. In non-human sources, *S.* Derby (13.8%) and *S.* Anatum (8.5%) are the most frequently isolated strains.

Health authorities lack accurate information on outbreaks of cryptosporidiosis, and few studies have been conducted. In studies with children and cancer patients, a prevalence of 29.6% was reported, compared with 11.4% in apparently healthy individuals (Garza Almanza and Morales Vallarta 2002). In 2002, 28 cases of leptospirosis were confirmed (OPS 2002).

Drinking-water infrastructure covered 88% of the population in 2000; 23 states had coverage higher than 85%, while 5 states had coverage lower than 70%. In 2000, 95% of the drinking-water was disinfected. Sewage disposal services covered 76% of the population in 2000; 5 states had coverage higher than 85%, 17 states had between 70% and 85% coverage, and the remaining 10 states had coverage lower than 70%. In 2000, 76% of the population had access to sewerage services and excreta disposal: 90% in urban areas and 37% in rural areas. There is an official standard for handling hospital waste, and the majority of waste is incinerated (OPS 2002).

7.2.2 Costa Rica

The rates for acute diarrhoeal diseases rose from 2903 per 100 000 population in 1996 to 3633 per 100 000 in 1999. In 1999, the mortality rate from acute diarrhoeal diseases was 2.8 per 100 000 population (OPS 2002). In 2002, 293 cases of leptospirosis were reported (SNVSCR 2003). Water supply service coverage was

97.5% in 1999. Coverage with sanitary sewerage and *in situ* excreta disposal reached 96.1% of the population. However, sewage disposal via sanitary sewerage lines was 26%, and only 4% of the sewage received sanitary treatment (OPS 2002).

7.2.3 Cuba

Cuba has reported a low incidence of gastrointestinal diseases, and a surveillance system is operating continuously to detect cases that may occur.

Cryptosporidium spp. are internationally distributed intestinal parasites that have been recently recognized as an important cause of diarrhoea, malabsorption, and weight loss and as a possible life-threatening factor for inmunologically compromised patients, such as those suffering from acquired immunodeficiency syndrome (AIDS). In a study in which 24 patients were examined during 1995 and 1996, all the parasite-affected individuals belonged to the 4th human immunodeficiency virus (HIV) infection group. Diarrhoea and weight loss were the most frequent clinical symptoms associated with such parasitism in the 24 patients (Cassola *et al.* 1999). In another study, Martínez *et al.* (2002) reported that the protozoan *Cyclospora cayetanensis* was isolated in samples of fresh faeces in 20 patients (0.2%) selected from 7956 patients with watery diarrhoea and other clinical manifestations at the Parasitology Department of "William Soler" University Pediatric Hospital from January 2000 to July 2001.

7.2.4 Dominican Republic

The estimated mortality rate in children (0–4 years) was 40 per 1000 live births in 1995–2000. Under-registration of deaths in infants was estimated to be 60% in 1998. In that year, conditions arising in the perinatal period accounted for 64.5% of infant deaths, 13% of communicable diseases, and 9.4% of acute diarrhoeal diseases. In 1998, communicable diseases constituted the leading cause of death (40%) in the group aged 1–4 years, followed by external causes (24.6%). In 1999, the leading causes of morbidity in infants were acute respiratory infections (668.8 per 1000), acute diarrhoeal diseases (329.3 per 1000 live births), and parasitoses (138.5 per 1000 live births). The leading causes of morbidity in children 1–4 years of age were acute respiratory infections (221.2 per 1000 population) and acute diarrhoeal diseases (69.4 per 1000 population).

A 1999 survey showed that 65.5% of schoolchildren were infected with *Blastomyces hominis* (27%), *Entamoeba coli* (26.7%), and *Giardia lamblia* (17.7%), among others. Cases and small outbreaks of leptospirosis were confirmed, and toxoplasmosis infections were reported in pregnant women in some areas of the country (OPS 2000, 2002).

The General Bureau of Epidemiology has the normative responsibility for a decentralized surveillance system (a component for early warning and one for prevention and control). At the central level, it includes units of surveillance, health situation analysis, and computer support. Potable water and sanitation are the responsibility of the National Potable Water and Sewerage Institute. Services in the communities are the responsibility of more than 20 associations of rural water supply systems. In 2000, 71.4% of the population had drinking-water services. The coverage of excreta disposal systems was 89.5%, while coverage of sewerage services was only 20.1% (OPS 2002).

7.2.5 Nicaragua

Acute diarrhoeal diseases are among the main types of notifiable diseases. Children under 5 years of age are hit hardest by these diseases, accounting for 73% of the total reported. The morbidity rate was 484 cases per 100 000 population in 1997 and 415 cases per 100 000 in 1998, with mortality rates around 7 per 100 000 in the same years (OPS 2002).

Potable water supply availability was 89.4% in 1998; the urban coverage was 89.5%, and the rural coverage 33.7%. Of the water samples collected in 1999, 4% contained over 50 faecal coliform bacteria per 100 ml. The percentage of the population without access to adequate excreta disposal service dropped to 21.1% in 1998. Only 4.7% of the urban population was still without service, compared with 31.7% of the rural population. Only 34% of collected wastewater received any type of treatment.

7.2.6 El Salvador

In weeks 50–52 of 2000, an increase of diarrhoea in children was caused by rotavirus type 1. In 1999, there were 40 cases of leptospirosis reported, 0.65 per 100 000 population (OPS 2002).

7.2.7 Panama

There is no information available on waterborne zoonoses in Panama.

7.2.8 Honduras

According to the National Survey of Epidemiology and Family Health of 1996, the infant mortality rate was estimated at 36 per 1000 live births (53% neonatal) between 1991 and 1995. Acute respiratory infections and acute diarrhoea with dehydration were the leading causes of death in children under 5. The annual average number of cases of diarrhoea for this period was around 200 000, 85% in

children under 15. Between 1998 and 1999, a laboratory diagnostic capability was developed for leptospirosis, and in 1998, the first diagnosis was made 4 days after the occurrence of Hurricane Mitch. In 1999, 39 cases of leptospirosis were diagnosed (OPS 2002). Water and sewerage services and sanitation in general have shown limited progress in the last 5 years. Investment over the last 2 years in this sector has focused on repairing infrastructure damaged by Hurricane Mitch. In 1999, access to potable water at the national level was 80.9%; 71.1% of the population was served by some form of excreta disposal (OPS 2002).

7.2.9 Guatemala

In 1999, there were 385 633 reported cases of acute diarrhoeal disease (incidence: 3470 per 100 000 population) and 3244 deaths from this cause (29.2 per 100 000). In 2000, morbidity was up 21.6% from that in 1999, with 468 981 reported cases (4220 per 100 000). In 1999, children under 5 years of age were most affected, with 238 434 cases, or 61.8% of the total. In 2000, five cases of leptospirosis were documented (OPS 2002). The water supply coverage reached 92% of the population in urban areas and 54% in rural areas, while sanitation coverage was 72% and 52%, respectively. In urban areas, 47% of the population disposes of solid waste through collection services (OPS 2002).

7.3 SUMMARY AND CONCLUSIONS

In general, there are many differences between countries in regard to the quality, extent, completeness, and reliability of the available information. There have been very few reports of cases of some emerging zoonotic pathogens, such as *Cryptosporidium* and *Campylobacter*. This could be related to deficiencies in the diagnostic ability in most of the countries, making it very difficult to establish the presence of these pathogens. Most of the countries are able to deal with the principal agents, such as *Salmonella*; however, in the case of protozoa, viruses, and fastidious bacteria, such as *Campylobacter*, improved technical training, infrastructure, and economic resources will be required to establish a well organized surveillance system that includes human and animal information.

It is important to develop new research directions that would permit improved capabilities to detect and fight against emerging and re-emerging zoonotic diseases in Mexico and Central America. These would include the following:

- Use all the scientific and technical knowledge related to the detection, identification, treatment, and control of these diseases.
- Extend research to the study of ecological and environmental factors that influence their transmission.

- Create an international network that includes human and animal resources, in order to share information and experiences between all the member countries and to use this information to make decisions about the control of the zoonotic diseases transmitted by water.

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8

Waterborne outbreaks caused by zoonotic pathogens in the USA

G.F. Craun, R.L. Calderon, and M.F. Craun

8.1 INTRODUCTION

In 1971, the Centers for Disease Control and Prevention, the US Environmental Protection Agency (EPA), and the Council of State and Territorial Epidemiologists began a collaborative surveillance programme to collect and report data on the occurrence and causes of outbreaks associated with contaminated drinking-water and recreational water. To be defined as a waterborne outbreak, at least two persons must experience a similar illness after the ingestion of or contact with water, and epidemiological evidence must implicate water as the probable source of the illness. There are two exceptions: single-case outbreaks of chemical poisoning or dermatitis when water quality information indicates contamination by the chemical, and single-case reports of laboratory-confirmed primary amoebic meningoencephalitis. The surveillance

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system records information about the epidemiology of the outbreak, etiologic agents, types of water system, system deficiencies, water sources, and water quality. Because the reporting of waterborne outbreaks is voluntary, the statistics do not reflect the true incidence of outbreaks or illnesses associated with the reported outbreaks. However, the information is considered useful for evaluating the relative degrees of risk associated with different types of source water and water systems and assessing the adequacy of current source water protection strategies, water treatment technologies, and drinking-water regulations (Levy *et al*. 1998; Barwick *et al*. 2000; Craun *et al*. 2002; Lee *et al*. 2002).

In this chapter, we review information available for waterborne outbreaks in the USA that were caused by zoonotic agents.

8.2 PRINCIPAL WATERBORNE ZOONOTIC PATHOGENS

8.2.1 Protozoa

Cryptosporidium parvum is likely infectious for all species of mammals; young animals are most prone to infection and illness (Sterling and Marshall 1999). Many mammalian hosts can also carry *Giardia intestinalis* (Schaefer 1999). Because humans are significant carriers of infection for both of these protozoa, the extent to which infections in animals contribute to human infection and illness is uncertain. Recent findings emphasize the need for more information about the species and genotype of *Cryptosporidium*; various isolates may be virulent to varying degrees in humans (Okhuysen *et al*. 1999). *Cryptosporidium* and *Giardia* have been found in drinking-water and recreational water, and a significant number of outbreaks have been reported in the USA when human sewage and wild or domestic animals have contaminated surface water and groundwater sources and water distribution systems. In recreational waters, faecal contamination from bathers has been an important source of exposure.

Blastocystis hominis has been found in monkeys, apes, pigs, dogs, cattle, sheep, and ducks (Garcia 1999a). It has also been identified in stool specimens from ill persons in several waterborne outbreaks in the USA, but whether *B. hominis* was the cause of the reported illness is unclear, because its pathogenicity is debated. In 2000, an outbreak of undetermined etiology affected two persons using an untreated well water system in Florida; stool specimens from one person tested negative for *Giardia* and positive for *B. hominis* (Lee *et al*. 2002).

Balantidium coli is widely distributed in pigs in warm and temperate climates and in monkeys in the tropics (Garcia 1999b). Human infection is generally found in warmer climates but can occur sporadically in cooler areas. Waterborne transmission has been reported in areas of the Caribbean where people live in

close proximity to pigs and sanitation conditions are poor. In one outbreak, a hurricane caused widespread contamination of individual water systems. *Toxoplasma gondii* infects virtually all warm-blooded hosts, but cats and other felines are the only definitive hosts (Dubey 1999). Intermediate hosts include rodents, sheep, pigs, cattle, and birds. A waterborne outbreak was reported in US soldiers who drank from a jungle pond while on manoeuvres in Panama. In 1995, a waterborne outbreak in British Columbia, Canada, was traced to feline contamination of a surface water reservoir source for a community system.

Humans may be the only natural host of *Cyclospora cayetanensis*, but similar organisms have been observed in chimpanzees and baboons (Ortega 1999). In 1990, a waterborne outbreak affected the hospital staff at a residence dormitory in Chicago. Contamination of the building's plumbing system was traced to a storage tank at the dormitory, but the source of contamination was not identified.

Outbreaks of a clinical syndrome consistent with schistosome cercarial dermatitis, commonly called swimmer's itch, have been reported in several states. The parasites belong to the family Schistosomatidae, and the disease is associated with non-human schistosomes that infect birds as the final or definitive host. In the Midwest, as many as 20 species of non-human schistosomes can cause swimmer's itch (Blankespoor 1999). Final or definite hosts include ducks, geese, gulls, starlings, and rodents. Most outbreaks reported in the USA have affected bathers in lakes and ponds. One outbreak was associated with swimming in ocean water where local snails were found to contain cercariae of *Austrobilharzia variglandis*, an avian schistosome.

Although roundworms, whipworms, and tapeworms may be transmitted by contaminated, untreated drinking-water, this is not their usual mode of transmission (Fredericksen 1999; Little 1999a, 1999b; Smith *et al*. 1999a, 1999b). *Ascaris lumbricoides* is a large roundworm found in humans. *Trichuris trichiura* is a nematode that infects the large intestine; it is commonly known as whipworm. Humans are the reservoir for both of these helminths, but pigs, dogs, cats, and chickens that feed on human faeces can also act as transport hosts, redistributing ova to other sites. *Ascaris* and *Trichuris* ova have been found in surface water and groundwater and may be a source of waterborne exposure for persons who consume untreated water in areas where sanitation is especially poor. Humans and pigs are believed to be the only reservoirs for the tapeworm *Taenia solium*. Water contaminated with faeces from either source poses a threat of infection. *Spirometra mansonoides* is a tapeworm that lives in the intestines of dogs and wild and domestic cats in the USA. Persons may become infected by drinking water contaminated by copepods or eating raw or inadequately cooked flesh of an animal containing the sparganum stage of the worm. The worm does not develop to the adult stage in humans, but a larval stage can invade cutaneous tissues and the brain. Most reported cases have occurred in the south-eastern states. No waterborne outbreaks of *A. lumbricoides*, *T. solium*, *S.*

mansonoides, or *T. trichiura* have been reported in the USA. *Gnathostoma spinigerum*, a roundworm commonly found in wild animals and humans in the Far East, is not known to occur in the USA. Persons may be infected by drinking water contaminated by copepods or eating raw or inadequately cooked fish.

Microsporidia produce a spore stage that survives in the environment. These protozoa are relatively ubiquitous in the environment and have been found in bird and mammal hosts, including dogs and cats (Cali 1999). Several species can infect humans. The disease and symptoms vary considerably. Although microsporidia may be transmitted via water, no waterborne outbreaks have been documented in the USA.

8.2.2 Bacteria

A significant number of waterborne outbreaks in the USA have been caused by *Escherichia coli* O157:H7. This pathogen is widely distributed in cows and other ruminants, where it may not cause animal disease, but can readily be spread to humans through contaminated food and water (Pruimboom-Brees *et al*. 2000). Drinking-water outbreaks in the USA have primarily been associated with inadequately disinfected or untreated groundwater and distribution system contamination. *Escherichia coli* O6:H16 and *E. coli* O121:H19 have also been implicated in waterborne outbreaks.

A wide range of domestic and wild animals, including poultry, pigs, cattle, sheep, dogs, cats, and rodents, can serve as reservoirs for *Campylobacter jejuni*, an important cause of diarrhoea throughout the world (Fricker 1999a). Although *Campylobacter* are susceptible to water disinfection, outbreaks can occur when water sources are inadequately disinfected. For example, in a 1978 outbreak in Vermont, 3000 persons became ill when an unfiltered surface water source was inadequately chlorinated; in a 1983 outbreak in Florida, 871 persons became ill when the disinfection of a groundwater source was interrupted. Outbreaks in the USA have also been attributed to the contamination of untreated groundwater sources, distribution system mains, and storage reservoirs.

Humans and a wide range of domestic and wild animals, including poultry, cattle, birds, dogs, cats, rodents, and turtles, can serve as reservoirs for *Salmonella* (Covert 1999). In the USA, waterborne outbreaks have been caused by various subspecies of *Salmonella*, including Typhimurium, Enteritidis, Bareilly, Javiana, Newport, and Weltervreden. *Salmonella* outbreaks have also been traced to contaminated wells and water storage reservoirs. In 1993, an outbreak of *S.* Typhimurium resulted in 650 illnesses and 7 deaths in Missouri; contamination was traced to a water storage tank that allowed access by birds.

Animals are the principal reservoir for *Yersinia*, and humans and pigs are important reservoirs for *Y. enterocolitica* (Fricker 1999b). Only two waterborne outbreaks of *Y. enterocolitica* have been reported in the USA. Both outbreaks

were traced to contaminated, untreated groundwater. Contaminated farm wells are suspected as the cause of sporadic, individual cases that are not reported as outbreaks.

Reservoirs for *Leptospira interrogans* include many wild and domestic animals, including rats, dogs, racoons, swine, and cattle (Benenson 1995). Contact of mucous membranes and the skin, especially if abraded, with contaminated water is one mode of transmission. Waterborne outbreaks have been associated with recreational activities in the USA. In 1998, a large outbreak of leptospirosis occurred among participants in a triathlon held in Illinois; 375 persons became ill, and 28 persons were hospitalized (Barwick *et al*. 2000). The illness was epidemiologically associated with swimming in a lake that received runoff from residential areas, agricultural land with cattle and pigs, and a wildlife refuge. Animal testing, however, did not reveal a specific animal source.

Numerous wild animals, especially rabbits, muskrats, beavers, and some domestic animals, can serve as reservoirs for *Francisella tularensis* (Benenson 1995). Tularaemia is not usually spread by the waterborne route, but two small waterborne outbreaks were reported in the USA during the period 1946–1960.

8.2.3 Viruses

Some human enteric viruses can infect other animals, and animal reservoirs may be important. The three serotypes of reoviruses found in humans and other mammals are indistinguishable, and human reoviruses are pathogenic in newborn mice (Sattar and Springthorpe 1999). Groups A, B, and C rotaviruses are found in humans and animals, and the interspecies transmission of rotavirus, including human infection by a bovine strain, has been reported (Abbaszadegan 1999). One waterborne outbreak of rotavirus was reported in the USA; sewage from a septic tank contaminated a well. Hepatitis E viruses (HEV) of pigs and rats are very similar to human HEV, and HEV may be zoonotic (US EPA 1999). Human strains of HEV have experimentally infected pigs, and porcine strains have experimentally infected primates (Benenson 1995; Craun *et al*. 2003). In developing countries, the seroprevalence of HEV infection can be as high as 60%. Pregnant women are at high risk of severe illness and death; the mortality can be as high as 20%. In developed countries, the seroprevalence is less than 5%, and very few cases of disease are reported. In a study of sporadic HEV cases reported in the USA, almost half of the ill persons had travelled to endemic areas or received blood transfusions (US EPA 1999).

Noroviruses and hepatitis A virus have caused a number of waterborne outbreaks in the USA. These viruses are not considered zoonotic. Although human sources of faecal contamination are the cause of most, if not all, waterborne viral outbreaks in the USA, it is important to recognize that viruses

are diverse and complex and have the ability to infect different hosts by genetic changes and expression of different phenotypic properties (Craun *et al*. 2003).

8.2.4 Acute gastroenteritis of unknown origin

For reporting purposes, outbreaks of unknown or undetermined etiology that have a symptom profile consistent with gastrointestinal disease are categorized as acute gastrointestinal illness of unknown origin (AGI) outbreaks. These AGI outbreaks may be caused by commonly identified and well known etiologic agents or less frequently identified and unrecognized waterborne agents, including zoonotic agents. In many AGI outbreaks, an agent could not be identified because the search was limited to those organisms easily cultured or clinical specimens were not collected in a timely manner. In some outbreaks, the etiologic agent was not isolated because the appropriate laboratory analysis was not available. For example, investigators may have suspected a viral etiology but did not collect clinical specimens because laboratory facilities were not available to conduct viral analyses. In several recent outbreaks, however, an etiology could not be established even though there was extensive laboratory analysis of both human specimens and water samples, including appropriate tests for newly recognized bacterial, viral, and parasitic pathogens. This serves as a reminder that although several newly recognized waterborne agents have been uncovered in recent years, additional agents are yet to be determined.

8.3 WATERBORNE OUTBREAKS REPORTED IN THE USA

During 1971–2000, 648 outbreaks were reported in public water systems, and 103 outbreaks were reported in individual water systems (Table 8.1). Almost 600 000 persons were reported ill in the 1010 reported outbreaks. Public water systems are classified as community or non-community systems. A community system serves year-round residents (15 or more service connections or an average of 25 or more residents). A non-community system is used by the general public for 60 or more days per year and has at least 15 service connections or serves an average of 25 or more persons (e.g., factories, schools, restaurants, parks with their own water systems). Of the 751 reported outbreaks in drinking-water systems, 665 (89%) outbreaks were of a known or suspected infectious etiology; 86 (11%) outbreaks resulted in an acute illness, primarily nausea, vomiting, and abdominal pain, after ingestion of a chemical agent in water.

Table 8.1. Waterborne outbreaks and illness by type of system, all causes, 1971–2000

Water system type	Outbreaks	Cases of illness	Emergency visits and hospitalizations	Deaths
Non-community	340	54 893	984	4
Community	308	517 944	5928	65
Untreated recreational water	143	13 898	192	28
Treated recreational water	116	7 842	50	0
Individual	103	1 600	98	3
All water systems	1010	596 177	7252	100

During 1971–2000, an additional 259 outbreaks were associated with recreational activities in various water venues. One hundred and forty-four outbreaks (56%) were associated with recreation in untreated water (e.g., lakes, streams, springs), and 112 (43%) occurred in locations where water was treated (e.g., swimming and wading pools, interactive fountains). Three (1%) outbreaks were associated with both treated and untreated recreational water.

An etiologic agent was identified in 60% of the reported outbreaks (Table 8.2). Almost half (49%) of the bacterial outbreaks were associated with contaminated recreational water. Most (80%) protozoan outbreaks were reported in recreational water and community water systems. Protozoa were more frequently identified than bacteria in outbreaks reported in community systems, but bacteria were identified more frequently than protozoa in non-community system outbreaks.

Table 8.2. Number of waterborne outbreaks by type of water system and etiology, 1971–2000

Water system type	Unidentified agents	Protozoa	Viruses	Bacteria	Chemicals
Non-community	228	31	27	43	11
Community	98	96	20	40	54
Treated and untreated recreational water[a]	40	98	18	97	5
Individual	39	16	9	18	21
All water systems	405	241	74	198	91

[a] An outbreak attributed to algal toxins is not included. An outbreak of both *Shigella* and *Cryptosporidium* is included in the protozoa category.

8.3.1 Waterborne outbreaks caused by zoonotic agents

A significant number of outbreaks and illnesses were caused by zoonotic agents (Tables 8.3 and 8.4). Zoonotic agents can be found in human sewage as well as domestic and wild animal faeces. Because the source of the faecal contamination was either not investigated or not identified in many of the zoonotic outbreaks, it was not possible to evaluate the importance of animal versus human sources for the agents. Reported statistics should be evaluated with this limitation in mind.

Table 8.3. Waterborne outbreaks caused by zoonotic agents by type of system, 1971–2000

Water system type	Outbreaks of zoonotic agents	% of all reported outbreaks[a]	% of outbreaks of identified etiology
Non-community	46	14	41
Community	118	38	56
Untreated recreational water	43	30	79
Individual	25	24	28

[a] Includes outbreaks of unidentified etiology.

Table 8.4. Severity of outbreaks by type of system, zoonotic agents and all causes, 1971–2000

Water system type	Cases of illness	Emergency visits and hospitalizations	Deaths
Non-community	6 033	117	2
Community	454 704	4 865	61
Untreated recreational water	1 799	69	0
Individual	383	29	0
Total	462 899	5 080	63

Zoonotic agents caused 118 outbreaks in community systems. These outbreaks represent 38% of the 308 outbreaks reported in community systems or 56% of the 210 community system outbreaks where an etiology was identified. In outbreaks associated with untreated recreational waters, zoonotic agents caused 30% of the outbreaks or 79% of the outbreaks of identified etiology. Zoonotic agents were responsible for most of the illnesses (79%), hospitalizations (71%), and deaths (88%) that were reported in outbreaks caused by contaminated drinking-water and untreated recreational water. Two-thirds of the illnesses (403 000 cases), 50 deaths, and 4400 hospitalizations were reported in a single drinking-water outbreak of cryptosporidiosis in Milwaukee in 1993.

8.3.1.1 Drinking-water outbreaks

Giardia, *Campylobacter*, *Cryptosporidium*, *Salmonella*, and *E. coli* were the zoonotic agents most frequently identified in outbreaks caused by contaminated drinking-water (Table 8.5). *Giardia* was identified in 66% of all drinking-water zoonotic outbreaks and in 70%, 62%, and 56% of the zoonotic outbreaks in community, non-community, and individual systems, respectively. *Cryptosporidium* was identified in only 8% of the zoonotic outbreaks and in 9%, 4%, and 8% of the zoonotic outbreaks in community, non-community, and individual systems, respectively. *Campylobacter* was identified in 10% of the zoonotic outbreaks. Non-typhoid *Salmonella* caused 8% of the zoonotic outbreaks, and *E. coli* O157:H7 caused 6% of the outbreaks.

Table 8.5. Drinking-water-borne outbreaks of zoonotic agents, 1971–2000

		Type of water system[a]			Water source[b]		
Etiologic agent	Total	C	NC	I	GW	SW	M/U
Giardia	126	83	29	14	31	90	5
Campylobacter	19	9	7	3	12	3	4
Cryptosporidium	15	11	2	2	8	5	2
Salmonella	15	11	2	2	11	2	2
E. coli O157:H7	11	4	4	3	8	2	1
Yersinia	2	–	1	1	2	–	–
E. coli O6:H16	1	–	1	–	1	–	–
E. coli O157:H7 and *Campylobacter*	1	–	1	–	1	–	–
Total	190	118	47	25	74	102	14

[a] C = community; NC = non-community; I = individual.
[b] GW = groundwater; SW = surface water; M/U = mixed or unknown.

Most (71%) outbreaks of giardiasis occurred in systems using surface water, whereas most (53%) outbreaks of cryptosporidiosis occurred in groundwater systems. Bacterial pathogens were identified in 49 (26%) of the zoonotic outbreaks and 20%, 34%, and 36% of the zoonotic outbreaks in community, non-community, and individual systems, respectively. Most (71%) outbreaks of zoonotic bacteria were reported in groundwater systems.

Outbreaks caused by protozoan and bacterial zoonotic agents were evaluated to determine the water system deficiencies that were responsible for the outbreak (Table 8.6). Inadequate disinfection as the only treatment of surface water and inadequate or interrupted treatment of surface water caused over half (52%) of the outbreaks of giardiasis and cryptosporidiosis. Nineteen

per cent of the outbreaks of giardiasis and cryptosporidiosis were due to contaminated, untreated, or inadequately treated groundwater; 11% were associated with contamination entering the distribution system. Although untreated surface water was responsible for 10% of the outbreaks of giardiasis and cryptosporidiosis, almost all of these outbreaks occurred in the early 1970s before EPA regulations required treatment.

Table 8.6. Number of waterborne outbreaks by deficiencies in drinking-water systems, 1971–2000

Type of contamination	*Giardia*, *Cryptosporidium*	*Campylobacter*, *E. coli*, *Salmonella*, *Yersinia*
Distribution system contamination	16	11
Inadequate disinfection; only treatment, surface water[a]	52	3
Inadequate, interrupted, or bypass of filtration; surface water	22	–
Untreated groundwater	14	14
Untreated surface water	14	2
Inadequate or interrupted disinfection; groundwater[b]	13	11
Water not intended for drinking; contaminated faucet or ice; unknown	10	8
Total	141	49

[a] Includes two outbreaks with surface water and groundwater sources.
[b] Includes three outbreaks where groundwater was filtered.

The three most important deficiencies identified for outbreaks of *Campylobacter*, *Salmonella*, *E. coli*, and *Yersinia* enteritis were use of contaminated, untreated groundwater (29%), distribution system contamination (22%), and inadequate treatment of contaminated groundwater (22%). Few bacterial outbreaks were attributed to untreated or inadequately treated surface water.

8.3.1.2 Outbreaks associated with untreated recreational water

Schistosomatidae caused 30% of the outbreaks of zoonotic etiology reported in untreated recreational waters (Table 8.7). *Escherichia coli* and *Leptospira* each caused 30% and 16% of the outbreaks; 23% of the outbreaks were caused by either *Giardia* or *Cryptosporidium*. Most (84%) outbreaks were associated with recreational activities in lakes or ponds.

Table 8.7. Outbreaks and illnesses, untreated recreational water, by zoonotic agent and water venue, 1971–2000

Etiologic agent	Total number of outbreaks	Lakes or ponds	River, springs, and other
Schistosomatidae	13	12	1
E. coli O157:H7	12	11	1
Leptospira	7	4	3
Giardia	6	4	2
Cryptosporidium	4	4	–
E. coli O121:H19	1	1	–
Total	43	36	7

The source of contamination was identified in 24 recreational water outbreaks (Table 8.8). Faecal contamination by bathers was identified in 11 of the *E. coli* outbreaks. An avian source was identified in seven outbreaks of schistosome dermatitis and suspected in six outbreaks. Animals, including dogs, cattle, and water buffalo, were suspected sources of *Leptospira*, but could be identified in only one outbreak.

Table 8.8. Identified causes of outbreaks of zoonotic agents associated with untreated recreational water

Source of contamination or deficiency	*Cryptosporidium* and *Giardia*	Schistosomatidae	*E. coli*	*Leptospira*
Animals, birds	2	7		1
Faecal accident, ill bathers	1		5	
Children in diapers	1		3	
Bather overload or crowding			3	
Seepage or overflow of sewage	1			
Total	5	7	11	1

8.4 SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Protozoan and bacterial pathogens are important causes of waterborne outbreaks in the USA. A protozoan pathogen was identified in 24% of all reported waterborne outbreaks, and a bacterial pathogen was identified in 20% of the outbreaks. No etiologic agent was identified in 40% of all outbreaks, and some of these outbreaks may have been protozoan or bacterial. Zoonotic protozoa were more frequently identified as the cause of outbreaks in drinking-water

systems (74%) and untreated recreational water (53%) than zoonotic bacteria. Most drinking-water outbreaks of zoonotic bacteria (71%) and cryptosporidiosis (53%) occurred in groundwater systems, but most drinking-water outbreaks of giardiasis (71%) occurred in surface water systems.

Reported outbreaks of zoonotic agents associated with recreational waters increased during 1991–2000, but drinking-water outbreaks of zoonotic agents did not. From 1991–1995 to 1996–2000, there was an almost 3-fold increase in the reporting of recreational water outbreaks of cryptosporidiosis. During the same period, the number of reported drinking-water outbreaks of cryptosporidiosis decreased by more than one-half.

8.4.1 Waterborne risks of zoonotic protozoa

Either *Giardia* or *Cryptosporidium* caused 60% of the outbreaks of identified etiology in community systems, but only 31% and 37% of the outbreaks of identified etiology in non-community and individual systems. Almost half of the drinking-water outbreaks of giardiasis and cryptosporidiosis occurred in the summer.

Current water filtration and disinfection practices and EPA regulations have reduced the risk of outbreaks associated with *Giardia* and *Cryptosporidium* in surface water systems. However, recent serological-epidemiological evidence suggests that the role of protective immunity is important to consider when assessing *Cryptosporidium* waterborne disease risks (Frost *et al*. 1997, 2000a; Craun *et al*. 1998). The severity and persistence of symptoms of cryptosporidiosis are related to both the immnocompetence of the host and previous infection (Okhuysen *et al*. 1998). Surface water sources may be a significant source of frequent, low-level exposure to *Cryptosporidium* oocysts, and the risk of symptomatic or severe illness among persons consuming water from these systems may be reduced because of protective immunity. Serological studies have found elevated levels of *Cryptosporidium* infection without an apparent increase in disease risk in populations with surface water systems that meet current water quality standards and regulations (Frost *et al*. 2002). Outbreak investigations also provide evidence of the importance of protective immunity for cryptosporidiosis (Frost *et al*. 2000b). Outbreak surveillance generally focuses on the occurrence of clinically detected disease, and waterborne outbreaks are usually detected only when water treatment deficiencies or distribution system contamination contribute to increased levels of exposure and cause increased symptomatic illness. A significant number of outbreaks of cryptosporidiosis occurred after groundwater systems were contaminated by surface water or sewage, resulting in a high incidence of clinical illness. Populations using groundwater sources have lower *Cryptosporidium* infection levels than populations using surface water sources,

and the high incidence of clinically detected cryptosporidiosis may be due to a low level of protective immunity in these populations. Additional evidence for protective immunity is provided by several waterborne outbreaks that were recognized in populations using surface water systems only because illness was reported by visitors; disease surveillance activities detected no increased illness among the residents (Frost *et al*. 1998, 2000a).

Naegleri fowleri, a naturally occurring environmental protozoan, caused most of the outbreaks of identified etiology in untreated recreational waters. Schistosomatidae, *Giardia*, and *Cryptosporidium* were the zoonotic protozoa that were identified in untreated recreational water outbreaks. These three protozoa caused 42% of the outbreaks associated with untreated recreational water. Birds such as ducks and geese are important reservoirs for the transmission of Schistosomatidae. Although wild and domestic animals can be reservoirs for *Giardia* and *Cryptosporidium*, important sources of contamination identified for untreated recreational waters included faecal contamination from bathers, septic tanks, and other sources.

8.4.2 Waterborne risks of zoonotic bacteria

Campylobacter, non-typhoid *Salmonella*, *E. coli*, and *Yersinia* were the zoonotic bacteria that caused outbreaks in public and individual water systems. Zoonotic bacteria caused fewer outbreaks than zoonotic protozoa. Zoonotic bacteria caused 15%, 16%, and 21% of the outbreaks of identified etiology in community, non-community, and individual water systems, respectively. Most of these outbreaks occurred in systems that use groundwater. A significant number of outbreaks were associated with groundwater contaminated by surface water or sewage. Outbreaks occurred because the source water was inadequately protected, treatment was not provided for contaminated sources, or disinfection was inadequate or interrupted. Bacterial pathogens are susceptible to water disinfection, but only if adequate disinfectant concentrations and contact times are maintained. Outbreaks of bacterial etiology were also associated with the contamination of distribution systems through cross-connections, back-siphonage, main breaks, main repairs, inadequately protected storage tanks, or uncovered reservoirs.

Bacterial zoonotic agents (*E. coli* O157:H7 and O121:H19 and *Leptospira*) caused 38% of the outbreaks associated with untreated recreational water. Animals are important reservoirs for the transmission of *Leptospira*. Although animals are also reservoirs for *E. coli* O157:H7 and O121:H19, faecal contamination from bathers, septic tanks, and other sources was identified as the important cause of outbreaks in untreated recreational waters.

8.4.3 Recommendations

The contamination of groundwater by *Cryptosporidium* or bacterial zoonotic agents is a frequent cause of outbreaks, and caution is urged for populations that use groundwater sources that may be subject to surface water runoff or sewage contamination. Sources of faecal contamination should be identified and action taken to prevent surface water and sewage from entering the well or spring. If contamination cannot be prevented, the well or spring should be treated to effectively remove and inactivate *Giardia* and *Cryptosporidium*. When groundwater is disinfected, the disinfection must be continuous and applied in concentrations and at contact times sufficient for anticipated contamination levels.

The importance of outbreaks associated with distribution system contamination has increased in recent years, and zoonotic agents have caused many of these outbreaks. Distribution systems become contaminated through cross-connections, main breaks and repairs, and poorly covered or uncovered storage tanks. More attention should be given to protecting the distribution system from contamination. It is especially important to prevent storage tanks and reservoirs from being contaminated by birds and rodents and water mains from being contaminated by runoff from animal feedlots, grazing lands, and food processing plants. Zoonotic agents can enter water mains that are leaking or under repair. Back-siphonage and intrusion of zoonotic agents can also occur in areas of low water pressure and through cross-connections, especially in rural and agricultural areas, at agricultural fairs, and in food processing plants. Hydraulic modelling of the system can help identify vulnerable areas of the system, and monitoring water pressure and loss of chlorine residuals in the system can help detect the possible entry of microbial contaminants into the system.

Important sources of contamination of untreated recreational waters include poor hygiene practices of bathers, septic tank and other sewage discharges, and contamination by wild and domestic animals. Additional public education is needed to prevent faecal contamination of recreational waters by bathers. Bathers should be warned about the risks of swimmer's itch, gastroenteritis, leptospirosis, and primary amoebic meningoencephalitis, how these diseases are transmitted, and the potential sources of contamination. Facilities for changing diapers should be available at all bathing sites. Prohibited activities while in the water should include the changing of diapers, rinsing diapers, and cleaning infants after bowel movements. Infants, children, and adults with symptoms of vomiting or diarrhoea should refrain from bathing activities while ill. Contamination from sewage discharges and surface water runoff should be reduced, and water quality monitoring can assist public health officials in closing beaches when needed.

Better surveillance to detect possible waterborne outbreaks, more complete investigation of sources of contamination, and improved laboratory capabilities should be provided to help identify additional zoonotic agents that may be transmitted by contaminated drinking-water and recreational water. Serological-epidemiological studies of *Cryptosporidium* infection should continue to be conducted to provide additional information about protective immunity, illness severity, and outbreak risks.

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9

Symptoms, treatments, and health consequences of waterborne zoonotic diseases

S. Kanarat

9.1 INTRODUCTION

Zoonotic agents can be transmitted from animals to humans either directly or indirectly. Indirect transmission means that the agents are passed from animals to humans via food, water, environment, vectors, etc.

Waterborne zoonotic agents include bacteria, protozoa, viruses, and helminths, but bacteria and protozoa are the zoonotic agents that are most often implicated in waterborne disease outbreaks. From 1986 through 1990, 20 waterborne outbreaks due to intestinal protozoa were reported in the USA

 Waterborne Zoonoses: Identification, Causes and Control. Edited by J.A. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D.O. Cliver, G.F. Craun, R. Fayer, and V.P.J. Gannon. Published by IWA Publishing, London, UK. ISBN: 1 84339 058 2.

(Burke 1993). Surveillance carried out by Levy *et al*. (1998) showed that *Cryptosporidium parvum*, *Giardia lamblia*, and *Escherichia coli* O157:H7 were the zoonotic agents found to be frequent causes of waterborne disease outbreaks in the USA. In developing countries, waterborne disease is a major problem.

In this chapter, the symptoms, treatments, and health consequences of major waterborne zoonotic diseases caused by bacteria — campylobacteriosis, *E. coli* (gastroenteritis and infective), salmonellosis, and leptospirosis — and by protozoa — cryptosporidiosis, giardiasis, and toxoplasmosis — are reviewed and discussed. These diseases are gastrointestinal tract infections, except for leptospirosis and toxoplasmosis, which are systemic infections. Therefore, diarrhoea is the common symptom of these diseases except for leptospirosis and toxoplasmosis. Mild cases of bacterial gastrointestinal infections are self-limiting, and antibiotic treatment is not recommended, especially for enterohaemorrhagic *E. coli* infection. Most of the diseases are effectively curable. The severity of the symptoms depends on the infective organisms as well as the health status of the infected person. Patients with acquired immunodeficiency syndrome (AIDS), immunocompromised persons, the old, and the young experience more severe symptoms. Most of the diseases give rise to health consequences.

9.2 BACTERIAL WATERBORNE ZOONOTIC DISEASES: SUMMARIES

9.2.1 Campylobacteriosis

9.2.1.1 Causative agent

Cases are usually caused by *Campylobacter jejuni* or, to a lesser extent, *C. coli* (Reynolds *et al*. 1993; IFST 1995; Nielsen *et al*. 1997; Wooldridge and Ketley 1997; Anonymous 1999; Myint *et al.* 1999a; Nadeau *et al*. 2002).

9.2.1.2 Symptoms

The incubation period is 2–5 days, with a range of 1–10 days. The illness normally lasts for 2–3 days, but severe cases can persist for up to 3 weeks. The infectious dose may be very low — i.e., only a few hundred cells. Symptoms include diarrhoea, which is the most consistent and prominent manifestation of campylobacteriosis, and the stool is often bloody. Other symptoms may include fever, nausea, vomiting, abdominal pain, and headache. Normally, hospitalization is not required. Mild cases are self-limiting; however, the disease can be severe and life threatening. The most severe infections occur in the very

young, the elderly, and the malnourished. Death is more common when there are underlying diseases (e.g., cancer, liver disease, and immunodeficiency disease) (Reynolds 1993; IFST 1995; Myint *et al*. 1999a, 1999b; http://www.dhs.sa.gov.au/pehs/Youve-got-what/specific-conditions/campylobacter.htm; http://www.about-campylobacter.com/page3.htm).

9.2.1.3 Treatment

Antibiotic treatment is not recommended, unless the infected person is severely ill. Erythromycin is the drug of choice, with ciprofloxacin as an alternative in adults. In case of septicaemia, gentamicin is used, but erythromycin, chloramphenicol, and tetracycline may also be used (IFST 1995).

9.2.1.4 Consequences

Long-term consequences from a *Campylobacter* infection can sometimes occur. Some people may develop a rare disease called Guillain-Barré syndrome, which affects the nerves of the body. Although rare, it is the most common cause of acute generalized paralysis in the western world. It begins several weeks after the diarrhoeal illness in a small minority of *Campylobacter* victims, and it occurs when a person's immune system produces antibodies against components of *Campylobacter* and these antibodies attack the body. Guillain-Barré syndrome begins in the feet and spreads up the body. Prickling sensations give way to weakness, which may lead to paralysis. The disease lasts for weeks to months and often requires intensive care. Full recovery is common; however, some victims may be left with severe neurological damage. Approximately 15% of Guillain-Barré victims remain bedridden or wheelchair bound at the end of 1 year. Two therapies, intravenous immunoglobulin infusions and plasma exchange, may improve the rate of recovery.

It is estimated that approximately 1 in every 1000 reported campylobacteriosis cases leads to Guillain-Barré syndrome. As many as 40% of Guillain-Barré syndrome cases reported in the USA occur following campylobacteriosis. Miller Fisher syndrome is another related neurological syndrome following campylobacteriosis, and it is also caused by immunological mimicry. In Miller Fisher syndrome, the nerves of the head are affected more than the nerves of the body.

Another potential associated chronic condition is an arthritis called Reiter's syndrome. This reactive arthritis most commonly affects large weight-bearing joints such as the knees and the lower back. It is strongly associated with a particular genetic make-up; persons with the human lymphocyte antigen B27 (HLA-B27) are most susceptible.

Campylobacter may also cause appendicitis or infect the abdominal cavity (peritonitis), the heart (carditis), the central nervous system (meningitis), the gall-bladder (cholecystitis), the urinary tract, and the bloodstream (http://www.about-campylobacter.com/page3.htm).

9.2.2 *E. coli* (gastroenteritis and infective)

9.2.2.1 Causative agents

Causative agents include:

(1) enterotoxigenic *E. coli* (ETEC);
(2) enteroinvasive *E. coli* (EIEC);
(3) enteropathogenic *E. coli* (EPEC); and
(4) enterohaemorrhagic *E. coli* (EHEC).

9.2.2.2 Symptoms

The incubation period ranges from 1 to 5 days, and the duration of the illness is 3–5 days (Myint *et al*. 1999c). Symptoms vary from mild to severe, depending on the strain and the underlying health of the host. Symptoms include diarrhoea, vomiting, stomach-ache, and fever. EHEC infection often causes severe bloody diarrhoea and abdominal cramps; especially in children under 5 years of age and the elderly, a complication called haemolytic uraemic syndrome (HUS) may occur in about 2–7% of the EHEC infections. Persons who have only diarrhoea usually recover completely (Mims *et al*. 1993; Myint *et al*. 1999a, 1999b; Shanson 1999a; http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.htm).

9.2.2.3 Treatments

Antibiotics are not recommended for gastrointestinal infection. Fluid replacement may be necessary, especially in young children. When septicaemia is suspected, systemic antibiotics are indicated, and the rational choice depends on the results of the sensitivity of the epidemic strains. For infection with EHEC, antibiotic treatment is not recommended, since treatment with antibiotics may precipitate kidney complications. Antidiarrhoeal agents should also be avoided. Treatment of HUS is urgent and may require dialysis (Mims *et al*. 1993; Shanson 1999a; http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.htm).

9.2.2.4 Consequences

Following an attack of *E. coli* gastroenteritis, some infants develop a disaccharidase and lactose intolerance, which may become clinically manifested as chronic diarrhoea (Shanson 1999a). About one-third of persons with HUS have abnormal kidney function many years later, and a few require long-term dialysis. Another 8% may have other lifelong complications, such as high blood pressure, seizures, blindness, paralysis, and the consequences of having part of their bowel removed (http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.htm).

9.2.3 Salmonellosis

9.2.3.1 Causative agent

The causative agent for salmonellosis is non-typhoidal salmonellae.

9.2.3.2 Symptoms

The incubation period of intestinal salmonellosis is 8–72 h, and the duration of the illness is 2–7 days (Brooks *et al*. 1991a; Mahon and Manuselis 1995a; Myint *et al*. 1999a, 1999c; Shanson 1999a; http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_g.htm). Symptoms are characterized by an abrupt onset of diarrhoea, abdominal pain, prostration, chills, fever, and vomiting. The vast majority of cases are self-limiting, but in the young, the elderly, and those with impaired immune systems and underlying diseases, symptoms may be severe. Bacteraemia and septicaemia may occur if the strain is invasive. Vomiting is rare, and fever is usually a sign of invasive disease (Brooks *et al*. 1991a; Mims *et al*. 1993; Reynolds *et al*. 1993; Myint *et al*. 1999a; Murray *et al*. 2002a; http://netvet.wustl.edu/species/primates/primzoon.txt; http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_g.htm; http://www.tc.umn.edu/~devo0028/zoonos.htm).

9.2.3.3 Treatments

Diarrhoea is usually self-limiting and resolves without treatment. Fluid and electrolyte replacement may be required in the very young and the elderly. Antibiotics should not be used, except where there is evidence of invasion and septicaemia, as they do not reduce the symptoms or shorten the illness, but may prolong excretion of *Salmonella* in the faeces. Antidiarrhoeal agents are also restricted, as they encourage adherence and further invasion (Brooks *et al*. 1991a; Mims *et al*. 1993; Mahon and Manuselis 1995a; Murray *et al*. 2002a). In case of bacteraemia, the disease should be treated with an effective antibiotic

selected by susceptibility tests. Fluoroquinolones (e.g., ciprofloxacin), chloramphenicol, trimethoprim/sulfamethoxazole, or broad-spectrum cephalosporin can be used (Brooks *et al*. 1991a; Murray *et al*. 2002a).

9.2.3.4 Consequences

Species of *Salmonella* that normally cause diarrhoea may become invasive in patients with particular predispositions. The organisms are not contained in the gastrointestinal tract but invade the body to cause septicaemia; consequently, many organs become seeded with salmonellae, sometimes leading to necrotizing cholecystitis, haemorrhage, osteomyelitis, pneumonia, thrombophlebitis, endocarditis, abscesses, or meningitis (Mims *et al*. 1993; Mahon and Manuselis 1995a).

9.2.4 Leptospirosis

9.2.4.1 Causative agent

There are three main serogroups of *Leptospira*: *L. canicola* (dogs are hosts; pigs are reservoirs), *L. icterohaemorrhagiae* (rats are reservoirs), and *L. hebdomadis* (cattle, mice, and voles are reservoirs) (Shanson 1999b). Any of the pathogenic serovars may cause benign leptospirosis, but Weil's disease, the severe form, is usually due to the serovar *icterohaemorrhagiae*.

9.2.4.2 Symptoms

The incubation period of leptospirosis is usually from 10 to 12 days, but may range from 3 to 30 days after inoculation. Duration of the illness varies from less than 1 week to 3 weeks. The severity of leptospirosis depends on many factors, such as strains and the general health of the host. The initial clinical sign is similar to an influenza-like illness, with fever and myalgias. During this phase, the leptospires are present in the bloodstream, and the organisms can frequently be isolated in cerebrospinal fluid. The fever and myalgia may remit after 1 week, or the patient may develop a more advanced disease, including aseptic meningitis or generalized illness with headache, rash, vascular collapse, thrombocytopenia, haemorrhage, and hepatic and renal dysfunction resulting in jaundice and nitrogen retention. Uncomplicated cases have a very low mortality rate. The icteric form is more severe and is associated with mortality approaching 10% (Gillespie 1994a; Mahon and Manuselis 1995a; Murray *et al*. 2002b).

9.2.4.3 Treatments

Leptospirosis usually responds to treatment with antibiotics if they are administered in large enough doses early in the infection. For severe infection, penicillin or ampicillin is given intravenously for up to 7 days. For mild infection, ampicillin, amoxicillin, or deoxycycline is taken orally. Patients allergic to penicillin may be treated with streptomycin, tetracycline, or erythromycin. Leptospirosis is usually not fatal, particularly in the absence of icteric disease. When there is impairment of kidney function, as sometimes happens in Weil's disease, renal dialysis may be required to counteract the uraemia, which is the main cause of death. Tetracycline should not be used if there is evidence of renal failure (Brooks *et al*. 1991b; Mahon and Manuselis 1995a; Greenwood *et al*. 1997a; Shanson 1999b; Murray *et al*. 2002b).

9.3 PROTOZOAN WATERBORNE ZOONOTIC DISEASES: SUMMARIES

9.3.1 Cryptosporidiosis

9.3.1.1 Causative agent

Cryptosporidiosis is caused by *Cryptosporidium parvum*.

9.3.1.2 Symptoms

Infection with *Cryptosporidium* organisms may result in asymptomatic carriage. In symptomatic individuals, the incubation period varies from 3 to 14 days (Myint *et al*. 1999a; Shanson 1999a). Disease in previously healthy individuals is usually mild, self-limited enterocolitis characterized by watery diarrhoea without blood. Spontaneous remission after an average of 10 days is characteristic. Patients with immunocompromised conditions (e.g., patients with human immunodeficiency virus [HIV], AIDS) are characterized by 50 or more stools per day and tremendous fluid loss, which can last for months to years. The symptoms may include abdominal cramps, weight loss, nausea, vomiting, and fever (Mims *et al*. 1993; Gillespie 1994b; Colley 1995; Greenwood *et al*. 1997b; Guerrant 1997; Carpenter *et al*. 1999; Shanson 1999a; Murray *et al*. 2002c; http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis).

9.3.1.3 Treatments

Currently, there are no broadly effective therapies for cryptosporidiosis. Therapy consists primarily of supportive measures such as fluid and electrolyte replacement. Spiramycin may help control the diarrhoea in some patients in the

early stages of AIDS who have cryptosporidiosis, but is ineffective in patients who have progressed to the later stages of AIDS. Azithromycin and paromomycin appear to offer some benefit (Anonymous 1984; Gillespie 1994b; Greenwood *et al*. 1997b; Guerrant 1997; Shanson 1999a; Murray *et al.* 2002c; http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis).

9.3.2 Cyclosporiasis

9.3.2.1 Causative agent

The causative agent of cyclosporiasis is *Cyclospora cayetanensis.*

9.3.2.2 Symptoms

The incubation period of cyclosporiasis varies from 1 to 14 days, with an average of 7 days. The organisms infect the small intestine and usually cause watery diarrhoea, mild nausea, anorexia, and abdominal cramping. Other symptoms may include weight loss, myalgias, fatigue, malaise, flatulence, and bloating. In immunocompetent hosts, diarrhoea is self-limiting, but may be prolonged and last for weeks. The clinical illness is typically prolonged and severe and is associated with a high rate of recurrence in immunocompromised patients, particularly those infected with HIV. Biliary tract infection with *Cyclospora* has been reported in two patients with AIDS (Murray *et al*. 2002c).

9.3.2.3 Treatments

Trimethoprim-sulfamethoxazole in combination is reported to be effective for the treatment of *Cyclospora* infection in both immunocompetent and immunocompromised patients. In HIV-infected patients, the high rate of recurrence can be attenuated by long-term suppressive therapy with trimethoprim-sulfamethoxazole. Metronidazole, norfloxacin, quinacrine, nalidixic acid, tinidazole, and diloxanide furoate have also been used in various trials, but the effectiveness of any one of these agents has not been proved (Shlim *et al*. 1991; Connor *et al*. 1999; Murray *et al*. 2002c).

9.3.2.4 Consequences

Guillain-Barré syndrome, reactive arthritis, acalculous cholecystitis, and biliary infection have been reported to be sequelae of *Cyclospora* infection (Connor *et al*. 2001; Murray *et al*. 2002c).

9.3.3 Giardiasis

9.3.3.1 Causative agent

Giardiasis is caused by *Giardia lamblia* (*G. duodenalis*).

9.3.3.2 Symptoms

Giardia lamblia is the most reported intestinal parasite. The incubation period ranges from 1 to 4 weeks, with an average of 10 days; 50% of infected individuals are asymptomatic. Symptomatic disease ranges from mild diarrhoea to a severe malabsorption syndrome (Gillespie 1994b; Mahon and Manuselis 1995b; Myint *et al*. 1999c; Shanson 1999a; Murray *et al*. 2002c). The onset of the disease is sudden and consists of self-limiting, foul-smelling, watery diarrhoea, abdominal cramps, flatulence, and steatorrhoea. The stool may be semisolid, greasy, and bulky. These symptoms may lead to weight loss and dehydration (Brooks *et al*. 1991c; Gillespie 1994b; Murray *et al*. 2002c; http://www.cdc.gov/ncidod/dpd/parasites/giardiasis/factsht_giardia.htm). Spontaneous recovery generally occurs after 10–14 days (Myint *et al*. 1999a, 1999b, 1999c; Murray *et al*. 2002c). Multiple relapses may develop in chronic patients. Patients with immunoglobulin A deficiency or achlorhydria seem not only to be prone to the infection but also to develop chronic infection (Mahon and Manuselis 1995b; Shanson 1999a; Murray *et al*. 2002c; http://netvet.wustl.edu/species/primates/primzoon.txt).

9.3.3.3 Treatments

Quinacrine hydrochloride by the oral route can treat 90% of *Giardia lamblia* infections. Metronidazole and furazolidone are alternatives. Tinidazole is widely used for 1-day treatment (Brooks *et al.* 1991c; Mims *et al*. 1993; Mahon and Manuselis 1995b; Greenwood *et al*. 1997b; Murray *et al*. 2002c).

9.3.4 Toxoplasmosis

9.3.4.1 Causative agent

Toxoplasma gondii is the causative agent of toxoplasmosis.

9.3.4.2 Symptoms

Toxoplasma gondii can infect populations of all ages. It has emerged as the second most common opportunistic infection in AIDS patients, leading to a 75% mortality rate (Jalan 1998). In immunocompetent individuals, the *Toxoplasma* infection is mostly asymptomatic. It may cause mild influenza-like disease with

enlargement of lymph nodes, fever, fatigue, muscle pain, headache, malaise and anaemia (Mims *et al*. 1993; Myint *et al*. 1999c; http://martin.parasitology.mcgill.ca/jimspage/biol/toxoplas.htm). The initial infection is self-limiting, becomes chronic (latent), and poses no serious risk unless the host is immunocompromised, in which case it frequently involves the central nervous system and lungs and is often fatal. The central nervous system can be severely affected in patients with congenital infections, those treated with immunosuppressant drugs, and patients with AIDS. In these cases, acute focal or diffuse meningoencephalitis with extensive areas of brain necrosis and vascular involvement may be observed. *Toxoplasma* proliferates in the ependymal and subependymal regions and spreads widely. Morphological changes include lymphocytic infiltration of the meninges, destructive lesions of both brain and white matter, and focal periventricular and periaqueductal calcification. In immunodeficient adult patients, the major finding is necrotizing encephalitis; large abscesses can occur. Symptoms of toxoplasmosis in immunocompromised patients include myocarditis, chorioretinitis, meningoencephalitis, and death.

9.3.4.3 Ocular toxoplasmosis

The lesions include acute chorioretinitis with severe inflammations and necrosis, necrotizing retinitis, and granulomatous chorioretinitis (Mims *et al*. 1993; Myint *et al*. 1999c; http://martin.parasitology.mcgill.ca/jimspage/biol/toxoplas.htm).

9.3.4.4 Congenital toxoplasmosis due to acute symptomatic or asymptomatic infections of the mother during pregnancy

Up to 8% of pregnant women get acute *Toxoplasma* infection, and only 10% of them are symptomatic. The acute disease contracted during pregnancy is a serious danger to the offspring, as 40% of the infected pregnant women transmit *Toxoplasma* organisms to the placenta and fetus, leading to *Toxoplasma* infection in the newborn. There are indications from several studies that there is a greater chance of severe illness among the infants whose mothers become infected with *T. gondii* during the first two trimesters. However, infections during the third trimester are more common, but usually result in subclinical disease. The later in pregnancy the acute infection, the more likely the involvement of the placenta at the time of delivery. If treatment of the mother is disregarded and the onset of the infection is during the first trimester, the chance of infection in the newborn is 10–15%, and the consequences will be severe in two-thirds. If the onset is during the second trimester, about 30% will be infected at birth, nearly 10% of which will be severe. If the disease is contracted during the last trimester, approximately 60% of

the neonates will be infected, and virtually none will be severe (Jalan 1998). If the pregnant woman is infected before conception, there is no risk of transmission of the organism to the fetus. Maternal antibodies acquired from the infection prior to pregnancy prevent fetal infection. The effect of the illness acquired during pregnancy on the occurrence of spontaneous abortions and stillbirths is not certain.

At birth, 60–75% of infants with congenital toxoplasmosis have subclinical infections. Among the minority symptomatic infected infants, hydrocephalus or microcephalus, chorioretinitis, convulsion, and intracerebral calcification may be present at birth, but are usually not evident until the infants are a few months of age. The brain is the site of most evident abnormality, together with the retina, the area most commonly and most severely diseased. In the severe form, neonatal sepsis, fetal hydrops, and congenital nephrotic syndrome may occur; however, the disease in the early days of life may be unapparent, only to manifest itself in a few days or weeks. It may have general characteristics of neonatal sepsis, and death results in a short time (Jalan 1998).

9.3.4.5 Treatments

The drugs used for treatment of *Toxoplasma* infection are pyrimethamine, sulfadiazine, trimethoprin, and spiramycin. The standard treatment of toxoplasmosis is to use the combination of pyrimethamine and sulfonamide (sulfadiazine) in equal parts. Both pyrimethamine and sulfonamide are toxic. During treatment, examining for crystalluria and haematuria is required in order to modify the dose of sulfonamide used. In most countries, the favoured agent for the treatment of acute toxoplasmosis during pregnancy is spiramycin. Spiramycin is a relatively safe drug that concentrates in the placenta and may reduce the risk of maternal/fetal transmission by 60% without having any effect on the fetus. The benefits to the fetus and newborn of spiramycin treatment provided the mother during pregnancy appear to be significant. Based on isolates of the organism from the placenta at birth, the advantages seem to be most marked if spiramycin is begun during the first two trimesters. If the fetus is shown to be infected, the combination of pyrimethamine, sulfonamide(s), and folic acid is added for the duration of the pregnancy. The use of pyrimethamine, sulfadiazine, pyrimethamine, and clindamycin in combination for the treatment of toxoplasmosis encephalitis gives comparable results (Jalan 1998).

9.3.4.6 Recommended treatments for infants

Remington and Desmonts (1990) recommend treatment of newborn infants with congenital toxoplasmosis as follows:

(1) Include a 21-day course of 1 mg of pyrimethamine per kilogram of body weight orally once every 1–4 days (preferably every 3–4 days) together with 25–50 mg of sulfadiazine per kilogram of body weight orally twice each day (triple sulfonamides may be substituted).
(2) Follow by a 30- to 45-day course of 50 mg of spiramycin per kilogram of body weight orally twice each day. Periods of antimicrobial therapy should be alternated for 1 year.
(3) Because pyrimethamine is a folic acid antagonist, 5 mg of folic acid should be provided orally twice each week while it is being used.
(4) For those infants with high concentrations of protein in their cerebrospinal fluid (CSF) or active chorioretinitis, it is suggested that 1 mg of prednisone per kilogram of body weight be given orally twice each day until the active retinal inflammation is resolved or the elevated concentrations of CSF protein are no longer present.

Indications for treatment are as follows:

(1) newborn infants with overt *Toxoplasma* infection;
(2) active chorioretinitis;
(3) elevated concentrations of CSF protein;
(4) infants known to be infected but without clinical evidence are included among those to be treated according to the regimen described above; and
(5) for apparently healthy infants with equivocal laboratory findings and uncertain historical information, if the mother is known to have contracted toxoplasmosis during the pregnancy and the newborn infant lacks clinical or laboratory evidence of the disease, one cycle of pyrimethamine with sulfadiazine and folic acid followed by spiramycin is suggested while further investigation is carried out (Jalan 1998).

Treatments of paediatric patients for toxoplasmosis are as follows:

(1) Sulfadiazine (100 mg/kg of body weight per day) and pyrimethamine (1 mg/kg of body weight per day) are given twice a week.
(2) Folic acid (5 mg) is given twice a week.
(3) Sulfadiazine and pyrimethamine should be alternated with spiramycin (100 mg/kg of body weight per day), given in 6-week cycles.
(4) Therapy for small children consists of sulfadiazine or trisulfapyrimidine (150 mg/kg of body weight per day in divided doses) and pyrimethamine (1 mg/kg of body weight per day in divided doses).
(5) A double dose is used for the first 3 days.

(6) Prednisolone (1–2 mg/kg of body weight per day) should be added to the therapy in newborns with a high protein concentration in the CSF or chorioretinitis.
(7) Treatment should be continued for 6 months for both congenital and acquired toxoplasmosis.

9.3.4.7 Consequences

In pregnant women, acute toxoplasmosis contracted during pregnancy leads to *Toxoplasma* infection of the infant, resulting in seizures, mental retardation, other manifestations of severe brain damage, or death in severe cases (Jalan 1998).

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Section IV

Epidemiological data, case-studies, and outbreaks

G.F. Craun

This section presents information about surveillance activities, outbreak investigations, and epidemiological studies of endemic disease and illustrates how these activities can help public officials identify current and emerging zoonotic agents, assess the importance of waterborne, foodborne, and other modes of transmission, and evaluate control strategies. For example, in Scotland, zoonotic agents constitute a significant disease burden, with farm animals and birds being the major reservoirs from which human infection occurs. Studies also show that just because an agent is considered zoonotic, it should not be assumed that all sources of infection derive from contact with animals. In the case of cryptosporidiosis, human reservoirs may be as important as animal reservoirs, and for campylobacteriosis and salmonellosis, foodborne transmission is just as important as animal and environmental sources.

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Surveillance in New Zealand shows that zoonotic diseases that are potentially waterborne currently constitute about 80% of reported diseases, and epidemiological studies have reported a number of waterborne associations, including increased rates of giardiasis and water treatment efficacy in one large city; campylobacteriosis outbreaks and town water supplies where the water supply is subject to animal runoff; cryptosporidiosis mortality and the quality of rural water supplies; and increased cryptosporidiosis in rural communities coincident with the onset of calving. Rates of campylobacteriosis, giardiasis, salmonellosis, and cryptosporidiosis have also been increasing concurrent with pastoral agricultural activity in New Zealand.

Local, national, and international surveillance activities can help officials detect outbreaks or epidemics, identify new and emerging disease threats, and assess temporal trends. Surveillance may consider laboratory-confirmed, physician- or self-reported disease, or disease symptoms and the collection of other information, such as water quality data or information about disease or infection in animal populations. Because zoonotic agents can be transmitted by contaminated drinking-water, recreational water, or food and by direct or indirect contact with infected humans and animals, epidemiological analyses are needed to evaluate waterborne transmission. The timely investigation of an outbreak by a multidisciplinary team (e.g., epidemiologist, engineer, water quality specialist) with appropriate laboratory assistance can provide information about the mode of transmission, the etiologic agent, sources of contamination, and deficiencies in water and wastewater treatment technologies and watershed protection programmes.

Several countries systematically collect information obtained from the investigation of waterborne disease outbreaks to guide research as well as evaluate controls and regulations for drinking-water and recreational water. For example, in the USA, zoonotic protozoa (*Giardia* and *Cryptosporidium*) and bacteria (*Campylobacter*, *Salmonella*, *Escherichia coli* O157:H7, and *Yersinia*) are important causes of drinking-water outbreaks. The most important water system deficiency identified for outbreaks of giardiasis and cryptosporidiosis was inadequate or interrupted treatment of surface water; however, the contamination of groundwater and distribution systems is an increasingly important deficiency. Outbreaks caused by *Campylobacter*, *Salmonella*, *E. coli* O157:H7, and *Yersinia* were associated with the use of untreated or inadequately disinfected groundwater and distribution system contamination. These data have helped officials develop control programmes to improve the quality of surface water sources and have called attention to the need for improved protection of groundwater sources and distribution systems from contamination.

Surveillance activities cannot provide information about the burden of waterborne zoonotic disease. Not all outbreaks will be recognized or investigated, and not all cases of disease will be reported. The sensitivity of a surveillance system to detect disease or an outbreak may be quite poor, depending upon the available resources, type of surveillance, and etiologic agent. Asymptomatic and mild illnesses are not reported in most surveillance systems, and these may be waterborne. In addition, some percentage of sporadic cases and endemic disease may be waterborne. Appropriately designed epidemiological studies can provide a quantitative assessment of the endemic waterborne zoonotic risks and a benchmark for microbial risk assessment modelling. Several studies are currently under way, and a microbial risk assessment framework has been developed and used to assess waterborne risks of cryptosporidiosis and campylobacteriosis.

Although information from current surveillance activities and epidemiological studies can be helpful in developing general control strategies that may be applicable for many countries, it is likely that a particular zoonotic agent may be more important in some countries than others. This will depend on socioeconomic conditions, general sanitation, and animal husbandry and agricultural practices. Thus, local and national surveillance and epidemiological activities can provide benefits for all countries. Information about the effectiveness of current prevention and control programmes may lead to changing systems of animal husbandry, water source protection and treatment, and food production. This, in turn, can reduce the disease burden, improve productivity, and increase the economic well-being of the population.

10

Epidemiological studies and surveillance

G.F. Craun, D.G. Till, and G. McBride

10.1 INTRODUCTION

Epidemiology is the study of the distribution and determinants of disease and the application of this knowledge to the prevention and control of health problems. Epidemiologists view disease primarily at the population level, describing its incidence and prevalence, temporal trends, geographic clustering, and other patterns. They also evaluate associations between disease risks and exposures (e.g., waterborne, foodborne), demographic characteristics, or behaviours (e.g., risk factors). Epidemiological studies and surveillance activities can provide information about the waterborne risks of zoonotic agents and assist public health officials in developing control measures to reduce these risks. However, it is important to understand the inferences that can and cannot be made from

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surveillance and epidemiological information, so that expectations of their outcomes are realistic. This understanding is necessary when designing surveillance activities and epidemiological studies and using the information to frame policies.

10.2 EPIDEMIOLOGICAL CONCERNS

Waterborne diseases are usually caused by exposure to enteric pathogens that are transmitted by the faecal–oral route and occasionally by exposure to pathogens in urine (e.g., *Leptospira*). The pathogens are excreted by infected animals or persons, who may or may not exhibit symptoms. Transmission of these pathogens can occur in the form of contaminated water, food, or fomites and contact with infected persons or animals. Because of these multiple sources of exposure, waterborne transmission must be established by epidemiological investigations that evaluate the various modes of transmission. Although ingestion is the principal exposure route, some waterborne pathogens may be transmitted by dermal contact or inhalation of contaminated aerosols. Many of the important waterborne pathogens are of domestic and wild animal origin, and some have significant animal reservoirs (e.g., *Campylobacter*). Confirming the route of waterborne transmission of disease in a single patient is extremely difficult and, in most cases, practically impossible. Thus, for all of the infectious diseases that may be caused by contaminated water, except dracunculiasis and primary amoebic meningoencephalitis, at least two cases of illness must be reported in order to conduct an epidemiological investigation and determine the mode of transmission.

Zoonotic infections can be transmitted by contaminated drinking-water, recreational water, or food, contamination during food preparation or production, and direct or indirect contact with infected humans and animals. The relationship between animal reservoirs, human sources of infection, and the contamination of food complicates the assessment of waterborne zoonotic risks. Other important complicating factors and epidemiological issues include the following:

- Zoonotic infection can be transmitted not only through contaminated water but also through contaminated food and in other ways.
- Even when the primary source of infection is contaminated water, risks of secondary spread for many agents may increase the waterborne burden.
- Persons in a community are not equally susceptible to infection and disease, and susceptibilities between communities may be even greater.
- Waterborne zoonotic infections do not always result in clinical disease.

- Protective immunity may be important for some waterborne diseases.
- The incidence and prevalence of various zoonotic waterborne pathogens are subject to geographical and socioeconomic factors.
- The importance of any zoonotic waterborne disease can change as selective pressures in communities and parts of the world change.
- Surveillance activities can provide important information about zoonotic agents and disease.
- An outbreak investigation of the increased incidence of infection or disease is required to assess whether water is the mode of transmission.
- Analytical epidemiological studies are required to assess whether endemic or sporadic disease or infection is waterborne and to provide a benchmark for risk assessment modelling and calculations.

An epidemiological investigation may lead to inconclusive results if all of the risk factors or sources of exposure are not considered or if random or systematic error has occurred. Investigations may fail to observe an association with water or underestimate the risk because of low statistical power or non-differential exposure misclassification (e.g., obtaining incomplete information about water contact and consumption). Systematic error, such as recall bias, can also cause misleading results (Craun *et al*. 2001).

Waterborne infections that do not result in clinically recognized disease will be difficult to identify and may not be considered in the risk estimate. However, asymptomatic persons can be a source of contamination and infection. Studies may consider only the primary mode of transmission (e.g., water), but secondary transmission can occur. Persons who are infected by contaminated water may infect others. Transmission can be direct or indirect. The transmission of waterborne diseases to familial, institutional, or other contacts by a primary case has been confirmed epidemiologically in outbreaks caused by *Escherichia coli* O157:H7 and *Cryptosporidium*. The impact of waterborne zoonotic diseases will be underestimated when asymptomatic cases and secondary transmission are not considered. However, it will be difficult to detect secondary transmission when the primary infection results in mild illness or no symptoms at all.

Host susceptibility is important to consider when assessing waterborne risks. Host susceptibility can vary both within a community and between communities. Persons with increased risk of disease and severity of disease include the very young and the elderly, pregnant women, undernourished individuals, and patients with compromised immunity due to diseases, such as acquired immunodeficiency syndrome (AIDS), and medical interventions, such as organ transplant and cancer treatment. Zoonotic agents may have a greater impact on persons who are malnourished or already suffering from other disease. An important implication of varying host susceptibility is that

information about the importance of waterborne transmission of zoonotic agents and the risk of infection and disease cannot necessarily be extrapolated from one country or community to another. Possible protective immunity should also be considered when assessing waterborne risks and developing control and regulatory strategies. For example, sero-epidemiological studies suggest that immunity is important when assessing waterborne *Cryptosporidium* risks (see chapter 8). However, not all waterborne pathogens confer protective immunity, and some may confer only short-lived protective immunity (see chapter 3).

The incidence and prevalence of waterborne zoonotic risks are subject to geographical, climatic, and socioeconomic factors. Although most pathogens are distributed worldwide, some are not. Outbreaks of some diseases, such as cryptosporidiosis, may be regional. In addition, the incidence and prevalence of these agents and diseases can change as changes occur in communities and regions. Changes in populations of zoonotic pathogens in the environment occur primarily as the result of selection by factors including susceptible hosts, reservoirs of infection, and conditions that favour or prohibit the transmission of the pathogens. Rapid growth in populations of humans and animals can accelerate changes in prevailing pathogen populations, result in larger numbers of hosts, and provide closer contact among animal and human hosts. The frequent movement of humans and animals over long distances from one environment and community to another offers ideal opportunities for new strains of pathogens to find environments and hosts in which they can survive. For many zoonotic agents, animal reservoirs need not exhibit any clinical illness, yet they still excrete large numbers of agents to water sources. Examples include *Campylobacter*, *Giardia*, *Cryptosporidium*, *Leptospira*, and *E. coli* O157:H7.

10.2.1 Disease models

The relationship between the host, agent, and environment is described by the epidemiological triad, a relatively simple, but important, model of disease transmission (Figure 10.1). The host, agent, and environment co-exist independently, and infection occurs only when there is interaction between the host and the agent or environment. The presence (or absence) of the agent is necessary for infection to occur (or be prevented). The environment must support the agent, and the agent must be transmitted to a susceptible host in an appropriate time, manner, and sufficient dose to cause infection and disease.

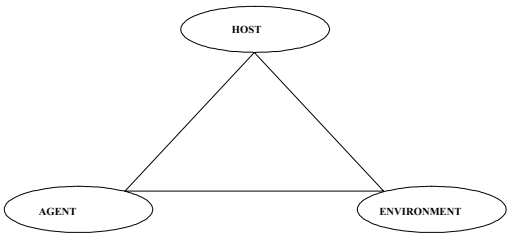

Figure 10.1. Host–agent–environment relationship.

The zoonotic agent and all relevant social, physical, and biological environments that allow the agent to survive (e.g., climate, reservoirs of infection) and maintain opportunity for contact with the host (e.g., personal behaviour, agricultural practices, hygiene and sanitation practices) present opportunities for exposure. If exposed, the human host may become infected, and the pathogen may multiply inside the host or pass through its life cycle. The person becomes infectious to others and may excrete pathogens into the environment. The resulting disease may be asymptomatic, mild, or severe, depending upon host susceptibility. Genetic traits and other host factors may also be important. The complexity between the agent, host, and environment is important to recognize when attempting to assess waterborne zoonotic risks, and a more complex disease model is usually necessary. Figure 10.2 is a model that more completely describes the modes of transmission, sources of contamination, and disease consequences for *Cryptosporidium*. The model illustrates that a more serious disease may occur among immunocompromised persons, whereas the disease process for an immunocompetent person may result in mild or asymptomatic infection.

10.2.2 Risk models

In New Zealand, a risk analysis and modelling effort for zoonotic agents is being guided by the results of epidemiological studies and surveillance systems (see chapter 29). However, it should be recognized that these efforts can also provide input to the design of epidemiological studies and surveillance systems, put zoonotic risks into a wider perspective, and instruct public policy. The recent and ongoing risk analysis upon which New Zealand guidelines for fresh water are now based serves to highlight the extent of *Campylobacter* infection and its ecology. Figure 10.3 shows the complex relationship of reservoirs, amplifiers, and transmission routes for *Campylobacter*. Campylobacteriosis is the main component of the reported disease burden in New Zealand (see chapter 12).

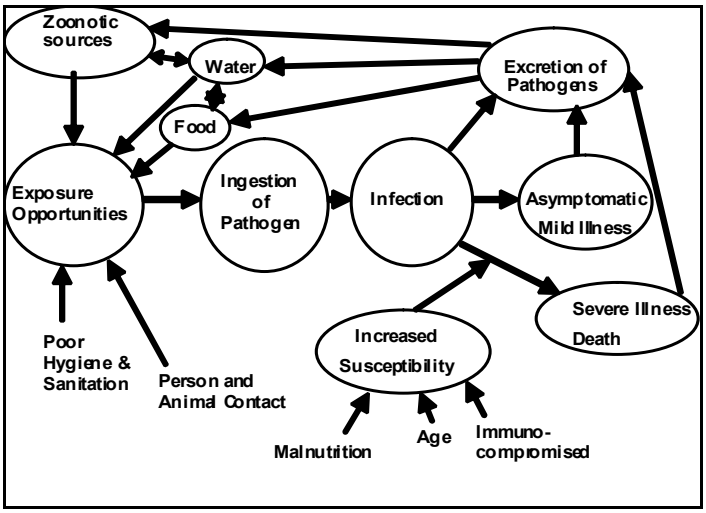

Figure 10.2. A disease model for *Cryptosporidium.*

10.3 EPIDEMIOLOGICAL SURVEILLANCE

Detection of a zoonotic agent in water can provide important information about sources of contamination, but a disease-based surveillance system is necessary to detect possible waterborne outbreaks and help public health officials assess temporal trends. Because of their poor sensitivity, surveillance activities are of little value in determining the disease burden (Hunter 2003a). For example, studies have found that the sensitivity of surveillance can vary from as little as 0.06% for norovirus to 7.9% for *Campylobacter* and 31.8% for *Salmonella* (Hunter 2003a). The primary value of surveillance is in identifying sudden changes in disease incidence, detecting new or emerging etiologic agents, and providing a starting point for epidemiological studies.

The sensitivity of the surveillance activity can vary substantially from one country to another, and the large variation in reported waterborne disease between European countries is an example (Hunter 2003a). The surveillance systems of many European countries were judged incapable of detecting waterborne disease, and the situation in the developing world is even more problematic (Hunter 2003a; Stanwell-Smith *et al*. 2003). Since surveillance systems can measure various disease outcomes, it is important that officials be clear about the disease that is to be surveyed. This requires a case definition. Case definitions can be based on laboratory-confirmed diagnoses or disease symptoms. In a symptom-based surveillance system, cases of possible

waterborne diseases will be included in the statistics gathered for gastroenteritis. The emphasis on gastroenteritis is appropriate, since these are the most common symptoms of waterborne disease. However, a focus on gastrointestinal illness ignores respiratory infections, eye, ear, throat, and skin irritations, and systemic disorders that may be zoonotic and transmitted by water. Surveillance can include the entire population or select subgroups within the population. Surveillance may also consider infections among a specified subgroup. However, this can be quite expensive, and many infections are not readily diagnosed. Outbreaks as well as cases can be the principal outcome of interest in a surveillance system, and waterborne outbreak surveillance is currently being conducted in several countries (Stanwell-Smith *et al*. 2003).

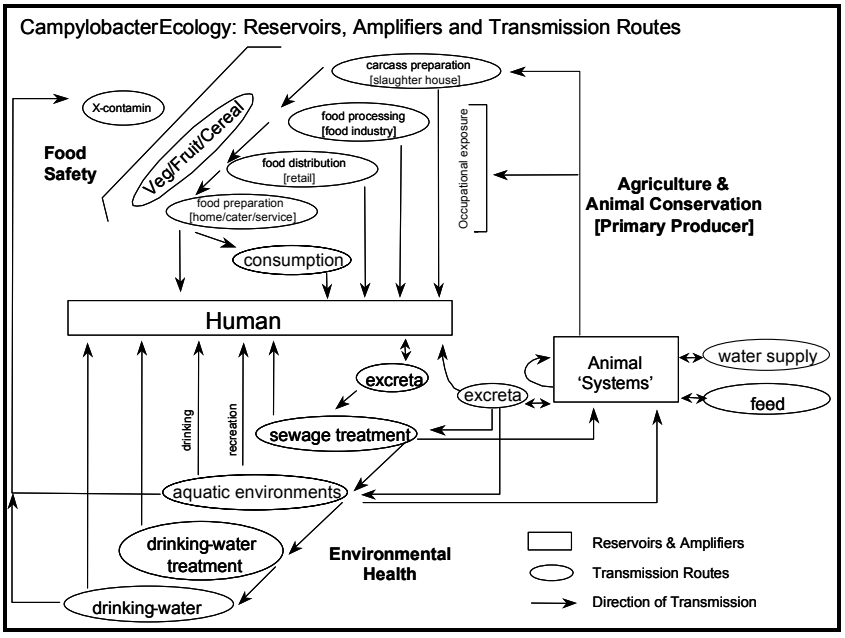

Figure 10.3. A risk model for *Campylobacter* (from New Zealand Ministry of Health and Dr A. Hudson, Environmental Science Research).

Surveillance can be further classified as local, national, and international, and it is important to describe the objectives of the surveillance. For example, the primary purpose of local and national surveillance may be to detect outbreaks early enough to implement control measures to prevent further disease. Another purpose may be to identify patterns of disease or measure the effectiveness of prevention and control programmes (Hunter 2003a). Collection of additional

information about cases (e.g., age, gender, residence, and risk factor information) can help officials better interpret the surveillance information, but privacy concerns may limit the information that can be collected. Water quality information may also assist officials in interpreting the surveillance information (Morris *et al*. 1996), and information about host factors can also be important, especially in areas with high levels of malnutrition, immune deficiency, or significant mortality from waterborne pathogens. International surveillance is important to warn of the potential spread of an ongoing epidemic, identify outbreaks among travellers, detect emerging pathogens, and recognize potential future global problems (Hunter 2003b).

Figure 10.4 illustrates the patterns of disease that may occur in a community and how the sensitivity of surveillance can affect the detection of outbreaks and disease patterns. The outbreak (and endemic or sporadic case) detection level will vary depending upon the agent, type of surveillance activity, and available resources. Although certain surveillance activities may be more sensitive in detecting outbreaks or cases, outbreak investigations and epidemiological studies are still required to evaluate the waterborne transmission risks. The surveillance must provide information early enough to enable investigators to respond quickly and take appropriate action.

Evidence of waterborne transmission of endemic or sporadic diseases in the absence of a detected outbreak requires large-scale, complex epidemiological studies conducted by a multidisciplinary team of investigators. Analytical epidemiological studies can provide a quantitative estimate of waterborne risk that can serve as a benchmark for risk assessment modelling and calculations. Recent studies have identified endemic waterborne gastroenteritis risks in some locations (Payment *et al*. 1991, 1997; Schwartz *et al.* 1997, 2000; Schwartz and Levin 1999), but not in others (Colford *et al*. 2001; Hellard *et al*. 2001).

Historically, for many developed countries, waterborne disease was originally a major health problem. Partly as a result of improved water and sanitation in these countries as part of their development, waterborne surveillance activities in these countries have received a low priority. In developing countries, surveillance activities may not exist. The potential for increased transmission of zoonotic disease with the emergence of such zoonotic agents as *Cryptosporidium*, with oocysts resistant to some water disinfectants, and such highly infective agents as *E. coli* O157:H7 has raised questions about the need to improve surveillance activities in all countries. Improving surveillance, conducting epidemiological studies to assess the burden of waterborne, foodborne, and other transmission risks for zoonotic agents, and identifying sources of contamination can lead to appropriate control strategies.

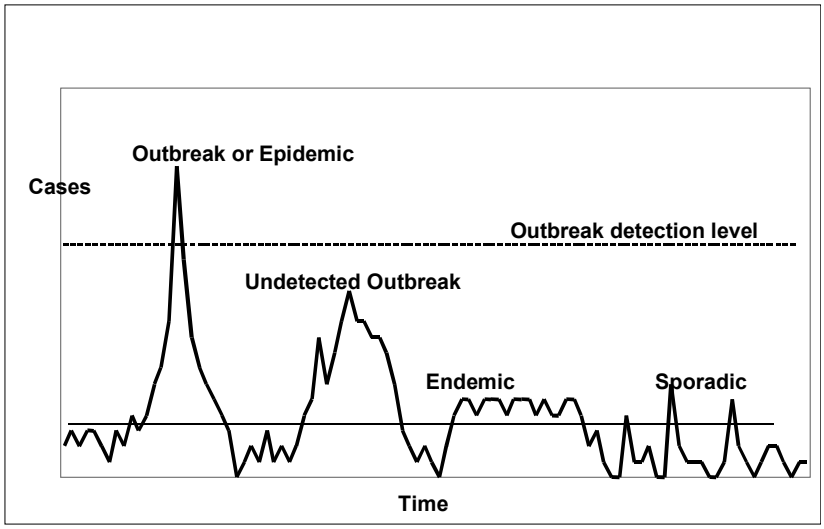

Figure 10.4. Epidemic versus endemic disease (adapted from Frost *et al*. 2003).

10.3.1 Waterborne disease surveillance

Waterborne disease outbreak surveillance can help identify important waterborne zoonotic agents, sources of contamination, and water system deficiencies. Much of what is known about the epidemiology of waterborne disease comes from studies of outbreaks. Outbreaks have provided the best evidence that a particular disease can be transmitted by water. They have also provided information about failures in water treatment and distribution and sources of contamination for source and recreational waters. Outbreaks, however, cannot provide a true measure of the waterborne disease burden. Outbreaks of waterborne disease are regularly detected only in those countries with surveillance systems. Many outbreaks go unrecognized; even when outbreaks are detected, investigations often do not identify all of the cases that may have occurred, especially from secondary transmission. In addition, as previously discussed, endemic and sporadic cases of illness may be due to waterborne exposures, and little information is currently available about these risks.

The sensitivity of surveillance activities to detect outbreaks, the investigative response, and reporting requirements will largely determine how many outbreaks are included in the surveillance system and the amount of information that is available for analysis. Outbreak detection has been improved by several enhanced surveillance activities, including designation of an outbreak

coordinator who routinely contacts health units, physicians, and clinical laboratories about cases; frequent, routine computer analyses of cases and laboratory reports; gastroenteritis surveillance in sentinel populations; and monitoring sales of antidiarrhoeic medications. Not all of these methods will be effective in all locations (Frost *et al*. 1995, 2003; Quigley *et al*. 2003; Stanwell-Smith *et al*. 2003), and the timely investigation by a multidisciplinary team (e.g., epidemiologist, engineer, water quality specialist) with appropriate laboratory assistance will be necessary to obtain complete information about the cause of the outbreak (Craun *et al*. 2001).

In the USA, waterborne disease outbreak surveillance has been conducted by the Centers for Disease Control and Prevention and the Environmental Protection Agency since 1971 (see chapter 8). The current surveillance system in the United Kingdom was established in the early 1990s (Stanwell-Smith *et al*. 2003). The Communicable Disease Surveillance Centre (CDSC) maintains surveillance data for England and Wales, and the Scottish Centre for Infection and Environmental Health maintains surveillance for Scotland. An improved waterborne outbreak surveillance system in Sweden was established in 1980 (Stanwell-Smith *et al*. 2003). In England and Wales, surveillance is almost entirely based on laboratory-confirmed diagnoses. Someone with diarrhoeal disease presenting himself or herself to a family doctor is likely to have a stool sample taken. This sample is also likely to be screened for a wide range of pathogens. In the USA, it is much less likely that a person will visit a physician for diarrhoeal disease and have a stool sample taken. Even though the sensitivity of the surveillance systems may vary, waterborne outbreak data from the USA, the United Kingdom, and Sweden illustrate the change in the epidemiology of reported waterborne outbreaks and identification of new etiological agents. The last three decades have seen a dramatic increase in reported outbreaks associated with zoonotic agents, especially *Cryptosporidium*, *Giardia*, and *Campylobacter*. These pathogens were identified as agents of disease only during the late 1970s, and they would not have been identified before then even if they were a significant cause of waterborne disease. In the USA and England and Wales, *Cryptosporidium* was the agent most frequently identified; in Sweden, *Campylobacter* was the most frequently identified cause of waterborne outbreaks (Stanwell-Smith *et al*. 2003).

10.3.2 Veterinary surveillance

Very few countries have a surveillance system where outbreaks of animal infection and disease are identified at a local or national level and reported to health authorities. Such a notification system is an apparent necessity if any true assessment of waterborne disease from zoonotic agents is to be comprehensive.

In England and Wales, *Cryptosporidium* surveillance data have been collected for more than 10 years by both the CDSC and the Veterinary Service (Nichols 2003). The CDSC *Cryptosporidium* surveillance data show a clear seasonal trend for laboratory-confirmed cases of cryptosporidiosis, with peaks in the spring and late summer/autumn. The spring increases may be due to direct or indirect exposure to oocysts derived from newborn lambs and calves that are frequently infected with *Cryptosporidium*, as the incidents of cryptosporidiosis reported by the Veterinary Service show similar trends, especially with sheep, whereas the late summer/autumn increase may reflect infection while travelling abroad (Nichols 2003).

10.4 INTERNATIONAL COLLABORATION

There is great variation in both the nature of surveillance systems and reported waterborne disease problems from country to country. This is largely due to differences in surveillance and reporting. With increasing population migration, ease of international travel, and movement of food products from country to country, international surveillance becomes more important to alert officials about outbreaks in travellers, impending epidemics, and emerging zoonotic pathogens. The statistics may warn of potential animal husbandry, drinking-water protection and treatment, or food production and preparation practices that should be avoided. Close collaboration is important to ensure that international surveillance systems have a common basis for comparison purposes and that the information is readily shared. Because of the possibility for confusion and misunderstanding, the principles of any international surveillance system should be agreed upon (Hunter 2003b). The principles for the European Enter-Net System were recently published. Since international efforts are no better than the component national systems, considerable efforts will need to be made to bring many national systems up to an acceptable minimum effort.

10.5 CONCLUSIONS

The percentage of diarrhoeal and other diseases attributable to contaminated water or waterborne zoonotic agents is largely unknown, because many countries do not have effective surveillance systems to detect waterborne disease. Even in countries with surveillance systems, outbreak investigation activities have frequently been unable to identify sources of infection and etiologic agents. In addition, few countries have conducted epidemiological studies to assess the burden of endemic waterborne disease risks. Although outbreaks probably represent a small proportion of the cases of waterborne zoonotic disease, local surveillance systems should be designed to inform

officials of an outbreak. Investigative responses should be timely and adequate to identify mistakes that need to be corrected. Information from a national waterborne outbreak surveillance system can lead to improved public health protection and help assess the adequacy of current practices and regulations.

National and international surveillance can help identify new and emerging threats. Surveillance activities focus on disease or symptoms of disease, and officials should consider the collection of additional information or integration of other information (e.g., water quality data) to help identify possible associations of disease with water exposures. The follow-up of suspected associations by appropriate analytical epidemiological studies can provide a quantitative assessment of the endemic waterborne zoonotic risks and a benchmark for microbial risk assessment modelling.

In view of the importance of zoonotic and other waterborne diseases that have been identified in those countries with surveillance systems, the absence of local and national surveillance systems seems unacceptable. The value of surveillance and epidemiological studies is that they can lead to improvement in the health and productivity of the population. The greatest health improvements can be made in the developing world, but populations in developed countries can also benefit from surveillance and epidemiological follow-up. Surveillance and epidemiological information provided about zoonotic agents can lead to changing systems of animal husbandry, water source protection and treatment, and food production, reducing the disease burden and increasing economic well-being.

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11

Zoonoses in Scotland — food, water, or contact?

W.J. Reilly and L.M. Browning

11.1 INTRODUCTION

In Scotland, zoonoses form a significant disease burden, with some of the highest reported rates in the world; in a human population of 5.1 million, the 229 laboratory-confirmed cases of *Escherichia coli* O157:H7 in 2002 represent a rate of 4.5/100 000. This is the approximate average rate for the last 10 years and 2–3 times that reported in England and Wales for the same period. Zoonoses are those infections we share with animals, and the role of humans in their maintenance and spread should not be underestimated

The most important of the zoonoses, both numerically and clinically, have farmed animals and birds as the major reservoirs from which human infection occurs through direct and indirect routes. As of December 2002, there were 1.9

 Waterborne Zoonoses: Identification, Causes and Control. Edited by J.A. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D.O. Cliver, G.F. Craun, R. Fayer, and V.P.J. Gannon. Published by IWA Publishing, London, UK. ISBN: 1 84339 058 2.

million cattle (dairy and beef), 5.5 million sheep, 0.9 million pigs, and 15 million poultry in Scotland (Scottish Executive Environment and Rural Affairs Department 2003a). These are not equally distributed; for example, the main cattle-rearing (including dairy), sheep, and pig production areas are in the South West region, with the least sheep in the North East and the most poultry in the South East (Figure 11.1, Table 11.1). These distributions do not directly correlate with the occurrence of human infection with zoonoses.

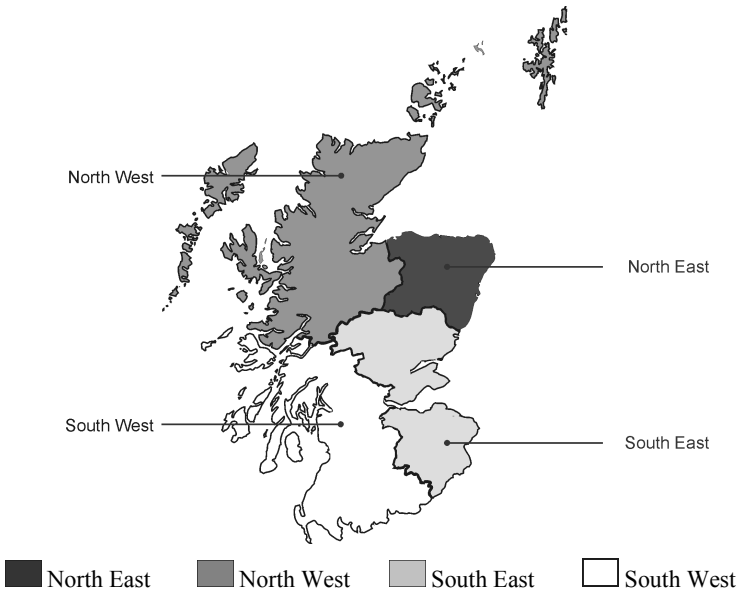

Figure 11.1. Main agricultural areas in Scotland.

Table 11.1. Distribution of livestock in Scotland, 2002

Agricultural area	Cattle	Sheep	Pigs	Poultry
North East	370 000	575 000	153 000	2 340 000
North West	234 000	1 221 000	291 000	245 000
South East	383 000	1 564 000	17 000	9 190 000
South West	914 000	2 100 000	489 000	2 518 000

Many rural parts of Scotland are not supplied by public water supplies, and these private water supplies may be untreated or inadequately treated, leading to opportunities for human infection in the resident or transient (tourist) population. In 2001, it was estimated that there were 18 500 private water supplies serving 83 900 people, and a further 62 600 people had access to

private water through hotels, campsites, etc. (Scottish Executive Environment and Rural Affairs Department 2001). There is a concentration of private supplies in the north-east (Figure 11.2). Not surprisingly, there are fewer in the most heavily populated areas in the central belt around the cities of Glasgow and Edinburgh. However, some of the largest unfiltered supplies also serve parts of this central belt population. Not all public supplies are treated to the same standard, and many do not include an integral filtration system capable of removing parasites such as *Cryptosporidium* spp. and *Giardia* spp.

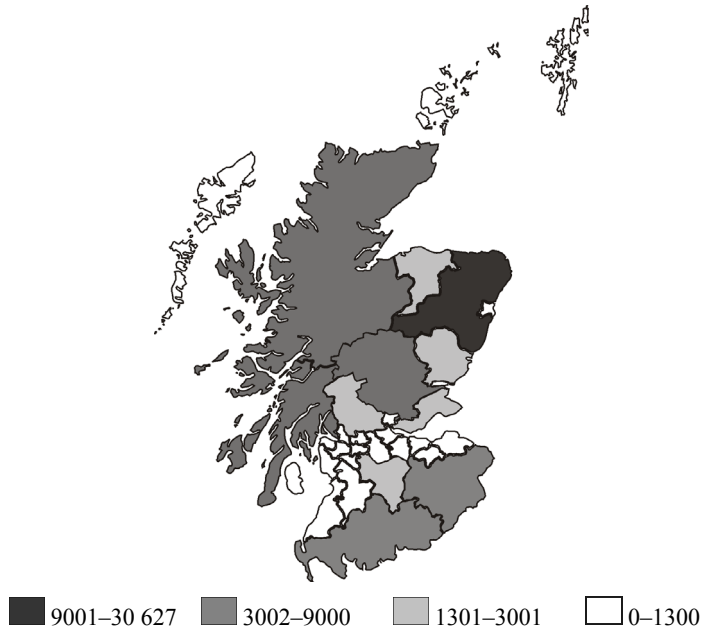

Figure 11.2. Distribution of private water supplies in Scotland.

11.2 ZOONOSES IN SCOTLAND

More than 15 zoonotic diseases are or have been regularly reported in Scotland (Table 11.2).

The most numerically significant of these are the gastrointestinal infections of campylobacteriosis, salmonellosis, cryptosporidiosis, giardiasis, and *E. coli* O157. The others, while of importance to the individual, do not constitute a major numerical threat. Of this second group, only leptospirosis is likely to have a waterborne route of transmission, and this is now an uncommon infection, probably as a consequence of vaccination in the dog and cattle populations. In

addition, occupational exposures such as in sewer workers are now less common following improved occupational hygiene.

Table 11.2. Laboratory-confirmed zoonoses in Scotland, 1998–2002

	1998	1999	2000	2001	2002
Cryptosporidium spp.	860	598	867	569	646
Giardia spp.	360	296	281	251	207
Campylobacter spp.	6381	5865	6482	5435	5121
Salmonella spp.	2109	1879	1720	1571	1149
E. coli O157	217	294	197	235	229
Lyme disease	11	7	37	28	85
Q fever	9	14	6	2	6
Leptospirosis	1	0	0	0	3
Listeriosis	14	7	11	15	20
Toxoplasmosis	19	24	20	16	32
Toxocariasis	0	0	0	0	0
Psittacosis	7	2	10	2	10
Tuberculosis[a]	11	5	9	2	0
Brucellosis	1	0	0	0	1
Hydatid disease	0	1	0	0	0

[a] *Mycobacterium bovis.*

11.3 SURVEILLANCE

Information on zoonoses is gathered through a variety of routes, including statutory notification, routine laboratory reporting, outbreak investigation, and research studies. Each of these brings a different perspective on the size and nature of the problem and is important to understanding the epidemiology of zoonoses. The major routes of human infection with zoonoses may vary from organism to organism and can be reflected in, for example, the different seasonal distributions. Waterborne infection plays a largely unquantified part in the epidemiology of most of the major zoonoses.

11.3.1 Statutory reporting

None of the five major zoonoses is a notifiable disease in people or animals in Scotland. While "food poisoning" is notifiable, there is no definition of "food poisoning" in the legislation. In the context of "food poisoning," food includes water. Any of the five major zoonoses could be identified as "food poisoning"

but would not be differentiated within the data. Thus, the useful epidemiological information available from notifications is limited.

Three zoonoses are notifiable: leptospirosis, Lyme disease, and toxoplasmosis (Table 11.3). This improves enumeration of the clinical problem but adds little to the understanding of the epidemiological picture other than reinforcing the relatively uncommon nature of these infections, and it is insufficiently sensitive to enable timely intervention.

Table 11.3. Statutory reporting of zoonoses in Scotland, 1998–2002

Year	Food poisoning	Leptospirosis	Lyme disease	Toxoplasmosis
1998	9186	4	11	3
1999	8517	0	11	1
2000	9263	3	27	1
2001	8525	2	17	1
2002	7685	2	44	3

11.3.2 Laboratory reporting

Routine reports from diagnostic laboratories provide the mainstay of surveillance of infections (including zoonoses). Weekly reports are received from all diagnostic and reference laboratories, giving a comprehensive overview of the numerical importance of each of the pathogens (http://www.show.scot.nhs.uk/scieh). This has been in place since the late 1960s and allows trends to be followed. Laboratory reporting also acts as an early warning system for emerging infections and incidents (Figure 11.3).

These data underpin our understanding of the epidemiology of the zoonoses. The biggest changes have occurred with the increase in *Campylobacter* infections and also the rise and subsequent fall in *Salmonella* infections. The descriptive information available from routine reporting also allows comparisons to be made and differences identified and assists in understanding the epidemiology of the different infections (e.g., the seasonal distribution). Over a 10-year period, the seasonality of the five major zoonoses is quite different. In Figure 11.4, for each of the zoonoses, the actual incidence for every 4-week period is compared with the 4-week average and the variation recorded as a percent deviation.

Cases of cryptosporidiosis peak earliest in the year, followed by campylobacteriosis, salmonellosis, and then giardiasis. *E. coli* O157 has a much more extended summer peak when the single large outbreak in 1996–1997 (500 cases) is excluded from the data set. *Salmonella* spp., *Campylobacter* spp., and *Cryptosporidium* spp. are more likely to cause clinical disease in animals during pregnancy and in neonates. Calving in the dairy herd occurs throughout the year in Scotland but peaks in the spring. The majority of beef cattle calve in the

spring, with a smaller peak in the autumn. Lambing is an extended period of some 6 months, with lowland flocks lambing as early as December, upland flocks from February/March, and hill flocks as late as May/June. With this complex pattern, it is difficult to correlate the different peaks of human infection with seasonality of calving and lambing.

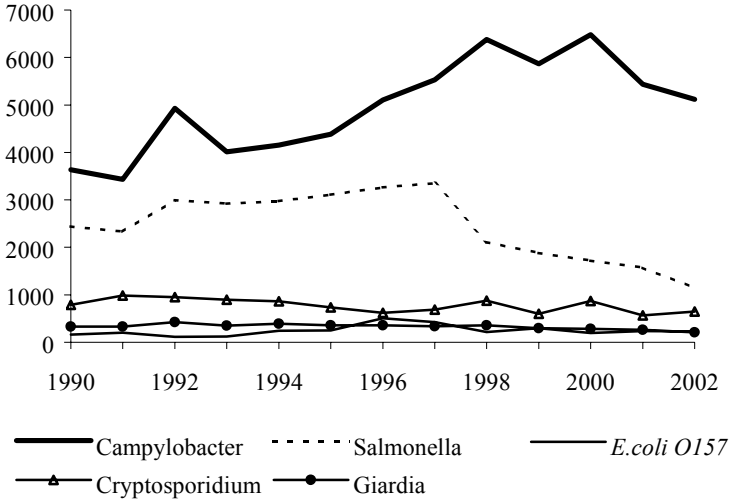

Figure 11.3. Zoonoses in Scotland, 1990–2002, laboratory-confirmed infections.

11.3.2.1 Salmonella

All isolates of *Salmonella* spp. are routinely typed in Scotland. This means that trends over time for different sero and phage types can be followed. Salmonellosis peaked in 1997 and has fallen by approximately 70% since then. The rise and subsequent fall were largely due to the emergence and decline in *S.* Enteritidis PT4, which was associated with foodborne infection through poultry meat and eggs (Figure 11.5) (SCIEH 2003).

While *S.* Typhimurium infections have also declined during this period, the epidemiology of two different phage types (DT104 and DT204c), both associated with infection in cattle, has been different (Figure 11.6).

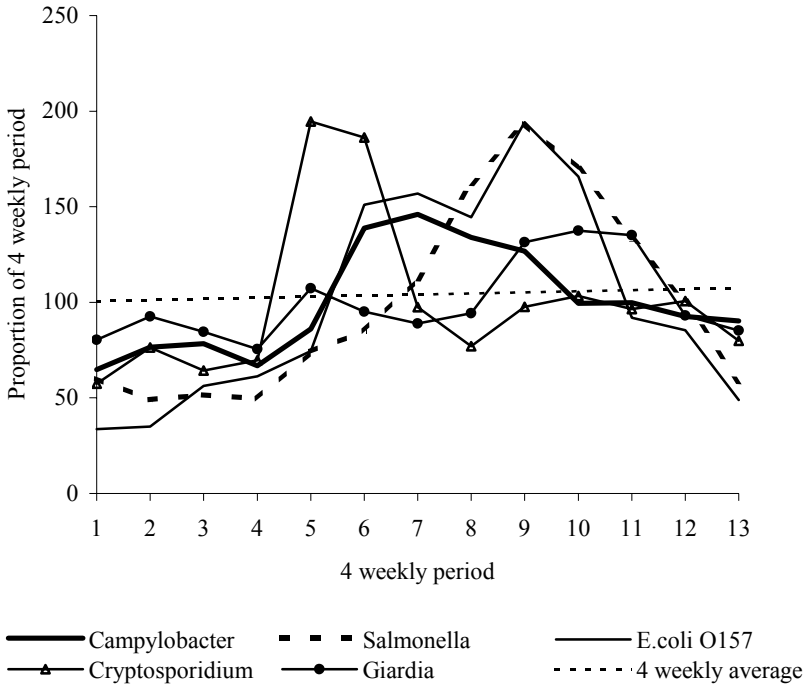

Figure 11.4. Zoonoses in Scotland, seasonal distribution, 1993–2002 (excludes data for *E. coli* O157 for 1996–1997).

S. Typhimurium DT104 caused considerable human infection during the 1990s. This was associated with infection in cattle initially, although it has subsequently spread to other animal species. The two epidemic curves were very similar. This organism causes infection and disease in cattle of all ages and was thus able to enter the food-chain when animals were slaughtered.

In contrast, *S.* Typhimurium DT204c was primarily an infection in younger animals and was therefore less likely to enter the human food-chain. There were considerably fewer human cases of infection with DT204c, and the epidemic curve in humans was much later and of much smaller size than that in cattle. These infections were much more likely to occur in people in direct contact with cattle. Although the total numbers of animal cases of DT104 and DT204c were very similar, the difference in human cases confirms that the route of exposure can have a significant effect on the number of human cases (Department for Environment Food and Rural Affairs 2000).

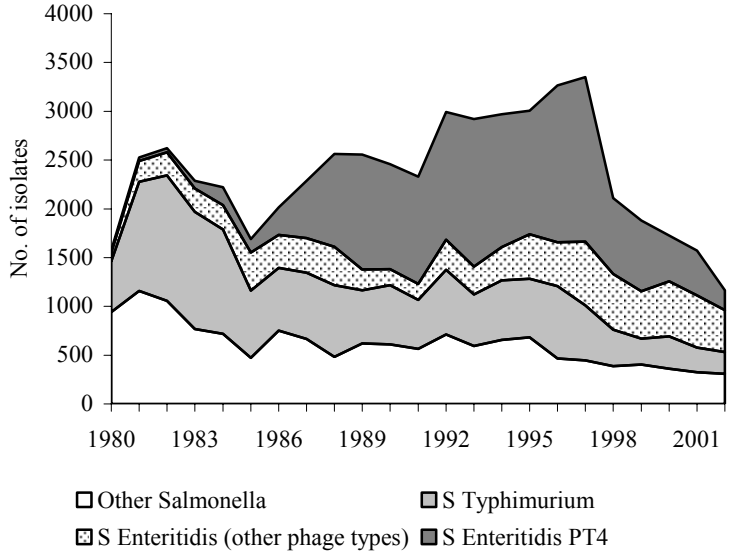

Figure 11.5. *Salmonella* isolates in Scotland, 1980–2002.

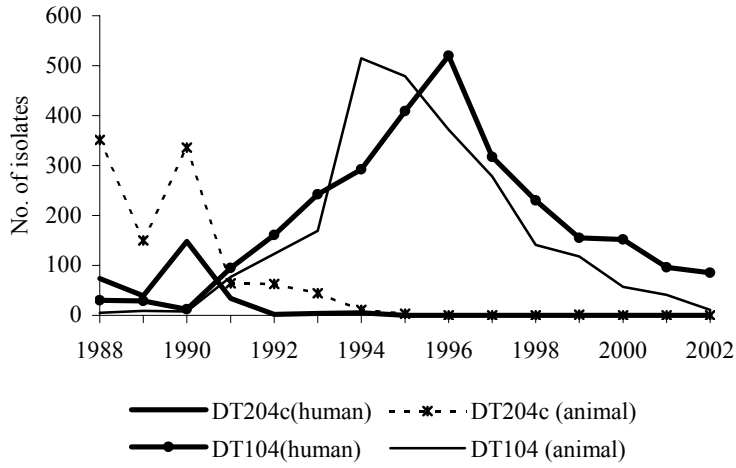

Figure 11.6. *S.* Typhimurium DT104 and DT204c in Scotland, 1988–2002.

There are significant differences in the rates of human infection with *Salmonella* spp. in different parts of Scotland, with the highest rates in the North East agricultural region (Figure 11.7). While some of the difference may be due to different protocols for collection of human stool samples and laboratory testing, it is also probable that the differences reflect different exposures.

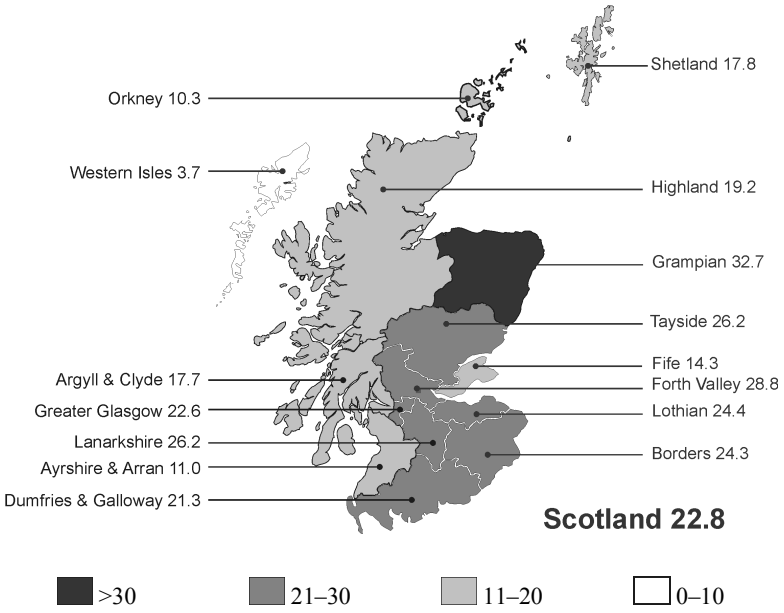

Figure 11.7. *Salmonella* spp. isolates from humans in Scotland, 2002, rates per 100 000.

While rates tend to be higher in areas with greater numbers of private water supplies, there is no clear association with livestock intensity. There is no direct correlation between the geographical distribution of human cases and the distribution of animal populations — i.e., the greatest cattle populations are in the South West, but the highest rate of human infection is in the North East.

Human exposure to *Salmonella* also occurs through direct contact with infected animals and their environment, as demonstrated in one study of the urban and rural parts of the South West agricultural region (Calvert *et al.* 1998). Environmental contamination such as pollution of water supplies is likely to play a significant part in the local spread of these infections between animal populations.

Improving surveillance and data collection have recently demonstrated that up to 20% of cases of salmonellosis in Scotland occur in travellers, and this contributes significantly to the late summer peak (see Figure 11.4).

11.3.2.2 Campylobacter

No routine typing of *Campylobacter* is currently undertaken in Scotland, as the available typing schemes, whether phenotypic or genotypic, have not added any benefit for public health purposes. Data are therefore available only on overall trends (see Figure 11.3). The majority of isolates from humans are *C. jejuni*, with *C. coli* accounting for approximately 5%. The number of confirmed infections has risen almost annually since the early 1990s, and only in the last 2 years has any reduction been observed.

As with *Salmonella* infections, there is considerable geographical variation, with the highest rates again seen in the North East agricultural region and major differences between the major conurbations of Greater Glasgow (59/100 000) and Lothian (134/100 000) (Figure 11.8).

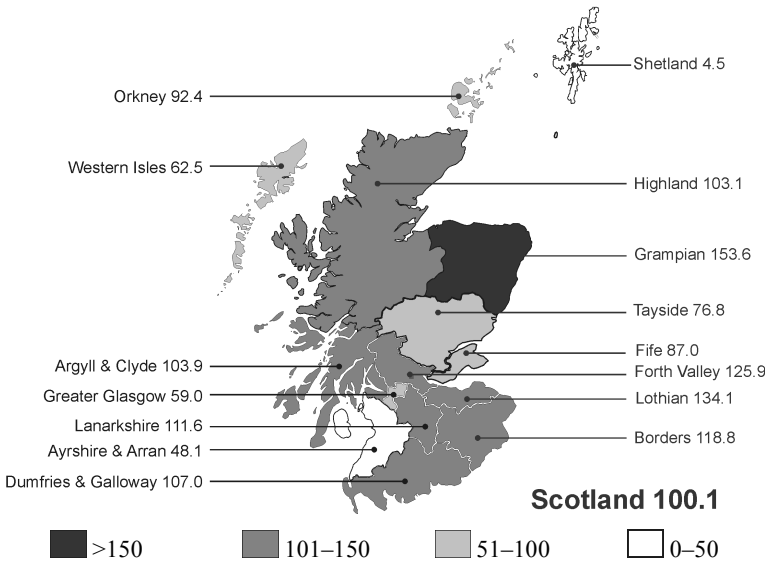

Figure 11.8. *Campylobacter* spp. isolates from humans in Scotland, 2002, rates per 100 000.

Few outbreaks of *Campylobacter* infection are reported, with most human cases appearing to be sporadic and not associated with any outbreak, which makes investigation of the source very difficult.

Campylobacter spp. are regularly isolated from livestock in the United Kingdom. Recent studies have shown faecal carriage of 13% *C. jejuni/coli* in cattle and 16% in sheep, compared with 87% in pigs (Newell 2000).

Comparison of strain types does show overlap between the strains in animals and those in humans.

Poultry meat (but not eggs) has been implicated as a major source of human infection with *Campylobacter* spp. (Food Standards Agency 2001a). A study published in 2002 by the Food Standards Agency demonstrated that almost 90% of fresh poultry on sale in Scotland was contaminated with *Campylobacter*, with *C. jejuni* accounting for 75% of the isolates and *C. coli* 25% (http://www.foodstandards.gov.uk/multimedia/webpage/111802). By implication, poultry is regarded as the major source of *Campylobacter* for humans. This hypothesis is supported by the fall in human cases that occurred in Belgium following the reduction in consumption of poultry meat as a result of the withdrawal of poultry meat because of concerns over contamination with dioxins (Vellinga and van Loock 2002). Reducing the levels of *Campylobacter* spp. in poultry presented for slaughter is currently the focus for strategic intervention by the Food Standards Agency. A major part of this strategy is the improvement of biosecurity on the poultry farms, including further exploration of the role of poultry water supplies in spreading the infection within and between farms (Food Standards Agency 2003).

11.3.2.3 E. coli O157

There are fewer cases of *E. coli* O157 than of the other enteric zoonotic infections, although the clinical severity can be much greater, with development of systemic complications such as haemolytic uraemic syndrome and the associated fatalities. Although the number of confirmed cases appeared to rise during the 1980s and peaked with a single foodborne outbreak in 1996–1997, this was largely due to improved laboratory diagnosis and ascertainment (Figure 11.9). There is an underlying rate of infection at about sporadic 200 cases per year, on top of which the cases from a variable number of outbreaks are imposed.

All isolates from humans, animals, food, and the environment are typed by the Scottish *E. coli* Reference Laboratory. Phage types have changed significantly over the last 15 years. Whereas phage types 2 and 49 predominated in the 1980s and were involved in the majority of outbreaks (meat and milk), phage type 21/28 now accounts for some 70% of human cases and is the most commonly identified type from the extensive surveys carried out in livestock.

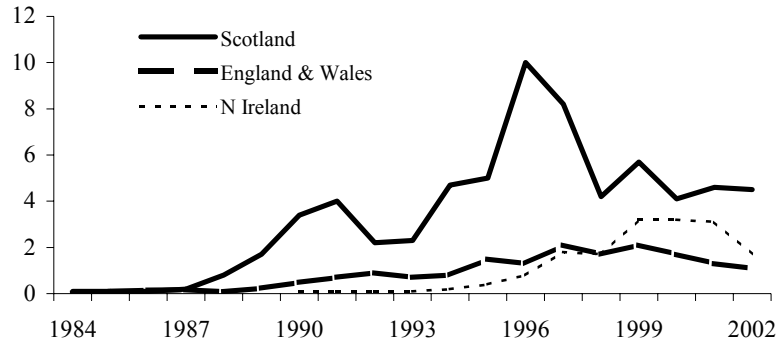

Figure 11.9. *E. coli* O157 isolates from humans in the United Kingdom, 1984–2002, rates per 100 000.

Recent investigations in cattle have demonstrated the phenomenon of "high shedders," which may be responsible for maintaining and spreading infection to both animals and humans, and at least one site of colonization in cattle at the recto-anal junction (Gally *et al.* 2003). This finding will now facilitate the identification of strategies to reduce carriage by livestock.

There is a significant difference between incidence of infection with *E. coli* O157 in Scotland and that in other parts of the United Kingdom that cannot be accounted for by differences in laboratory practices, surveillance systems, or infection rates in livestock (see Figure 11.9).

Such differences must reflect different exposures. There appear to be few differences in food habits between Scotland and other parts of the United Kingdom that could explain this variation. One hypothesis has been the difference in the number of untreated or inadequately treated water supplies, but this was not confirmed by the Scottish case–control study.

Very few cases of serotypes other than O157 are identified in humans, in contrast with what is found in many other parts of Europe — e.g., Italy, where serogroup O26 is the most common cause of haemolytic uraemic syndrome in children (Scheutz 2003). This appears to be a real difference, since, despite active case searching, only small numbers of other serogroups are found. It is not clear why this should be the case, given that other serogroups of verocytotoxigenic *E. coli* such as O26 have also been found in the cattle surveys.

There is significant geographical variation in rates of infection within Scotland (Figure 11.10).

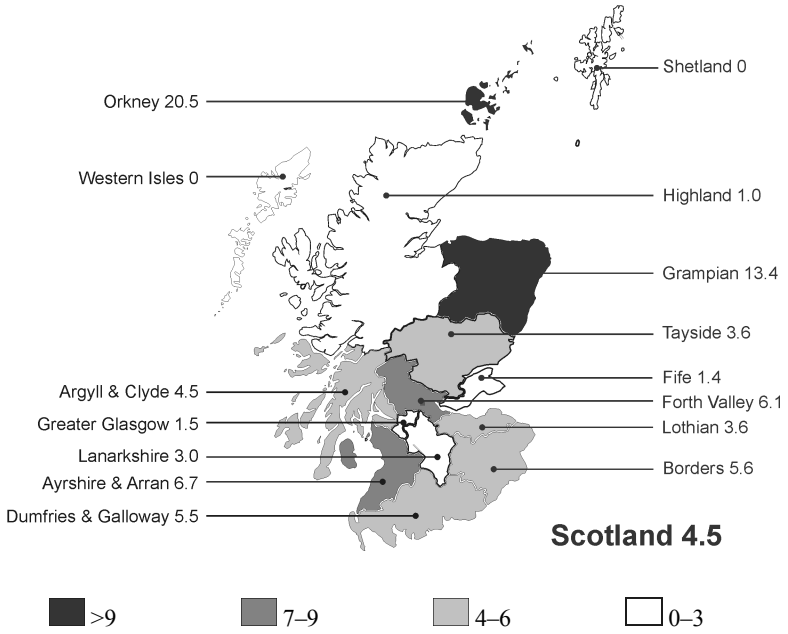

Figure 11.10. *E. coli* O157 isolates from humans in Scotland, 2002, rates per 100 000.

Studies in livestock in Scotland and the United Kingdom confirm that approximately 25% of cattle herds have animals excreting *E. coli* O157 of the same phenotypes and genotypes present in humans (Jenkins *et al.* 2002). Cases and outbreaks in people are always investigated; where there is an animal link, the livestock and environment are also examined. In both sporadic cases and outbreaks, an indistinguishable organism has been found in both the human and animal samples (including water). Most often, cattle are associated with human infection either by direct contact or through water, but sheep and occasionally horses have been involved. Some of these exposures have been through direct contact with the livestock and their environment, and some through water.

In recognition of the problem, the Scottish Executive established an *E. coli* O157 Task Force that made over 100 recommendations (Food Standards Agency 2001b), most of which were accepted by the government (Scottish Executive Environment and Rural Affairs Department 2003b). There is an active programme to ensure that appropriate public health advice is made available to those at risk. This includes agencies and departments such as health and agriculture, but also health and safety, education, and environment.

11.3.2.4 Cryptosporidium

Actual or potential contamination of water supplies with *Cryptosporidium* has been a major issue in recent years in Scotland, leading to considerable overhaul of many treatment facilities.

Most human cases are diagnosed by microscopy, and there is at present no routine further identification or typing other than by morphology. It is likely that improved laboratory diagnostics will be implemented within the coming year that will at the very least differentiate the different species. What has until recently been regarded as *C. parvum* is in fact several different species and in humans includes both *C. hominis* and *C. parvum*. These were previously thought to be both *C. parvum* Type 1 and Type 2, respectively. Type 1 is primarily but not exclusively recovered from humans, whereas Type 2 has been found in most livestock species (McLauchlin *et al.* 2000). Other species of *Cryptosporidium* are found in animals (e.g., *C. andersoni*) but are not thought to be human pathogens.

In a pilot project in Scotland, to differentiate strains of *C. parvum*, more than 1100 isolates from humans and animals were genotyped to the species level. Of these, some 295 human and 2 bovine isolates were Type 1 (now *C. hominis*). There were 403 human and 417 *C. parvum* Type 2 (now *C. parvum*) isolates. In the same study, 180 isolates were genotyped using a panel of seven mini and micro satellite markers (Mallon *et al.* 2003). Thirty-eight multilocus genotypes were identified. *Cryptosporidium hominis* comprised seven types in a single group, whereas 31 *C. parvum* isolates formed five groups. Groups 1 and 5 were recovered only from humans, whereas groups 2, 3, and 4 were found in both humans and animals. This study demonstrated that over 40% of human cases do not have an animal origin (*C. hominis*) and that the remainder could be either animal or human, but that even some isolates of *C. parvum* are not zoonotic.

Between 500 and 900 laboratory reports of *Cryptosporidium* are made each year in Scotland. This has not changed significantly in recent years, but can vary depending on the size of any outbreaks.

As with the other enteric zoonoses, there is considerable variation between different parts of the country (Figure 11.11). The highest rates are again reported in the north-east, where the number of private water supplies is the greatest. However, west and central Scotland, where there are “high-risk” water supplies, consistently record the lowest levels of infection.

There is a single public supplier of water in Scotland, and, as part of accountability, the water regulator requires all public water supplies to be assessed for risk (including *Cryptosporidium*) and, where appropriate, routine monitoring of the raw and treated water (Scottish Executive Environment and Rural Affairs Department 2000). This has led to an increasing number of

incidents where the oocysts of *Cryptosporidium* have been found in the final water, resulting in media scares. The lack of ability to differentiate between likely pathogenic and non-pathogenic *Cryptosporidium* makes risk assessment very difficult. Outbreaks of apparently waterborne cryptosporidiosis have been reported when no oocysts have been detected in the water, and no outbreaks occurred when oocysts have been detected.

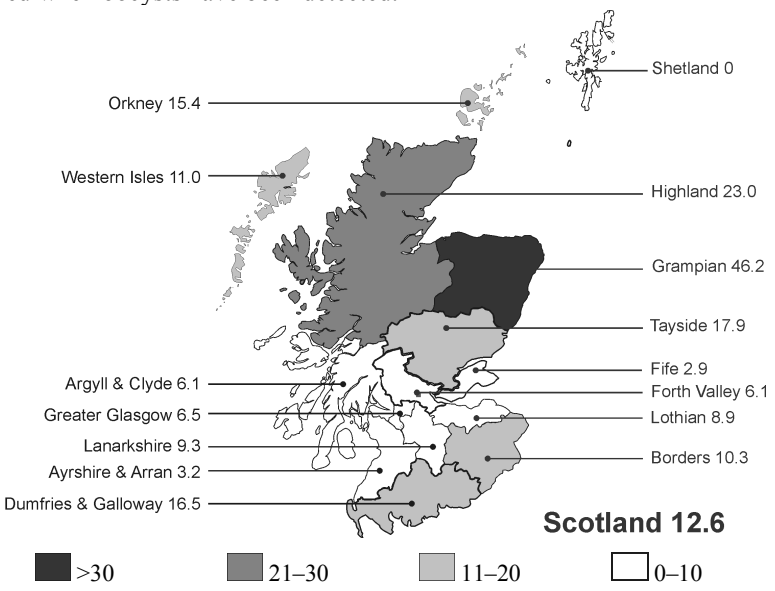

Figure 11.11. *Cryptosporidium* isolates in humans in Scotland, 2002, rates per 100 000.

In the last few years, the importance of recreational water in the epidemiology of cryptosporidiosis has been well documented, particularly involving swimming pools (BBC News 2002). Cases have been reported in returning holidaymakers, where anecdotal reports suggest that many hundreds of people have been infected. In July/August 2003, more than 120 cases in Scotland and a similar number in England were confirmed by laboratories in the United Kingdom, all associated with a single hotel swimming pool (Eurosurveillance 2003). It is most likely, in all of these swimming pool outbreaks, that human cases follow a human "faecal" accident in the pool and others are infected before the filtration plants can remove the parasite.

11.3.2.5 Giardia

Giardiasis is not a common infection reported in Scotland, and the number of laboratory reports has been declining in recent years. In 2002, there were only 207 reports, fewer than those for *E. coli* O157 and only one-third of the number

of reports of cryptosporidiosis. In many other parts of the world, there are more reports of *Giardia* spp. than of *Cryptosporidium* spp. (Heitman *et al.* 2002).

Little is understood about the epidemiology of giardiasis in Scotland. No livestock reservoir has been identified. Scottish Water does not report it in its routine water examinations.

There are again considerable geographical variations, but in this instance, in marked contrast to cryptosporidiosis, the north-east has one of the lowest rates and the south-east the highest (Figure 11.12).

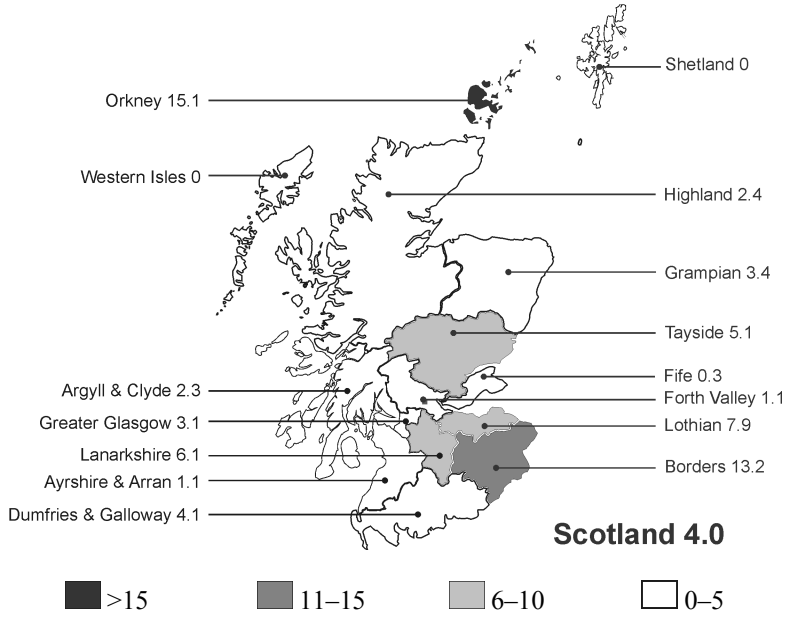

Figure 11.12. *Giardia* spp. isolates from humans in Scotland, 2002, rates per 100 000.

Another major difference between cryptosporidiosis and giardiasis is in the age of the patients affected (Figure 11.13). The proportion of cases in the under 15 years of age group is significantly greater for cryptosporidiosis, while the proportion in all age groups over 20 years of age is greater for giardiasis. Particularly striking is the difference in the under 5 years age group, which accounts for 40% of cases of cryptosporidiosis compared with only 20% of cases of giardiasis.

Approximately 14% of cases of giardiasis are reported as imported infections, compared with approximately 2% for cryptosporidiosis. Of these, about 50% are associated with travel to Asia, which is disproportionate to the number of travellers to that continent.

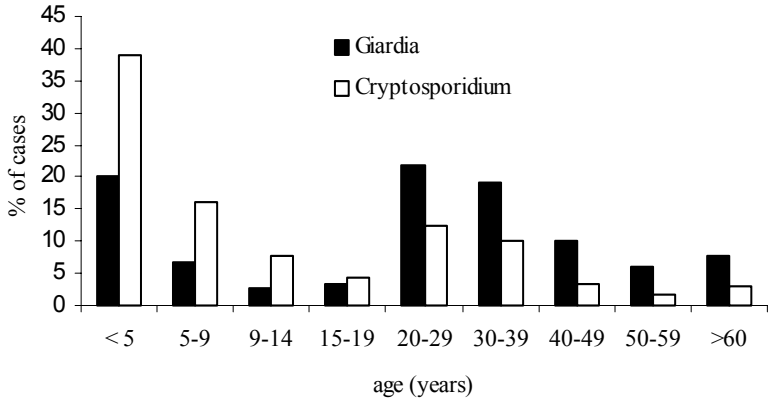

Figure 11.13. Proportions of cases, by age, for cryptosporidiosis and giardiasis, 1988–2002.

The contrast in the descriptive epidemiology between cryptosporidiosis and giardiasis is dramatic and suggests that the epidemiology of the two infections is different. The lack of an identified animal reservoir and the complete absence of any outbreaks of giardiasis indicate that person-to-person spread may be one of the most important routes. It will not be possible to implement controls for giardiasis until the routes of human infection are more clearly established.

11.3.3 Outbreak investigation

Since 1996, standardized data have been collected on all outbreaks of infectious intestinal disease (IID) in Scotland. This has helped estimation of the size of the problem and identification of the causes of outbreaks. Information on outbreaks of the major zoonoses is captured by this surveillance system.

Most case of IID are, however, not part of outbreaks, and the epidemiological information obtained from outbreak investigations may not be directly applicable to non-outbreak routes of infection. The proportion of cases associated with outbreaks varies by organism (Table 11.4).

Table 11.4. Cases of infectious intestinal disease, outbreak related, 1996–2002

	Total laboratory reports	Total from outbreaks	Proportion from outbreaks
Salmonella spp.	15 043	856	5.7%
Campylobacter spp.	39 924	78	0.2%
Cryptosporidium spp.	4 867	588	12.1%
E. coli O157	2 101	550	26.2%

Less than 0.2% of all cases of infection with *Campylobacter* spp. are recorded as part of an outbreak, compared with more than 26% of cases of infection with *E. coli* O157. Even with this latter infection, however, the majority of cases are not outbreak cases. The epidemiology of outbreak cases may be very different from that of sporadic cases, and it may be wrong to extrapolate the epidemiology of outbreaks to all cases. Nevertheless, for most infections, the data on outbreaks are the best that are available.

During 1996–2002, information on a total of 822 outbreaks of IID was gathered. Of these, the majority (425) were reported by the investigators to be associated with person-to-person spread, largely reflecting the increase in Norovirus infections in recent years, which are not thought to be zoonotic (Table 11.5).

Table 11.5. Outbreaks of infectious intestinal disease in Scotland, 1996–2002, by route of spread

Route	Number of outbreaks
Environmental	29
Foodborne	90
Multiple	127
Person to person	425
Water	19
Not known	132

Water was reported to be the major route of transmission in only 19 outbreaks, and these involved *Campylobacter* spp., *Cryptosporidium* spp., *E. coli* O157, and four outbreaks where the agent was not identified but was probably Norovirus and not a zoonosis (Table 11.6).

No waterborne outbreaks of either salmonellosis (from a total of 62 outbreaks) or giardiasis (no outbreaks) were reported during this period.

Table 11.6. Outbreaks of infectious intestinal disease in Scotland, 1996–2002

Mode of transmission	*Salmonella* spp.	*Campylobacter* spp.	*Cryptosporidium*	*E. coli* O157
Environmental	–	1	2	4
Foodborne	45	11	–	15
Multiple	8	–	1	13
Person to person	6	–	1	14
Water	–	6	4	5
Other	–	–	3	1
Not known	3	1	–	3
Total	62	19	11	55

Two recent outbreaks are typical of the type of waterborne outbreak and illustrate the problems of no or inadequate water treatment. These are described in the next two sections.

11.3.3.1 A waterborne outbreak of E. coli O157

In July and August of 2002, an outbreak of *E. coli* O157 PT21/28 occurred involving visitors to a caravan and campsite in the Scottish highlands. In total, 28 people were affected, with six laboratory-confirmed cases and two hospitalized. The campsite was supplied by a private water supply from a spring. This supply also served a small housing development of approximately 40 houses. Only two of the local residents were affected, the remaining cases occurring in the visitors. During investigation of the incident, an indistinguishable organism was isolated from those affected, from water on the campsite, and from water, mud, and cattle faeces from the vicinity of the spring source (Figure 11.14).

The incident was controlled by a "boil water" advisory and ultimately by introducing a piped mains water supply to the site. It is interesting to note the low attack rate in the resident population, who may well have been exposed to previous episodes of contaminated water and developed immunity to *E. coli* O157. The incident followed a period of heavy rain, which not only overwhelmed the rudimentary treatment works but also raised the level of surface water to an extent that surface water flowed into the local holding tank.

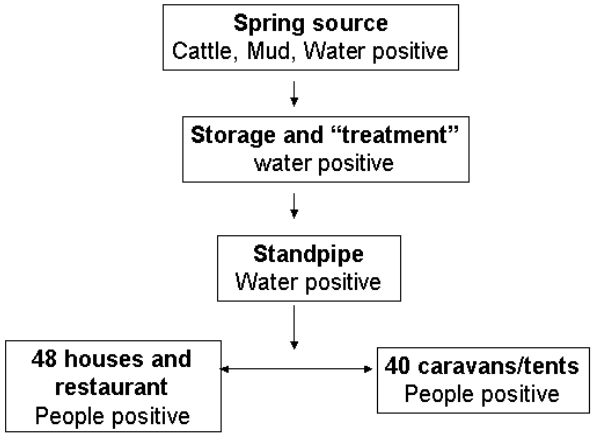

Figure 11.14. Schematic layout of the water supply in an outbreak of *E. coli* O157, 2002.

11.3.3.2 A waterborne outbreak of Cryptosporidium spp.

During a period of almost 4 weeks in May 2000, an outbreak involving 90 cases occurred in the urban population of Glasgow. The water supply to the affected community came from Loch Katrine reservoir through two 42-km-long aqueducts into two parallel storage reservoirs. This water system was constructed during the 1850s, and the water treatment consisted of straining prior to chlorination. The supply served some 700 000 people (Figure 11.15).

The catchment serving Loch Katrine comprises approximately 10 000 ha of hill and upland grazing, with a resident flock of about 8000 breeding sheep and 8000 lambs during May–October. There were very few cattle on the catchment. Sheep not only grazed the hill but also could access the loch side. The aqueducts traverse agricultural ground and were designed to collect water, delivering about 10% more water to the storage reservoirs than left Loch Katrine. This water supply had been associated with previous incidents of cryptosporidiosis and has been classified as high risk. The outbreak occurred after a period of very dry weather followed by very heavy rain. The hypothesis was that the weather conditions facilitated the runoff of oocysts from the land into streams, etc., into the reservoir and/or aqueducts, and from there to the water distribution system. Monitoring the raw and final water for the presence of *Cryptosporidium* did not demonstrate significant numbers of the parasite, although the monitoring was not in place until the incident was nearly over (Greater Glasgow NHS Board Outbreak Control Team 2001).

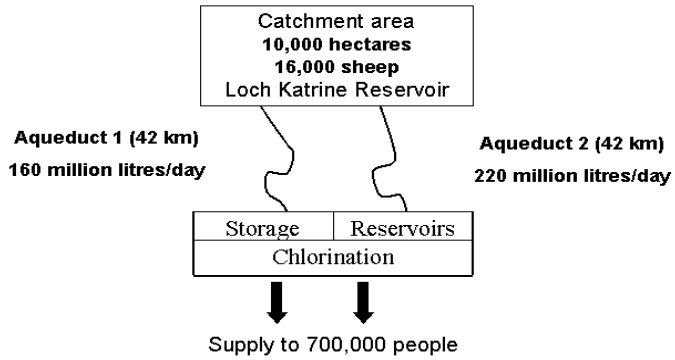

Figure 11.15. Schematic representation of the water supply to Glasgow.

No public health measures were put in place at the time of the incident, other than plans to improve the management of the catchment area to reduce the likelihood of contamination with oocysts. Ultimately, the decision was made by the Water Authority to remove all (16 000) sheep and cattle from the catchment, which was allowed to go fallow. Plans were also drawn up to improve the filtration of the storage reservoirs to a standard to remove *Cryptosporidium*, and it was planned to implement this by 2005.

Removal of the sheep was begun in the spring of 2001, but by 2002 had not been completed. In August 2002, the routine monitoring of water supply to the storage reservoir showed high and rising levels of oocysts (up to 11 oocysts per 10 litres). An incident management team took the decision to implement a "boil water" requirement on the part of the distribution system that could not be fed from other sources (Incident Management Team 2003). This applied to about 170 000 consumers and lasted until the whole supply could be replaced from an alternative source. No human cases of cryptosporidiosis were reported. Subsequent typing of the oocysts recovered from the sampling equipment showed that they were mainly *C. andersoni* and were probably not pathogenic to humans!

The major lessons from these two incidents reinforce the importance of securing the safety of the source but also of confirming the identity of the organism.

11.3.4 Enhanced investigation

In Scotland, a number of systems have been in place since 1996 to gather additional information on cases of *E. coli* O157. These give additional information on exposures, clinical presentation and disease outcomes, travel association, etc. Cases in humans result in follow-up of livestock populations where appropriate (e.g., in August 2003, a single case in a child was associated with infection in 2 of 10 horses in the stable). This continually adds to the knowledge base on which guidance on protecting public health is based.

To investigate the epidemiology of indigenously acquired sporadic cases, a case–control study was carried out. This excluded outbreak, secondary, travel-associated, and mixed infection cases. Over 180 cases and 545 controls were recruited and followed up by a single researcher using a telephone-administered questionnaire (Locking *et al.* 2001). The study was designed to test environmental and water exposures and also included foods as confounders. The most striking finding was the association with animal faeces (Table 11.7).

This study confirmed the importance of contact with animals and their environments in the epidemiology of *E. coli* O157. This finding has been increasingly recognized in other parts of the world.

Table 11.7. Risk factors for sporadic cases of *E. coli* O157, multivariate analysis

Variable	Odds ratio	95% confidence interval	*P*-value
Likely contact with animal faeces (excluding pets)	4.80	2.42–9.48	<0.0005
Contact with animal faeces (excluding pets)	3.65	1.81–7.34	<0.0005
Ate poultry/game	0.53	0.28–0.97	0.041
Contact with raw salad/vegetables	0.48	0.27–0.85	0.012
Drank bottled water	0.28	0.15–0.52	<0.0005

Surprisingly, this study did not find a statistically significant association with private water supplies. While cases were reported, the numbers did not reach statistical significance. Bottled water, in contrast, was inversely associated with infection! No food exposures were significantly associated with increased risk

The findings from this study and the reports from environmental outbreaks have helped to shape the public health policy in Scotland, with targeted intervention and continuing research, particularly in the areas of livestock carriage.

11.4 SUMMARY AND CONCLUSIONS

Zoonoses continue to be a source of human infection in Scotland. Numerically, the five enteric infections of campylobacteriosis, salmonellosis, cryptosporidiosis, giardiasis, and infection with *E. coli* O157 are the most important. The major routes of human infection vary between organisms. Just because an organism is zoonotic, it should not be assumed that all routes derive from animals. This is particularly the case with cryptosporidiosis, where it is likely that human reservoirs may be as important as animal reservoirs.

The foodborne route is probably the most important for campylobacteriosis and salmonellosis, although contact with animals and animal environments also plays a part. With cryptosporidiosis and *E. coli* O157, water is an important element in the transmission cycle, but direct animal contact is particularly relevant. The routes of infection with giardiasis remain largely unknown.

For both cryptosporidiosis and campylobacteriosis, the understanding of the problem is still considerably hampered by limitations of laboratory testing for strain differentiation. There will be little progress until molecular methods for genotyping are sufficiently robust and routinely available.

One of the concerns not addressed in this chapter is that of emerging infection, whether as a candidate for bioterrorism or a newly emerging disease. Water

supplies are particularly vulnerable. Anthrax scares have already occurred. New infections that may be animal derived have appeared or threatened — e.g., avian influenza, severe acute respiratory syndrome. In Scotland, current concerns include West Nile fever virus and the ability of climate change to maintain the life cycle of the intermediate vector. Rabies from bats has recently caused a fatality. Other infections are not yet defined as zoonoses — for example, the link between *Mycobacterium avium* ssp. *paratuberculosis* and Johne's disease in cattle, sheep, and goats and Crohn's disease in humans. If this link is established, this will become a major zoonosis in the United Kingdom. It is estimated that there is a prevalence of about 50 000 cases, with an annual incidence of about 3000–4000. Much of the current interest in this organism is targeted at the food route, particularly milk; given the robust nature of the pathogen, water transmission must be a strong possibility. There is a fundamental issue of asking whether water treatment will be an effective deterrent for certain organisms.

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12

Potential public health risk of *Campylobacter* and other zoonotic waterborne infections in New Zealand

D.G. Till and G.B. McBride

12.1 INTRODUCTION

New Zealand's land area of 250 000 km^2 and temperate climate support a substantial agricultural sector, including extensive and intensive animal husbandry. That sector is a major driver of the New Zealand economy. As of 2002, New Zealand supported a human population of 4 million and a husbanded animal stock of 51 million — including 39.5 million sheep and 9.5 million dairy

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and beef cattle. Dairy cow stock has steadily increased from 3.84 million in 1994 to 5.16 million in 2002, a 34% increase over 9 years; its current rate of increase is approximately 4% per year (Ministry of Agriculture and Forestry 2003). However, the major increase has been in the southern half of the South Island, with an increase of 137% over the 9-year period compared with 17% in the North Island. The dairy industry is the country's second largest earner (after the tourist industry) of overseas revenue (Lynch 2003).

At the same time, diseases that are zoonotic and potentially waterborne are of increasing concern, currently constituting about 80% of the total notified illnesses (Table 12.1). Table 12.1 also shows the predominance of campylobacteriosis, salmonellosis, cryptosporidiosis, and giardiasis in rates of reported diseases. Furthermore, some of these rates have been increasing. In particular, Figure 12.1 shows how annual campylobacteriosis cases have increased since campylobacteriosis was first declared to be notifiable, in 1980.

Table 12.1. Reported rates of potentially waterborne notifiable diseases, 1999–2002

	Rate (cases per 100 000 people per annum)			
Notifiable disease	1999[a]	2000[a]	2001[b]	2002[b]
Campylobacteriosis	225.6	232.5	271.5	334.2
Cryptosporidiosis	27.0	21.4	32.3	26.1
Giardiasis	49.6	46.6	42.9	41.4
Legionellosis	1.9	1.9	1.2	1.4
Leptospirosis	1.6	2.8	2.8	3.8
Salmonellosis	57.4	49.9	64.7	50.0
Typhoid	0.2	0.6	0.7	0.6
VTEC/STEC[c]	1.8	1.9	2.0	2.0
Yersiniosis	13.9	10.9	11.5	12.7
Total (potentially waterborne)	379.0	368.5	429.6	472.2
% Campylobacteriosis	59.5	63.1	63.2	70.7
Total (all sources)	501.7	560.7	545.0	577.9
% Campylobacteriosis	45.0	41.5	49.8	57.8
% Potentially waterborne	75.5	65.7	78.8	81.7

[a] Environmental Science Research (2001).
[b] Sneyd and Baker (2003).
[c] Verocytotoxin (Shiga toxin)-producing *E. coli*.

How much of this increasing disease burden is a consequence of zoonoses that are potentially or actually waterborne is a growing concern. This question is discussed generally, and in particular, as related to campylobacteriosis, the main component of the reported disease burden in New Zealand — where reported

campylobacteriosis is markedly greater than in comparable countries of similar socioeconomic status.

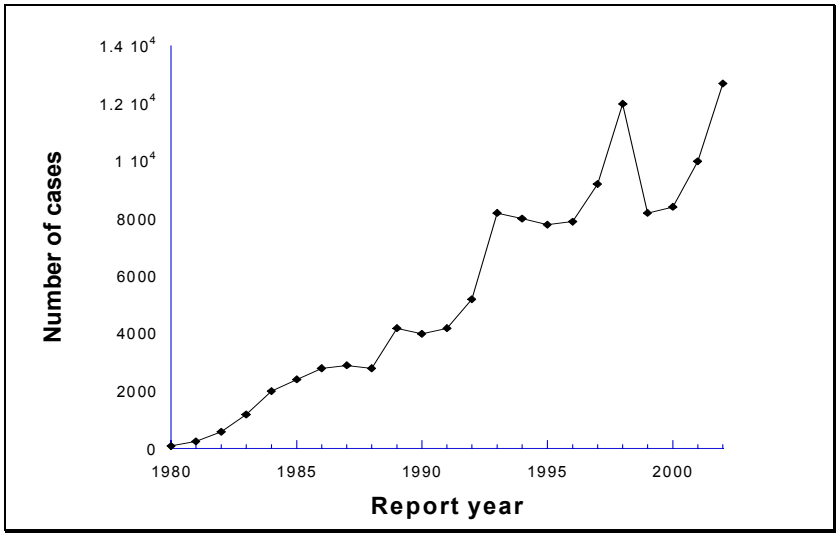

Figure 12.1. Annual campylobacteriosis notifications, 1980–2002 (Sneyd and Baker 2003).

12.2 SETTING

Until recent times, the focus of concern in New Zealand (as elsewhere) for water-related illness has been contamination by human effluent. For example, the Department of Health (1992) issued provisional microbiological water quality guidelines for recreational waters that included the advice that exposure to animal faecal microorganisms is much less of a risk than exposure to pathogens of human origin, reflecting the outcome of a study that was about to be published (Calderon *et al*. 1991). Accordingly, the effects of animal effluents were effectively set aside.

In New Zealand, environmental and public health scientists have been increasingly questioning this conclusion, supported by the following findings:

- an association between drinking-water treatment efficacy and giardiasis rates in a major city (Fraser and Cooke 1991);
- associations between town water supplies and campylobacteriosis outbreaks where the raw water supply is exposed to farm animal runoff (Brieseman 1987; Stehr-Green *et al*. 1991; McElnay and Inkson 2002);

- rates of campylobacteriosis have been increasing (Thornley *et al.* 2002; Sneyd and Baker 2003), coincident with the increase of pastoral agricultural activity;
- a relationship between ongoing cryptosporidiosis morbidity and the quality of rural water supplies (Duncanson *et al*. 2000);
- the detection of low levels of *Campylobacter* in some water supplies (Savill *et al.* 2001);
- frequent (59%) reports of contact with farm animals by notified cases of VTEC/STEC infection; 26.5% reported recreational contact with water. Of the 70 notified infections in 2002, 26.8% geocoded to rural areas. In comparison, just 12.6% of the New Zealand population is classified as rural (Sneyd and Baker 2003);
- a large rural epidemiological study demonstrating an association between water supplies and campylobacteriosis (Savill *et al*. 2002);
- regular timing of a cryptosporidiosis surge in rural communities coincident with the onset of calving (see Figure 12.2);
- a quantitative health risk analysis indicating that about 5% of all cases of campylobacteriosis could be attributable to contact with recreational fresh water (McBride *et al*. 2002); and
- deterioration of water quality in areas subject to intensive agricultural development (Hamill and McBride 2003).

As well as these New Zealand findings, some overseas results have supported such questioning. In particular, outbreaks of pathogenic *E. coli* and campylobacteriosis at Walkerton, Ontario, Canada (O'Connor 2002), and cryptosporidiosis at Milwaukee, USA (MacKenzie *et al*. 1994; Hoxie *et al*. 1997), have been associated with contaminated drinking-water obtained from rural catchments.

All of the above have led competent health authorities to issue statements of concern. At the recent New Zealand Geographical Society Annual Conference, Ministry of Health Medical Officers from two major dairy industry districts — Waikato in the North Island (Hood 2003) and Southland in the South Island (Poore 2003a) — presented papers on the implications to public health of the dairy industry and in particular of intensive dairying.

12.2.1 Epidemiological debate

In epidemiological studies, health effects among recreational water users have often been found to be a function of the degree of human faecal contamination (Prüss 1998). While this contamination has been measured by bacterial indicators (such as enterococci and *E. coli*), the etiological agents are generally

thought to have been non-zoonotic agents, especially Norovirus (e.g., Cabelli 1989). Few such studies have been carried out in waters with faecal contamination predominantly from animals. One such study carried out in rural fresh waters (Calderon *et al*. 1991) reported the absence of an association between the swimmers' illness risk and levels of bacterial indicators. While a large case–control epidemiological study of campylobacteriosis in New Zealand, with 621 cases and controls in four cities over an 8-month period, identified "raw or undercooked chicken" as the main risk factor (Eberhart-Phillips *et al*. 1997), animal and water-related risk factors were not fully considered. For these reasons, some health authorities have opined that water is not an important risk factor (Sneyd and Baker 2003: 85). Such views and findings have often formed the basis of the argument for concern being focused on human wastes.

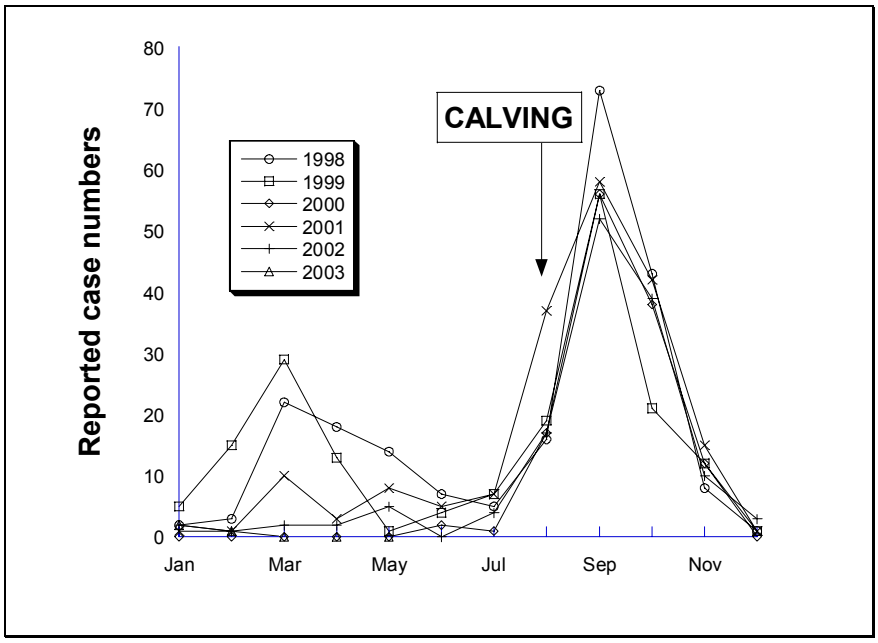

Figure 12.2. Cryptosporidiosis rates in a rural health region — Waikato, New Zealand (Hood 2003).

However, some countervailing arguments have been put forward. Firstly, the interpretation of the results from the Connecticut freshwater study, where the impact of animal wastes was considered (Calderon *et al*. 1991), the finding of no association of risks of gastrointestinal illness with densities of faecal indicator bacteria has been questioned, observing that their data could be

reinterpreted to imply that the faecally related health risks were very similar to those observed in studies on waters impacted by human effluents (McBride 1993).[1] Secondly, in the case–control campylobacteriosis study above where "raw or undercooked chicken" was proposed as the main risk factor (Eberhart-Phillips *et al*. 1997), weaker associations were also detected, including rainwater as a source of water at home.[2] More importantly, that study did not include consideration of exposure to contaminated recreational water.

Many epidemiological studies have not considered health effects for recreational waters impacted by animal wastes. Among those that do, Cheung *et al*. (1988, 1990) reported detecting associations between human health risk and animal wastes for marine waters in Hong Kong. Their study included two beaches impacted by livestock wastes (pigs). While these reported low swimming-associated gastrointestinal illness rates for beaches polluted by animal wastes, respiratory illness and skin infection rates were elevated, such that the total illness rate was very similar to those for the other beaches contaminated by human wastes. In a New Zealand study in which respiratory illness effects were detected, two beaches impacted by animal wastes were not separable from three others impacted by human wastes, but both were separable from the two pristine control beaches (McBride *et al*. 1998). The freshwater study by Calderon *et al*. (1991) also included animal waste impacts, as discussed above.

Given the potential importance of this issue, clarification is needed. Fortunately, there are a number of strands of work seeking to do so.

12.3 WHAT DO WE KNOW?

New Zealand has open pastoral agricultural systems — animals roam over pasture, usually having direct access to streams and lakes, and most are not housed (chickens and pigs being an exception). This situation, combined with the relatively low human population, means that much of the freshwater faecal contamination is of animal origin. In an attempt to quantify the extent of such contamination, a large national freshwater microbiological study was conducted in 1998–2000 (McBride *et al*. 2002; D.G. Till, A. Ball, and G.B. McBride, unpublished data). This study assayed six pathogens and five faecal indicators at 25 freshwater recreational sites at fortnightly intervals for 15 months, including

[1] In any event, inferring no association merely because the point-null hypothesis test has not been rejected is not logically permissible; some association will be present, however small. And if there are only a few samples available, that difference could be practically important, yet not "detected" by the test.

[2] Along with recent overseas travel, consumption of raw dairy products, and contact with puppies and calves.

two summer periods. A notable finding from this work is that 60% of all (726) samples were positive for the presence of *Campylobacter* species, and 8% of these exceeded the most probable number test's upper detection limit (110 organisms per 100 ml). The resulting distribution of *Campylobacter* has been used in a quantitative risk assessment to infer that about 5% of all campylobacteriosis cases could be attributable to water contact recreation (McBride *et al*. 2002). This result and associated risk profiles from that analysis have been incorporated into new national microbiological water quality guidelines for freshwater recreation (Ministry for the Environment and Ministry of Health 2003).

Surface runoff and point source pollution from pastoral agriculture can introduce pathogenic microorganisms such as *Campylobacter*, *Cryptosporidium*, and *Giardia* into streams and rivers (Geldreich 1996), compromising their suitability for contact recreation and as a drinking-water supply.

An investigation of two rural streams in the Waikato region of New Zealand was carried out to assess water quality with respect to faecal microbial contamination (Donnison and Ross 2003). The sampling site in each stream was surrounded by dairy farms, and sampling was approximately fortnightly over 1 year. Although *Campylobacter* concentrations were generally low in both streams, they were recovered from nearly all samples (i.e., 93%). These authors note that a constant presence of *Campylobacter* in rural streams may lead to cycling of these bacteria in farm animals and indirectly contribute to the high incidence of human infection in New Zealand.

In a further study, Ross and Donnison (2003) monitored a mole-tile drained farm supporting two separate dairy herds over a period of a year. In summer, the first herd was on the farm, and effluent that contained 10^3 *Campylobacter* per 100 ml was traced to the farm storage pond. The following spring, the second herd was present, and effluent that contained 10^5–10^6 *Campylobacter* per 100 ml was traced to the same pond. During this sampling, concentrations of this bacterial pathogen in the drainage water were similar to those in the applied effluent when irrigation caused preferential flow (optimal irrigation conditions). *Campylobacter jejuni* was the predominant species recovered. The summer (herd 1) sampling contained only one subspecies type (in effluent drainage water and soils). Several subtypes were observed in the spring (herd 2) sampling. Penner serotying revealed serotypes with well established links to campylobacteriosis in humans.

As previously noted, thermophilic *Campylobacter* are an important cause of gastrointestinal illness throughout the world (Blaser *et al*. 1983), and New Zealand has a higher incidence rate of campylobacteriosis than other countries of similar socioeconomic status (e.g., Queensland, Australia, has an incidence rate of about one-third that of New Zealand). Notified cases of campylobacteriosis for the whole of New Zealand are generally highest in spring and summer and decrease during winter, with some marked differences between urban and rural areas (Hearnden *et al*. 2003). The mechanisms behind these differences remain

uncertain (Skelly and Weinstein 2003). *Campylobacter* can survive in water for between 8 days (Buswell *et al*. 1998) and 4 months (Rollins and Colwell 1986), but are not capable of multiplying in water.

Some studies have focused on the survival and ecology of *Campylobacter* in aquatic systems (Buswell *et al*. 1998; Obiro-Danso *et al*. 2001), and the ability of *Campylobacter* to assume a viable but non-culturable (VBNC) form under adverse environmental conditions has been identified (Rollins and Colwell 1986). Koenraad *et al*. (1997) reviewed the epidemiology of *Campylobacter* in water-related environments and argued that direct monitoring of *Campylobacter* in recreational waters is needed because of a lack of correlation with indicator organisms and the low infectious dose of *Campylobacter*. Although such a correlation was established in the New Zealand study (McBride *et al*. 2002), this was mainly in catchments with high *Campylobacter* levels (Till *et al*. 2000).

12.3.1 *Campylobacter* ecology study

A New Zealand study conducted from June 2000 to June 2001 (Eyles *et al.* 2003) focused on the ecology of *Campylobacter* in a river that flows geographically through agricultural landscape in which the land use is predominantly farming (dairy, cattle, sheep, and deer). The study sought to identify probable environmental sources of *Campylobacter*, the period of maximum risk to recreational users (by recording the seasonal pattern in thermophilic *Campylobacter* concentration over a 1-year period), and the relationship between *Campylobacter* concentrations in the recreational area of the river and the incidence of notified cases of campylobacteriosis in the human population of the river catchment and a nearby city that utilized the recreational sites of the lower river.

The detection of thermophilic *Campylobacter* in streams and rivers is an indication of recent inputs of faecal matter (Jones 2001), through one of three pathways: surface runoff or subsurface drains from surrounding land during rainfall events, point source inputs (e.g., dairy shed effluent), and direct deposition of faecal material by livestock with access to stream channels. Two main seasonal peaks in *Campylobacter* flux were observed, one in winter and one in summer. In terms of the flux reaching the coastal environment, both winter and summer are associated with high loads. Consideration of the flux of *Campylobacter* reaching coastal areas is important in New Zealand, where feral shellfish gathering for consumption is popular. Shellfish have the ability to concentrate pollutants, including bacteria, viruses, and protozoa, from seawater. Teunis *et al*. (1997) observed a peak in *Campylobacter* levels in shellfish in winter and concluded that there was a significant risk of infection associated with the consumption of raw shellfish from Dutch waters. Wilson and Moore (1996) also detected higher levels

of *Campylobacter* in autumn and winter compared with summer months in Northern Ireland and detected *Campylobacter* when faecal coliforms and *E. coli* were absent, whereas overseas studies (Bolton *et al*. 1987; Carter *et al*. 1987; Jones *et al.* 1990; Brennhovd *et al*. 1992) have generally found higher concentrations of *Campylobacter* in surface waters in winter months. In the Eyles *et al.* (2003) study, concentrations of *Campylobacter* were slightly higher in summer, when recreational exposure is greatest, than in winter. Possible reasons why *Campylobacter* levels were higher in summer in this study are as follows: stocking levels are higher in summer following lambing and calving, minor flood events that occurred during summer may have played an important role in the transfer of faecal material from land to surface waters, and stock may be more likely to access streams and rivers to drink and cool themselves during summer. This provides a potential to increase bacterial concentrations from animal faecal material in the water column through both direct deposition and resuspension from sediments. Hunter *et al*. (2000) also observed higher faecal bacteria levels in streams within agricultural land use areas in the United Kingdom during summer low-flow conditions, attributable in part to higher stocking densities in summer months. The Eyles *et al.* (2003) study concluded that median levels of *Campylobacter* in the river were highest during summer months, the period of highest recreational use. A comparison between *Campylobacter* levels in the river and notified cases of campylobacteriosis in that district showed a drop in cases during a period when *Campylobacter* levels in the river were extremely low (February and March). This observed drop in cases occurring in late summer, a time of year when foodborne cases are usually high, suggests (although the study covered only one summer period) that waterborne transmission may play an important role in the epidemiology of campylobacteriosis in this region.

12.3.2 Deposition/yield studies

In a recent study in New Zealand, *E. coli* concentrations increased more than 100 times the background level after cattle had crossed a stream (Davies-Colley *et al*. 2002). The authors attributed this to direct faecal deposition, wash-off from legs, and disturbance of sediments by cattle hooves.

Stream sediments and banks have been shown to act as in-channel storage of microorganisms at low flows and then yield them to the overflowing water at higher flows (Nagels *et al*. 2002; Muirhead *et al*., in press). Such an observation has epidemiological importance when considering pathogen loadings to waterways, not only from runoff after rain, but also from storage reservoirs in sediment.

12.3.3 Potential public health impact of pastoral farming

The intensity of pastoral farming reflects the carrying capacity of the land. It is estimated that each dairy cow produces the same amount of effluent as 14 people (Johnson 2001). For 2002, that equates to a human population of approximately 70 million concentrated in rural areas in New Zealand, compared with New Zealand's present total population of approximately 4 million, of which only 12.8% (512 000) reside in rural areas. The potential public health risk could therefore be dependent on the zoonotic disease burden of farmed animals as related to the treatment and disposal of their effluent. About 10–15% of this is effluent dairy shed wastewater and is treated in a settling pond system. At present, this is disposed of by irrigation onto land rather than directly into waterways. Most of the effluent (85%) is deposited directly onto pasture, a potential non-point source of effluent to waterways (Poore 2003b).

Recently, a model has been developed to predict concentrations of *E. coli* in streams draining hill-country pastures grazed by sheep and beef cattle (Collins and Rutherford 2004). The long-term aim of this modelling is to aid assessment of the impact of land management practices upon faecal contamination of waterways. A daily record of grazing livestock is used in the current model to estimate *E. coli* inputs to a catchment. Scenario analysis suggests that excluding stock from streams and riparian retirement will improve microbiological water quality.

Calculating stock units as a measure of agricultural intensity over a catchment in relation to microbiological indicators offers a tool for assessing risk from potential waterborne zoonotic pathogens, assuming data for the amount and indicator/pathogen content of faecal material from farmed animals can be estimated.

12.4 WHAT DO WE NEED TO KNOW?

Current investigations in New Zealand are focusing on some of these items, but there are major gaps:

- rates and timing of *Campylobacter* shedding;
- the transport of *Campylobacter* from deposition areas through the landscape and waterways;
- the survival of *Campylobacter* in various components/strata of the landscape and waterways;
- quantifying the effectiveness of riparian retirement;
- the loading of zoonotic pathogens in animal wastes at given seasons, versus the health risk from human wastes; and

- more or better case–control studies, including molecular typing studies to determine the source of human *Campylobacter* infections.

Information on the last item may help to shed light on the relative health risk of animal versus human wastes. While the later contains many pathogenic viruses of public health concern that are not zoonotic, animal faeces containing high proportions of zoonotic bacterial and protozoan pathogens of public health concern could outweigh the perceived lack of pathogenicity.

Chapter 29 also discusses knowledge gaps for quantitative health risk assessments. Current research efforts are focusing on these issues.

12.5 MANAGEMENT RESPONSE

Recent microbiological water quality guidelines for recreational fresh water are based on a campylobacteriosis risk analysis (Ministry for the Environment and Ministry of Health 2003), as discussed in chapter 29. Much of these waters are in rural areas, so in promulgating these guidelines, health authorities have accepted that some water-related health risks may come from zoonotic microorganisms.

The issue now has a high profile in the agricultural community, especially following a major review that concluded that there is a link between agricultural land use and poor water quality, stream habitat, and impacted biotic communities (Parkyn *et al.* 2002).

The environmental impacts of farming not only are related to public health protection, but also have significant socioeconomic relevance, as, internationally, consumers are increasingly demanding proof that food is not produced through exploitation of the environment, workers, or animals. New Zealand's largest dairy company, Fonterra, has committed its 12 600 farmer/suppliers (there are only approximately 1000 other dairy farmers) to attend to non-point source pollution, including runoff of animal effluent and fertilizer into streams and rivers (NZWWA 2003). Fonterra has signed a "Dairying and Clean Streams Accord" with central and local government, which will give farmers up to 9 years to clean up their environmental performance in ways that can be objectively measured. Under the Accord, Fonterra will measure suppliers against a range of environmental standards, aimed particularly at cleaning up waterways adjacent to or on farms.

The dairy industry is promoting the integration of environmentally safe practices into farming, backed up by new multiple catchment studies (Wilcock 2003).

Regional government agencies are funding stream restoration projects and public education of the issues.

As a model, a recent New Zealand Ministry of Health pamphlet (attached as Appendix 12.1) describes a national approach to the coordination and integration of research activities of academic, industry, scientific, and administrative bodies to investigate causes of present and emerging enteric zoonoses in New Zealand.

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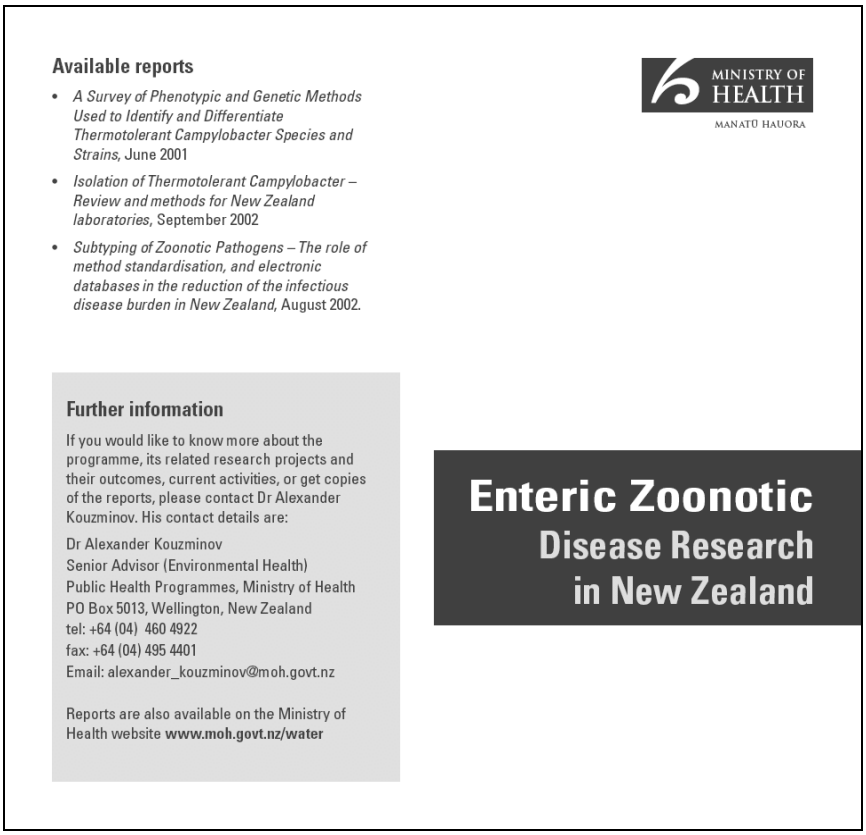
Appendix 12.1. Ministry of Health zoonoses pamphlet (2003)



Available reports

- *A Survey of Phenotypic and Genetic Methods Used to Identify and Differentiate Thermotolerant Campylobacter Species and Strains*, June 2001
- *Isolation of Thermotolerant Campylobacter – Review and methods for New Zealand laboratories*, September 2002
- *Subtyping of Zoonotic Pathogens – The role of method standardisation, and electronic databases in the reduction of the infectious disease burden in New Zealand*, August 2002.

Further information

If you would like to know more about the programme, its related research projects and their outcomes, current activities, or get copies of the reports, please contact Dr Alexander Kouzminov. His contact details are:

Dr Alexander Kouzminov
Senior Advisor (Environmental Health)
Public Health Programmes, Ministry of Health
PO Box 5013, Wellington, New Zealand
tel: +64 (04) 460 4922
fax: +64 (04) 495 4401
Email: alexander_kouzminov@moh.govt.nz

Reports are also available on the Ministry of Health website **www.moh.govt.nz/water**

MINISTRY OF HEALTH
MANATŪ HAUORA

Enteric Zoonotic Disease Research in New Zealand

Zoonoses are diseases, some severe, caused by micro-organisms that are transmitted from animals and birds to humans. In New Zealand, and other developed countries, enteric zoonotic diseases are major contributors to water- and food-borne disease, including gastroenteritis. We have a shared interest in developing strategies for their control.

In New Zealand the most significant micro-organisms causing zoonotic diseases are the bacteria *Campylobacter* spp., some strains of *Escherichia coli, Salmonella* spp., and the protozoa *Giardia* and *Cryptosporidium*.

Research co-ordination in New Zealand

Co-ordination of research on enteric zoonoses is challenging because it cuts across responsibilities of several government agencies. Research from a variety of scientific disciplines also needs to be integrated.

In May 2000 a joint interagency programme was developed as a result of a Ministry of Health initiative. The programme, 'Enhanced co-ordination and development of enteric disease research in New Zealand' has been implemented. It involves people from primary industries, researchers, research funding agencies, and policy advisors and regulators of food/water quality from central and local government. The programme focused initially on *Campylobacter* spp. but also covers co-ordination of research on other disease-causing organisms, including *E. coli, Salmonella, Listeria, Giardia* and *Cryptosporidium*.

The programme's mission is *to reduce the burden of enteric zoonoses in New Zealand.*

The quality and relevance of enteric disease research is being enhanced through collaboration between researchers in Crown Research Institutes and universities, and improved co-ordination of research funders who include central and local government, the Foundation for Research, Science and Technology, and primary industries.

Contact has been established internationally with research groups in USA, Canada, Australia, United Kingdom and Iceland.

This national and international co-ordination has been facilitated by the Enteric Zoonotic Disease Research Steering Committee and its two expert technical sub-committees: the Methodology Group and the Risk Management Group.

Research commissioned and co-ordinated by the Steering Committee has led to:

- better understanding of the ecology and transmission of *Campylobacter* in various reservoirs
- improved knowledge of amplification factors in these reservoirs, transmission pathways between reservoirs, and possible means of control of the disease in New Zealand
- development of quantitative risk assessment models for *Campylobacter* in New Zealand
- assessment of the effectiveness of interventions and remedial actions and how they may be improved to prevent water- and food-borne disease
- development of guidelines for more effective interventions to prevent water- and food-borne disease, including possible measures for implementation by local government.

Section V

Categories of waterborne disease organisms

D.O. Cliver and R. Fayer

Reports of case-studies as well as estimates of disease in local and regional populations provide us with patterns of disease that are subject to a variety of local environmental, societal, and biological influences. Environmental conditions are significantly influenced in turn by climate and human activities. All of these influences can be highly variable, resulting in very different patterns of disease burden among populations. Limiting discussion to the public health implications of waterborne zoonotic agents provides greater focus. Further limiting discussion to those agents that are emerging or are of renewed interest because they have resulted in increased disease burden provides even more focus. To select those agents for which a strategy can be developed for treatment or prevention, the impact of each agent must first be examined individually. The present section examines specific zoonotic agents from viruses to fungi,

 Waterborne Zoonoses: Identification, Causes and Control. Edited by J.A. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D.O. Cliver, G.F. Craun, R. Fayer, and V.P.J. Gannon. Published by IWA Publishing, London, UK. ISBN: 1 84339 058 2.

bacteria, protozoa, and helminths that pass in the faeces from an infected host to a susceptible host and are facilitated by waterborne transmission.

BACTERIA

Many bacterial zoonoses are known, but not many are known to be transmitted via water. The waterborne zoonotic bacteria are principally those shed in faeces by warm-blooded animals (birds and mammals), although some are also harboured by reptiles. Detection methods exist for the most important waterborne bacteria and are improving, but there is still good reason to use indicators of faecal contamination to monitor for water safety.

Escherichia coli

This bacterial species is found in the colons and faeces of all warm-blooded animals. It usually occurs as a commensal, causing no disease. Until 1982, most of the known *E. coli* types that caused disease in humans were human-specific and not associated with other animal species. In that year, *E. coli* O157:H7 was incriminated in human illnesses and was shown to have a reservoir in cattle. This was the first recognition of a genre now called verocytotoxin-producing *E. coli* (VTEC), Shiga toxin-producing *E. coli* (STEC), or enterohaemorrhagic *E. coli* (EHEC). All of these are capable of causing severe disease in humans, although they are typically shed by healthy cattle and other species. Human-to-human transmission, both by contact and via water, is also known.

Salmonella

The chapter on *Salmonella* explores the traits of this genus. Although salmonellae typically do not multiply in the environment, they survive remarkably well under a variety of environmental conditions. The peroral infectious dose tends to be large, unless a vehicle more protective than water is available to shield these bacteria from stomach acids, or perhaps if the consumer has low stomach acidity. Another zoonotic bacterial species considered in this chapter is *Yersinia enterocolitica*, which is most often associated with swine and may be transmitted to humans via water contaminated with swine manure. Both *Salmonella* and *Yersinia* are often shed by apparently healthy animals and are capable of causing severe disease in humans.

VIRUSES

Viruses are transmitted as organisms much smaller than bacteria and incapable of multiplying outside the host, but often associated with larger particles in the water environment. Nevertheless, they have a clear record of transmission via water and other environmental routes and seem to be quite efficient as waterborne pathogens. The difficulty of detecting viruses in water has inspired a continuing quest for valid indicators of their presence. The focal question, in the present context, is how likely they are to function as zoonoses.

The viruses transmitted via water are shed with faeces and infect by the oral route. Each has a specific repertoire of host cells, which it can invade to initiate an infection. Necessarily, the first susceptible host cells are situated in the intestinal lining; however, some viruses that infect perorally are later transmitted to other tissues (e.g., the liver), where their infection causes illness more significant than common gastroenteritis. In addition to tissue specificity, the viruses show strong specificities with respect to host species. No recorded waterborne outbreak of viral disease to date has been attributed to viruses produced by animals, although animal counterparts of most waterborne human viruses are known to exist. However, the viral replicative process is highly error-prone, so the possibility of mutation in host species certainly exists. For such a mutation to take effect, allowing transmission of virus from its previous host species to another, the mutant viral nucleic acid would have to be coated with protein that was specific for the same new host, and then the altered virus would have to be transported from an animal of one species to another (i.e., a human). The odds are greatly against this on any given occasion, but considering the numbers of viral particles produced in the course of a single infection and the numbers of viral infections that occur worldwide, it would be surprising if such host-range mutations did not occur.

PROTOZOA

Protozoan pathogens, including microsporidia, amoebae, ciliates, flagellates, and apicomplexans, originating in human or animal faeces have been found in surface waters worldwide. Many have been found infrequently or in low numbers or have been identified only by general morphological features that are not precise. The zoonotic protozoa that are emerging or are of renewed interest because their spread is associated with water include several species of microsporidia, the amoeba *Entamoeba histolytica*, *Giardia duodenalis* (*G. lamblia*), *Toxoplasma gondii*, and *Cryptosporidium* spp. Although *Cyclospora cayetanensis* is known to be a waterborne threat and has been detected in washings from vegetables contaminated with irrigation water, humans are the

only confirmed hosts for this species. The dearth of data on the prevalence of these zoonotic protozoa in surface waters is related to the lack of rapid and sensitive methods to recover and detect the encysted stages in the aquatic environment. Most of these protozoa, unlike bacteria and viruses that multiply exponentially under culture conditions, require animal assays or cell culture methods that are unavailable in many locations and take days or weeks to obtain results. The application of molecular techniques is permitting identification of species and genotypes in animals and humans and providing for the detection of low numbers of these protozoa in aqueous environments, enabling scientists to find these organisms in surface waters, drinking-water, and seawater environments where they have rarely or never been detected before. The ability to conduct epidemiological studies relating these organisms to human infections, animal sources, and water will now provide a basis for planning prevention and control strategies.

HELMINTHS

Major helminth zoonoses include nematodes such as ascarids, pinworms, hookworms, strongylids, angiostrongylids, capillarids, and guinea worms, flukes such as schistosomes and liver flukes, and tapeworms such as the beef, pork, and fish tapeworms, as well as cystic and alveolar hydatid tapeworms. Poor sanitation and poor water quality facilitate transmission among animals and humans. The life cycles of most of these helminths are very well known; if they are interrupted, infection and disease can be prevented. Recent observations indicate that human cases of fascioliasis (liver fluke disease) have increased in 51 countries, with estimates of 2.4–17 million or more people infected. Unlike previous assumptions, high prevalence in humans is not always found where fascioliasis is a significant veterinary problem. Temperature and rainfall pronouncedly determine seasonal incidence, and human infection is more frequently observed in years with heavy rainfall. Snail hosts and parasite life cycles have adapted to local environments to maximize parasite transmission, and contaminated water used for irrigation, washing foods, and beverages is now recognized as contributing to infection.

13

Verocytotoxin-producing *Escherichia coli* and other diarrhoeagenic *E. coli*

K. Mølbak and F. Scheutz

13.1 INTRODUCTION

Escherichia coli are facultative Gram-negative rods that inhabit the intestinal tract of humans and other animals. In the human bowel, most *E. coli* do not cause disease. However, *E. coli* is a very versatile bacterial species, and important subtypes of *E. coli* contain and express virulence factors that enable them to exhibit pathogenicity. Some subtypes of *E. coli* cause endogenous infections such as disease in the urinary tract (uropathogenic *E. coli*), invasive infections originating from foci adjacent to the gut, or infections of wounds contaminated with urine or faeces. Invasive *E. coli* is the most frequently

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encountered species in Gram-negative septicaemia and is also a common cause of meningitis. Zoonotic aspects of the uropathogenic and invasive *E. coli* will not be discussed in this chapter. Other strains of *E. coli* represent almost the entire range of means by which microorganisms damage intestinal function, cause secretion or inflammation, and thus produce gastrointestinal disease. These so-called diarrhoeagenic *E. coli* (DEC) are classified as shown in Table 13.1.

Table 13.1. Diarrhoeagenic *E. coli*

	Abbrev.	Definition	Type of disease
Verocytotoxin (Shiga toxin)-producing *E. coli*	VTEC or STEC	*E. coli* that produce verocytotoxin (Shiga toxin) VT1 and/or VT2	Diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome (HUS)
Enterotoxigenic *E. coli*	ETEC	*E. coli* that produce enterotoxins that are heat stable (STh, STp) and/or heat labile (LT)	Acute watery diarrhoea
Attaching and effacing *E. coli*	A/EEC	*E. coli* that attach to and efface the microvilli of enterocytes, but do not produce high levels of verocytotoxin	Acute or persistent diarrhoea
Enteropathogenic *E. coli*	EPEC	Subtype of A/EEC, usually of particular serotypes that mostly contain an EPEC adherence factor plasmid and often produce bundle-forming pilus (BFP)	Acute or persistent diarrhoea
Enteroaggregative *E. coli*	EAggEC	*E. coli* that exhibit a pattern of aggregative adherence to tissue culture	Acute watery, often protracted diarrhoea
Diffuse adherent *E. coli*	DAEC	*E. coli* that exhibit a pattern of diffuse adherence to tissue culture	Acute or persistent diarrhoea
Enteroinvasive *E. coli*	EIEC	*E. coli* that share virulence determinants with *Shigella* spp.	Acute, often inflammatory diarrhoea; dysentery

The classification of DEC is somewhat arbitrary, as certain subtypes contain unusual cocktails of virulence determinants, and these strains may not easily be classified. Most of the groups in the current classification include strains that

contain different virulence determinants and may therefore not be equally virulent. Not all of the strains that are currently classified as, for example, A/EEC, DAEC, or EAggEC may be human pathogens. Much remains to be understood about the pathogenesis, epidemiology, and public health significance of these types of DEC.

The term enterohaemorrhagic *E. coli* (EHEC) was originally defined as those serotypes that cause a clinical illness similar to that caused by *E. coli* O157:[H7] and contain similar virulence determinants, including a virulence plasmid that encodes enterohaemolysin. EHEC is now used as a term for VTEC that cause haemorrhagic colitis in humans. Waterborne transmission of VTEC from zoonotic reservoirs is well documented and will be discussed in this chapter.

ETEC is a leading cause of diarrhoeal disease in humans and domestic animals. ETEC is, however, not a zoonosis. Waterborne transmission of ETEC is an important route of infection in developing countries, but is caused by contamination of water by human faeces.

Typical EPEC strains produce BFP and exhibit a characteristic pattern of localized adherence to tissue culture. Typical EPEC strains belong to a group of specific "classical" EPEC serotypes. There is little evidence of an animal reservoir for EPEC, as A/EEC of animals belong to serotypes that are usually not linked to human disease. The epidemiology and public health importance of A/EEC that do not belong to the "classical" EPEC serotypes remain poorly understood; the same is true for EAggEC and DAEC.

EIEC is not regarded as a zoonotic infection, and waterborne transmission of *Shigella* and EIEC is caused by contamination with human faeces.

13.2 VEROCYTOTOXIN-PRODUCING *E. COLI*

13.2.1 Microbiology and epidemiology

More than 400 different serotypes of *E. coli* produce verocytotoxin, and most — but not all — of these have been linked to human illness. *Escherichia coli* O157:[H7] is the most widely recognized VTEC serotype. It was first recognized in 1982 in an outbreak of severe bloody diarrhoea that was linked to a fast-food restaurant chain and another outbreak in a nursing home in Canada. VTEC O157 is now recognized as an important cause of food- and waterborne illness in developed and some developing countries. Infection typically presents as a diarrhoeal illness, often with bloody stools. In approximately 8% of patients, infection progresses to HUS, a life-threatening condition characterized by microangiopathic haemolytic anaemia, thrombocytopenia, and renal failure. While O157:[H7] is the most commonly identified VTEC serotype in North America and the United Kingdom, non-O157 VTEC are much more common in most continental European countries and Australia. Important O groups within

non-O157 VTEC include O26, O103, O111, and O145; infections with these strains cause severe illness, including HUS.

13.2.1.1 Microbiology

Escherichia coli O157:[H7] is similar to most other *E. coli* in that it ferments lactose, but it does not usually ferment sorbitol rapidly, a feature that aids the diagnosis of *E. coli* O157:[H7] on the sorbitol-MacConkey plate. *E. coli* O157:NM strains that ferment sorbitol have been found in Europe, in particular in Germany. *E. coli* O157:[H7] usually has haemolytic activity on enterohaemolysin agar. Most non-O157 VTEC will readily ferment sorbitol, as will other *E. coli.* This is one of the factors that hamper routine screening for non-O157 VTEC.

E. coli O157:[H7] does not grow well at 44 °C, the usual temperature for measuring thermotolerant *E. coli* in water. *E. coli* O157:[H7] survives well in acidic environments, such as apple cider and fermented sausages. *E. coli* O157:[H7] has been shown to survive for several weeks in water, in particular cold water (Wang and Doyle 1998). It may also persist for long times in the environment, including soil, manure, slurry, etc.

E. coli O157:[H7] nearly always produce VT2; a high proportion of strains also produce VT1. In addition, *E. coli* O157:[H7] and most — but not all — other VTEC contain the LEE (locus of enterocyte effacement) pathogenicity island. This is a collection of genes that are also present in A/EEC and encode the ability to attach intimately to enterocytes, efface microvilli, and influence the formation of actin-rich cuplike pedestals on which the bacteria act. LEE contains, among other genetic loci, the *eae* gene, an outer membrane adhesin protein required for the attachment. It is likely that the clinically most important VTEC are those that both produce VT2 and contain LEE (*eae*) (Griffin *et al*. 2002; Ethelberg *et al*., in press).

13.2.1.2 Epidemiology

The distribution of *E. coli* O157:[H7] and other VTEC is global. Among countries with surveillance systems, the incidences of VTEC infections vary widely, reflecting differences in incidence, diagnostic activity, and reporting. High incidence has been reported from regions of Canada, Scotland, and Argentina. In most European countries and the USA, the annual incidence may range from one to four infections per 100 000 population. Few laboratories screen for non-O157 VTEC, which remain underdiagnosed. Human cases of VTEC infections generally peak in the summer months, with the highest incidence in young children.

Sources of human illness with *E. coli* O157:H7 include bovine products — in particular ground beef — contaminated produce and raw vegetables, contaminated juice, drinking-water and recreational water, and person-to-person transmission.

Generally speaking, non-O157 VTEC are thought to be acquired by similar routes, but much remains to be learned about the epidemiology of these bacteria. Three examples of "emerging types" include the following:

- Multidrug-resistant VTEC O118:H16 has been identified as an emerging pathogen for calves and humans in Belgium and Germany and has now been found in cattle in Latin America (Beutin *et al*. 1998; de Castro *et al*. 2003).
- VTEC O117:K1:H7 has been identified as a cause of prolonged diarrhoea in travellers returning from Asia, Africa, and Cuba and has also been found as a cause of childhood diarrhoea in developing countries (B. Olesen, C. Jensen, K.E.P. Olsen, V. Fussing, P. Gerner-Smidt, and F. Scheutz, unpublished data).
- In a cohort study of 200 children in Guinea-Bissau, 11 of 16 Stx2-producing strains also produced enterotoxin (STh and LT) (Valentiner-Branth *et al*. 2003). Infection with these unusual strains, which may be classified as both ETEC and VTEC, was associated with diarrhoea. Similar strains have been described in pigs, in which they may cause oedema disease. It remains to be shown whether these strains are zoonotic.

These cases have two things in common: all may mirror larger public health problems, and zoonotic waterborne transmission may play a role in the spread of these agents. These reports underscore the fact that currently available data from developing countries are inadequate to resolve many scientific questions.

13.2.2 Domestic and wild animals as reservoir for VTEC

Healthy ruminants, including cattle, sheep, deer, and goats, carry VTEC strains. Ruminants, in particular cattle, are considered to be the main reservoir for VTEC, in particular *E. coli* O157. Increasingly, *E. coli* O157 and other VTEC are identified in animals other than ruminants, including pigs, rabbits, opossums, and waterfowl. These findings may be due to transient carriage or may be indications that the reservoirs are wider than previously thought.

Non-O157 VTEC can cause disease in some domestic animals, such as diarrhoea in calves and oedema disease in pigs. Information is limited for other animal species. Non-O157 VTEC associated with disease in animals belong to a limited number of serotypes, some of which have been associated with human

disease. For example, VTEC causing disease in cattle are frequently serotypes O5:NM, O26:H11, O103:[H2], and O145:NM (Anonymous 1999).

In endemic areas such as the United Kingdom, *E. coli* O157 may be present in up to half of the cattle herds, but more sensitive methods are likely to find even higher rates. A variety of non-O157 VTEC are nearly always present in cattle and many other ruminants, but not all of these strains may be human pathogens, as stressed above.

Faecal shedding of *E. coli* O157:H7 appears to be highest in young weaned cattle and during the summer. Several production practices may contribute to the emergence of *E. coli* O157:H7 in cattle, including feeding practices and crowding.

13.2.3 Waterborne transmission of *E. coli* O157:H7 and other VTEC

Escherichia coli O157:H7 and possibly other VTEC, such as O26:H11, have a low infectious dose, which allows water to act as an efficient vehicle. In particular, watersheds that are vulnerable to infiltration by domestic or wild animals run the risk of contamination. Consequently, small water systems or wells that supply rural townships or camps have commonly been associated with waterborne outbreaks. This evidence is based on microbiological, environmental, and epidemiological studies of outbreaks (Table 13.2). In addition, recreational waters have often been implicated in outbreaks. Sources of contamination may be faeces from humans or other animals or sewage.

The presence of low numbers of target organisms in water makes microbiological confirmation difficult. Therefore, epidemiological evidence has been essential in outbreak investigations. Indeed, due to the low infective dose of *E. coli* O157:H7, a significant risk of infection may arise in waters that only just meet standards for index organisms. In situations where conventional methods have failed to demonstrate *E. coli* O157:H7, more sensitive culture methods, including polymerase chain reaction-based methods, have proven helpful (Bopp *et al.* 2003).

Despite the potential for contamination of water with VTEC O157, waterborne infection is relatively rare in industrialized countries, largely due to the susceptibility of the organism to water treatment processes (Chalmers *et al.* 2000). Little is known about this situation in developing countries.

Table 13.2. Examples of waterborne outbreaks with verocytotoxin-producing *Escherichia coli* (VTEC/STEC)

State or province, country	Year	Serotype	Additional pathogen	Short description	Most probable source	Number affected	Reference
Saitama, Japan	1990	O157:H7		Kindergarten outbreak	Well water	319 persons ill, 2 deaths	Kudoh *et al.* 1994
Missouri, USA	1990	O157:H7		Municipal water supply to a rural town. Shortly before the peak of the outbreak, 45 water meters were replaced, and two water mains ruptured.	Faeces of human or animal origin into water supply	243 persons ill, 2 cases of HUS, 4 deaths	Swerdlow *et al.* 1992
Oregon, USA	1991	O157:H7	*Shigella sonnei*	Prolonged outbreak from lake water. The unusually long duration supported the notion that *E. coli* O157 survive well in lake water and have a low infectious dose.	Faecal contamination from bathers	21 with *E. coli* O157 and 38 with *S. sonnei*	Keene *et al.* 1994
Scotland	1992	O157:H7		Children playing in a paddling pool	Human faecal contamination	1 case ill, 5 carriers, 1 case of HUS	Brewster *et al.* 1994
South Africa	1992	O157:NM		Community outbreak with multiple factors involved	Contamination of multiple water sources secondary to drought and cattle death	Approximately 41 000 physician visits	Effler *et al.* 2001

State or province, country	Year	Serotype	Additional pathogen	Short description	Most probable source	Number affected	Reference
The Netherlands	1993	O157:H7		Seminatural, shallow swimming lake	Water from ditches draining surrounding meadows with cattle	5 persons ill, 4 cases of HUS	Cransberg *et al.* 1996
New York State, USA	1994	O157:H7		Lake water at a country park	Faecal contamination from bathers or animals	12 persons ill	Ackman *et al.* 1997
Scotland	1995	O157:H7	*Campylo-bacter jejuni*	Stream water into which treated sewage discharged and contaminated the public water supply of the village	Faeces of animal origin into water supply	711 persons ill, 2 cases of HUS	Jones and Roworth 1996
Canary Islands	1997	O157:H7		Private water supply to four hotels	Contamination of water source with animal faeces	14 ill tourists from 5 countries	Pebody *et al.* 1999
Finland	1997	O157:H7		Freshwater lake	Human faecal contamination	5 primary and 8 secondary cases	Paunio *et al.* 1999
USA	1998	O157:H7		Improperly chlorinated swimming pool	Faecal contamination from bathers	18 persons ill	Friedman *et al.* 1999
Wyoming, USA	1998	O157:H7		Municipal water supply to a small rural town	Contamination of the water supply with surface water contaminated with elk or deer faeces	157 persons ill, 4 cases of HUS	Olsen *et al.* 2002

State or province, country	Year	Serotype	Additional pathogen	Short description	Most probable source	Number affected	Reference
Scotland	1999	O157:H7		Untreated private water supply at a campsite; outbreak went on for 7 weeks	Faecal contamination from sheep or deer	6 ill visitors	Licence *et al*. 2001
New York State, USA	1999	O157:H7	*Campylo-bacter jejuni*	Drinking water supply at Washington County Fair. At least one shallow well was contaminated with *E. coli* O157:H7. This well supplied unchlorinated water to several food vendors, who used the water to make beverages and ice.	Faeces from cattle or human origin into water supply	775 persons ill, 65 hospitalized, 11 cases of HUS, 2 deaths	Centers for Disease Control and Prevention 1999; Bopp *et al*. 2003
Connecticut, USA	1999	O121:H19		Freshwater lake	Transient local contamination from a sick child	11 persons ill, 3 cases of HUS	McCarthy *et al*. 2001
Washington, USA	1999	O157:H7		Freshwater lake	Faeces from ducks		Samadpour *et al*. 2002
California, USA	1999	O157:NM		Freshwater lake	Faeces from humans, cattle, or deer	7 ill persons	Feldman *et al*. 2002
Ontario, Canada	2000	O157:H7	*Campylo-bacter jejuni*	Contaminated municipal water supply in Walkerton, Ontario. Situation aggravated by heavy rains, flooding, a well subject to surface water contamination, and an overwhelmed water treatment system.	Faeces from livestock	2300 persons ill, 27 cases of HUS, 7 deaths	Anonymous 2000; Hrudey *et al*. 2003
Japan	2001	O26:H11		Secondary (untreated) water source	Faeces from wild animals	1 person ill, 5 other carriers	Hoshina *et al*. 2001

In a recent review of outbreaks with *E. coli* O157:[H7] in England and Wales between 1992 and 2001, 128 outbreaks were investigated; only 2% were waterborne, 26% were from person-to-person transmission, and 33% were foodborne (Gillespie *et al*. 2003). In the USA in 1982–2000, recreational water was incriminated in 6% of 228 outbreaks, and drinking-water in 3% (Rangel *et al*. 2003). However, outbreaks from drinking-water accounted for 29% of a total of 8466 cases affected in these outbreaks (primarily an effect of a single large outbreak, the Washington County Fair outbreak; Table 13.2). Hence, when waterborne outbreaks occur, the public health consequences may be devastating, as illustrated by the waterborne outbreak of gastroenteritis associated with the contaminated supply to the Washington County Fair in New York State in 1999 and in particular the Walkerton, Ontario, Canada, outbreak in 2000 (Table 13.2).

It has often been difficult to establish whether the contaminant was of bovine or human origin, since both cattle and humans may shed *E. coli* O157 and other VTEC. It is, however, important to stress that the shedding duration of VTEC in humans is much shorter than that for *Salmonella* in children and adults, and that ruminants may shed VTEC for long periods.

In the investigation of the Washington County Fair outbreak (Centers for Disease Control and Prevention 1999), an environmental analysis demonstrated a hydraulic connection between the septic system of a dormitory building and the water in the well. The lead hypothesis was that cow manure contaminated with *E. coli* O157:H7 was carried into the dormitory on muddy boots, washed into the septic system, and subsequently washed into the well.

In the investigation of the Walkerton, Ontario, outbreak (Anonymous 2000), ill persons were identified from 15 April until late June. It is possible that low numbers of bacteria were entering the Walkerton municipal water distribution system in April and early May. It was hypothesized, however, that heavy rainfall in mid-May was responsible for gross contamination of the distribution system, resulting in the majority of the illnesses. Mapping of the cases confirmed the widespread nature of the illnesses and supported the hypothesis that municipal water was the vehicle of the outbreak. Environmental testing of 13 livestock farms within a 4-km radius of three incriminated wells (including "Well 5") identified human bacterial pathogens in domestic animal manure on all but two farms. On nine farms, *Campylobacter* spp. were identified; on two farms, both *E. coli* O157:H7 and *Campylobacter* spp. were found — this included a farm adjacent to Well 5. The molecular subtypes and phage types of the *E. coli* O157:H7 and the *Campylobacter* spp. isolates from this farm were identical to those found in the majority of the human cases. While investigators could not prove that the pathogens were present before the outbreak, the evidence suggests that the pathogens that entered Well 5 were likely to have originated from cattle manure on this farm. A series of unfortunate

circumstances occurred to cause an outbreak of this magnitude. These included failure to enact a regulation requiring testing laboratories to notify the proper authorities promptly and directly about adverse results, heavy rains accompanied by flooding, *E. coli* O157:H7 and *Campylobacter* spp. present in the environment, a well subject to surface water contamination, and a water treatment system that may have been overwhelmed by increased turbidity.

In 1992, a large outbreak of bloody diarrhoea caused by *E. coli* O157 infections occurred in Swaziland (Effler *et al.* 2001). A total of 40 912 physician visits for diarrhoea occurred during October through November 1992. This was a 7-fold increase over the same period during 1990 and 1991. An investigation of this outbreak suggested that carriage of *E. coli* O157 by cattle, cattle deaths secondary to drought, and heavy rains that resulted in contamination of surface water were important factors contributing to the emergence of *E. coli* O157 in this community. Eating beef was also a risk factor for infection, which suggests that multiple sources played a role in this outbreak, possibly the largest VTEC outbreak ever recognized.

Most evidence of waterborne transmission is based on carefully conducted outbreak investigations, as summarized above. In addition, a limited number of case–control studies of sporadic illness have investigated risk factors for VTEC infection. In the USA, Slutsker *et al.* (1998) studied 73 patients with *E. coli* O157:H7 and 142 matched controls. In univariate analysis, an increased risk was associated with consumption of hamburger (matched odds ratio [MOR], 3.8; 95% confidence interval [CI], 1.9–7.9), undercooked hamburger (MOR, 4.5; 95% CI, 1.6–12.2), or hot dogs (MOR, 2.2; 95% CI, 1.1–4.4); eating at a fast-food restaurant (MOR, 2.3; 95% CI, 1.1–4.6); drinking unchlorinated well water (MOR, 2.4; 95% CI, 1.1–5.7); swimming in a pond (MOR, 5.4; 95% CI, 1.1–26.0); and having a household member with diarrhoea (MOR, 11.9; 95% CI, 2.7–53.5). In multivariate analysis, only eating undercooked hamburger remained associated with infection.

In Canada, a study by Rowe *et al.* (1993) included 34 children with HUS due to VTEC infections and 102 controls, who were otherwise healthy children with minor acute injuries. Children with HUS were significantly more likely than controls to have had close contact with an individual with diarrhoea in the 2 weeks before the onset of illness (74% vs. 29%, $P < 0.000\,01$; odds ratio, 7.0; 95% CI, 2.7–18.5). The onset of diarrhoea in the contacts occurred a median of 6 days (range, 1–>14 days) before the onset of diarrhoea in the HUS patients. These data provide evidence consistent with person-to-person transmission of VTEC in a substantial proportion of episodes of childhood HUS.

13.3 CONCLUSION

Diarrhoeagenic *E. coli* is an important cause of water-related diseases. Waterborne transmission of VTEC, in particular *E. coli* O157, from zoonotic reservoirs is well documented, primarily in developed countries. Recreational waters and private and municipal drinking-water supplies have been implicated as sources of outbreaks and causes of sporadic illness. Faeces from humans and other animals or sewage may be sources of infection; however, when faeces from ruminants are implicated, outbreaks tend to be prolonged and devastating, as underlined by the Walkerton outbreak and the outbreak in South Africa/Swaziland. In particular, watersheds, water systems, or recreational waters that are vulnerable to infiltration by domestic or wild animals or their waste run the risk of contamination. It is possible that waterborne transmission of *E. coli* O157 serves as a "prototype" of non-O157 VTEC transmission, but data to substantiate this hypothesis are insufficient.

A number of issues regarding VTEC remain poorly understood, in particular in developing countries:

- The public health significance of the different subtypes in developed as well as developing countries needs to be determined.
  - Why are there large differences in the reported incidence of different VTEC serotypes between different countries?
  - The burden of disease associated with possibly emerging specific subtypes of non-O157 VTEC needs to be determined.
- The different animal reservoirs and their relative importance need to be clarified.
- The importance of waterborne transmission from the zoonotic reservoirs for the most important non-O157 VTEC as well as *E. coli* O157 needs to be determined.

Diarrhoeagenic *E. coli* other than VTEC are endemic in developed and, in particular, developing countries. Zoonotic transmission is generally not thought to be an important route of infection for the non-VTEC DEC, but since non-VTEC DEC is a very heterogeneous group of bacteria and the rates of infection are high, it is likely that animal reservoirs may be of importance for some less studied subtypes of DEC. If this is so, these subtypes need to be identified, and their epidemiology, including waterborne transmission, needs to be better understood.

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14

Salmonella and other enteric organisms

D. Lightfoot

14.1 INTRODUCTION

Salmonellae are primarily intestinal parasites of humans and many other animals, including wild birds, domestic pets, and rodents; they may also be isolated from their blood and internal organs. They are found frequently in sewage, river and other waters, and soil (in which they do not multiply significantly). Thus, the presence of *Salmonella* in other habitats (water, food, natural environment) is explained by faecal contamination. Under suitable environmental conditions, they may survive for weeks in waters and for years in soils. They have been isolated from many foods, including the vegetables and fruit used by humans, and are important contaminants of animal protein feed

 Waterborne Zoonoses: Identification, Causes and Control. Edited by J.A. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D.O. Cliver, G.F. Craun, R. Fayer, and V.P.J. Gannon. Published by IWA Publishing, London, UK. ISBN: 1 84339 058 2.

supplements. They are pathogenic for many species of animals, giving rise to enteritis and typhoid-like diseases.

In most of the world, the prevalence of salmonellosis depends on the water supply, waste disposal, food production and preparation practices, and climate. Factors such as intensive rearing of animals, growing human populations, changes in the production of foodstuffs, and increasing movement and speed of movement of food as well as of human and animal populations have led to a continuing increase in the incidence of food poisoning worldwide.

14.2 DEFINITION

Salmonella are mainly motile (due to peritrichous flagellae), non-encapsulated, Gram-negative bacilli of the Enterobacteriaceae family. Most ferment glucose, maltose, and mannitol but do not utilize lactose. All pathogenic *Salmonella* other than *S*. Typhi produce gas. They do not hydrolyse urea or deaminate phenylalanine, usually form hydrogen sulfide on triple sugar iron agar, and use citrate as a sole carbon source. The many serovars in the group are closely related to each other by somatic and flagellar antigens, and most strains show diphasic variation of flagellar antigens. The type species is *Salmonella enterica* (Ewing 1986).

Salmonellae grow at temperatures ranging from 7 to 48 ºC, at pH 4–8, and at water activities above 0.93 (Baird-Parker 1991). Under special conditions, they may proliferate below 4 ºC (d'Aoust 1991) and withstand pH extremes (below pH 4) (Foster 1992). Salmonellae are capable of prolonged survival in faecal material, in slurry, or on pasture (Wray 1975).

The fact that salmonellae are able to survive and multiply readily in the environment is an important factor in the transmission and spread of salmonellosis. Examples quoted by Williams (1984) illustrate this: salmonellae will live for 28 months in naturally infected avian faeces; *S*. Heidelberg was recovered from contaminated poultry litter, grit, feed, and dust held for extended periods at room temperature (the poultry litter was positive at 7 months); *S*. Thompson survived 4–5 weeks in old poultry litter and 8–20 weeks in new litter; and *S*. Typhimurium survived in urban garden soil in England for at least 280 days.

Some serovars are known to be thermotolerant (Lui *et al*. 1969). A study by Humphrey and associates (1996) revealed marked differences between *S*. Enteritidis isolates and found that strains that were heat-tolerant were also more acid-tolerant and survived significantly better in the presence of hydrogen peroxide and on surfaces.

14.3 NOMENCLATURE

The genus *Salmonella* consists of two species: (1) *S. enterica*, which is divided into six subspecies — *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae*, and *S. enterica* subsp. *indica*; and (2) *S. bongori.* This nomenclature reflects the recent advances in *Salmonella* taxonomy (Le Minor and Popoff 1987; Reeves *et al*. 1989). There are 2501 serovars/serotypes in the genus *Salmonella* (see Table 14.1; Popoff 2001).

Table 14.1. Number of serovars in each species and subspecies of *Salmonella* (from Popoff 2001)

Species/subspecies	Number of serovars
S. enterica subsp.	
enterica	1478
salamae	498
arizonae	94
diarizonae	327
houtenae	71
indica	12
S. bongori	21
Total	2501

14.4 DISEASE/HOST SPECIFICITY/VIRULENCE

Most of the serotypes pathogenic to mammals, including humans, belong to *Salmonella enterica* subspecies *enterica* (i.e., subsp. 1). Some serovars have a habitat limited to a host species (host-adapted), such as humans (serovars Typhi, Paratyphi A), sheep (serovar Abortusovis), or fowl (serovar Gallinarum). Different syndromes can be caused by *Salmonella* serovars; for example, serovar Typhi causes typhoid in humans, serovar Typhimurium causes diarrhoea in humans and other animal species and a typhoid-like syndrome in mice, and serovar Abortusovis is responsible for abortion in ewes. Certain serovars, including Blegdam, Bredeney, Choleraesuis, Dublin (particularly associated with different extraintestinal infections in patients with acquired immunodeficiency syndrome [AIDS]), Enteritidis, Panama, Typhimurium, and Virchow, may also be invasive and cause pyaemic infections and localize in the viscera, meninges, bones, joints, and serous cavities. Most salmonellae, the ubiquitous serovars found in a number of animal species, tend to cause an acute, but mild, enteritis (Old and Threlfall 1998).

The estimated inoculum size (non-typhoidal *Salmonella*) required to cause symptomatic disease in healthy adult volunteers is 10^5–10^{10} organisms. The infectious dose varies depending on the age and health of the host, strain differences, and the vector. Evidence from particular outbreaks indicated that the infectious dose may be less than 100 organisms. The virulence mechanisms of *Salmonella* species are reviewed elsewhere (Old and Threlfall 1998).

14.5 EPIDEMIOLOGY

Salmonella and *Campylobacter* are the major bacterial causes of gastroenteritis worldwide. In many countries, the rate of salmonellosis is exceeded only by that of campylobacteriosis. The incidence of salmonellosis varies considerably between countries and within countries (case rates 10–>250/100 000 human population). For example, the overall case rate for salmonellosis in Australia in 2002 was 39.2, but the case rate for one of the territories, the Northern Territory, was 159.1 (Anonymous 2003). In the USA, 32 021 (case rate 11.7/100 000 population) *Salmonella* isolates were reported through the Public Health Laboratory Information System in 2000 (Anonymous 2002a). *Salmonella* causes an estimated 1.4 million illnesses each year in the USA (Mead *et al*. 1999). Due to a lack of national infectious disease surveillance schemes and/or the lack of national enteric reference laboratory facilities, the total worldwide burden of *Salmonella* infections is not known.

Many and varied factors contribute to the overall epidemiology of salmonellosis and the associated serovars, and the subject is very complex. *Salmonella* outbreaks, too many to mention, may directly or indirectly have an association with water. Two recently reported *Salmonella* outbreaks do, however, illustrate the relationship between animals, salmonellae, water, and, ultimately, foodborne disease outbreaks. The first relates to outbreaks of *S*. Poona infections in three US states associated with eating cantaloupe imported in the spring of consecutive years during 2000–2003. Possible sources of contamination included irrigation of fields with water contaminated with sewage, processing (cleaning and cooling) produce with *Salmonella*-contaminated water, poor hygienic practices of workers who harvest and process the cantaloupe, pests in packing facilities, and inadequate cleaning and sanitizing of equipment that came in contact with the cantaloupes. Iguanas have been proposed as the natural reservoir of *S*. Poona (Anonymous 2002c).

A traceback investigation identified a single sprout producer as the source of contaminated sprouts causing a cluster of *S*. Kottbus infections in California, USA. The contaminated batch of sprouts was linked to sprout seeds imported from Australia (Anonymous 2002b). *S*. Kottbus is a rarely reported cause of salmonellosis in the USA, but this serovar has been isolated from a number of

animal species in Australia. On the farm, sprout seeds may become contaminated through excretion from domestic or wild animals, runoff from domestic animal production facilities, use of improperly composted manure as fertilizer, use of untreated agricultural water, or improperly cleaned production or harvesting machines.

Aspects of animal salmonellosis will be dealt with in the following section.

14.6 ANIMAL RESERVOIRS

14.6.1 Livestock

14.6.1.1 Poultry

Some of the following information also applies to turkeys and ducks, but the discussion will focus on poultry (i.e., chickens), as they are one of the major sources of salmonellosis in humans.

Poultry and many other animals are often unapparent carriers, latently infected, or, less frequently, clinically ill. Poultry are commonly infected with a wide variety of *Salmonella* serovars. Infection is mostly confined to the gastrointestinal tract, and the birds often excrete *Salmonella* in their faeces and form a large reservoir and source of contamination for other animals and the environment. Horizontal spread of *Salmonella* may occur in a number of ways: during the hatching of chickens, from aerosols containing *Salmonella*, from *Salmonella*-contaminated feed or water, or from rodents. A definite correlation exists between the age of the chicken and the number of organisms required to induce infection detectable by shedding (range 10^2–10^{10}; Poppe 2000). When follicles in the ovary are infected or the developing eggs become infected with *Salmonella* in the oviduct, this is termed vertical transmission of infection. The poultry-specific serovars, *S*. Pullorum and *S*. Gallinarum, are the main serovars transmitted vertically. Other serovars that may cause a transovarian infection include *S*. Typhimurium, *S*. Enteritidis, and *S*. Heidelberg.

Considerable variation in the rates of carriage occurs, but *Salmonella* species have been isolated from up to 50% of poultry. The occurrence of the most common *Salmonella* serovars in domestic fowl varies between different countries and at different times. *S*. Typhimurium was among the most common serovars consistently isolated from poultry in many countries in the period from 1950 until the late 1970s. During the last 10–15 years, *S*. Enteritidis has replaced *S*. Typhimurium as the most common serovar in poultry in many countries worldwide (Poppe 2000). The prevalence of *S*. Enteritidis in poultry, particularly in eggs, has led to a worldwide epidemic of human *S*. Enteritidis infections (Tauxe 1999). Until recently, *S*. Enteritidis phage type 4 (PT4) has been the dominant strain worldwide. The challenge posed by *S*. Enteritidis is

related to the extraordinary biology of the infection in the avian host. It has now been clearly established that these strains can cause lifelong colonization of the peri-reproductive tissues of the hens from which the egg can be colonized before the shell is formed. A comprehensive monograph on all aspects of *S*. Enteritidis has been published (Saeed 1999).

Vertical transmission of infection may occur via litter, faeces, feed, water, fluff, dust, shavings, straw, insects, equipment, and other fomites contaminated with *Salmonella* or by contact with other chicks or poults, rodents, pets, wild birds, other domestic or wild animals, and personnel contaminated with *Salmonella*.

The faecal excretion by poultry, the transportation and disposal of slurry and manure from poultry-raising facilities, the transportation of slaughter offal to rendering plants, the cross-contamination of rendered meat meal and other poultry and animal by-products by dust, and contamination of equipment used in rendering plants and feed mills and for the transportation of all poultry-related by-products all contribute to spreading *Salmonella* in the environment.

Pigeons, sparrows, other birds, rodents, cats, dogs, and insects may be contaminated by contact with or the ingestion of spilled meat meal, feather meal, and other animal by-products outside the rendering departments at slaughtering plants and at poultry houses from conveyor belts, hoppers, and open trucks. This may lead to contamination of effluents, surface waters, creeks, rivers, lakes, pastures, and soils; to the colonization of many animal and bird species; and to contamination of animal feeds. It may also contribute directly to the recolonization of farm animals (Poppe 2000).

14.6.1.2 Cattle

Salmonella infections are an important cause of mortality and morbidity in cattle, and subclinical infections occur frequently. Infection is usually by the mouth, and numerous experiments have shown that oral doses ranging from 10^6 to 10^{11} of *S*. Dublin and from 10^4 to 10^{11} of *S*. Typhimurium are necessary to cause disease in healthy cattle (Wray and Sojka 1977). *S*. Typhimurium and *S*. Dublin are the major serovars isolated from cattle, although the distribution of these two serovars may differ between countries, and *S*. Dublin is thought not to be present in some countries.

Until about 1960, nearly all salmonellae were sensitive to a wide range of antimicrobial agents; since 1962, however, resistance, frequently plasmid-mediated, has appeared in salmonellae worldwide. The relative importance of antibiotic resistance and the serotype in which it occurs differ from country to country. For example, in the United Kingdom, resistance is common in serotypes associated with bovine animals (e.g., Typhimurium), but relatively

uncommon in serotypes associated with poultry. Within *S*. Typhimurium, resistance is found in only a few phage types associated with bovine animals (e.g., definitive phage types [DTs] 29, 193, and 204c). The acquisition of resistance by the particular strains of *S*. Typhimurium (DT29, DT193, and DT204c) seemed to coincide with the introduction and use of antimicrobial agents to combat infections in calves (Old and Threlfall 1998).

Of particular importance in this increase of incidence of multiresistance (to four or more antimicrobial agents) in *S*. Typhimurium since 1991 has been an epidemic in cattle and humans in England and Wales of multiresistant strains of *S*. Typhimurium DT104 of the R-type ACSSuT. Since 1992, a disturbing feature of infections with multiresistant strains has been the appearance of additional resistance to trimethoprim and ciprofloxacin (Threlfall 2000). In contrast to *S*. Typhimurium DT29 and DT204c, all resistance genes in DT104 are inserted in the chromosome (Threlfall *et al.* 1994). In the USA, the rate of occurrence of the resistant profile (ACSSuT) among human *S*. Typhimurium isolates jumped from 7% in 1990 to 35.3% in 1997 (Tauxe 1999).

The various sources of infection include introduction of infected animals into a herd, mixing of young susceptible animals and their subsequent travelling, "stress" (as in confined feedlot operations), which may either exacerbate disease or increase the susceptibility of cattle to *Salmonella* infections, persistence of salmonellae in animal accommodation after depopulation, animal wastes, pasture contamination, sewage sludge used as fertilizer, waterborne infection, contaminated foodstuffs, and introduction of infection onto farms by free-living animals (Wray and Davies 2000). Modern intensive cattle production systems produce large amounts of slurry, which has highlighted the risk of pasture contamination because of disposal problems. The subject was comprehensively reviewed by Jones (1992).

Cattle constitute an important reservoir for human infections via both direct contact with an infected animal and ingestion of *Salmonella*-contaminated meat.

14.6.1.3 Sheep

Salmonella infections in sheep have been recorded in most countries of the world. *S*. Abortusovis is the main pathogenic serovar (also host-specific) for sheep, causing abortions during the last 4–6 weeks of pregnancy. Environmental factors, including poor feeding, have been linked to the development of abortions caused by *S*. Abortusovis. In range sheep, the most common occurrence of salmonellosis is during times of drought. Salmonellosis in sheep is extensively reviewed by Wray and Linklater (2000). Hunter and associates (1976) found that *S*. Typhimurium spread from infected sheep into nearby watercourses and produced infections in animals drinking downstream.

14.6.1.4Pigs

Pork meat and pork meat products are a major source of foodborne salmonellosis. The organism now known as *S*. Choleraesuis was first isolated from pigs in 1886 and was associated with the disease swine fever. Today in the developed world, *S.* Choleraesuis is rarely identified; however, the prevalence of other *Salmonella* serovars has increased.

A number of experimental infection studies have demonstrated that, during acute disease, pigs will shed up to 10^6 *S.* Choleraesuis or 10^7 *S.* Typhimurium per gram of faeces (Fedorka-Cray *et al*. 2000).

14.6.2 Other animals

A wide variety of animals, including mammals, birds, reptiles, and insects (such as cockroaches), in the natural environment may be reservoirs of *Salmonella*, irrespective of the country or region. Even in urban environments, exotic animals kept as pets can be a source of *Salmonella*. Salmonellosis in humans has been attributed to pets, including tropical fish, terrapins, and lizards (Murray 2000). Cats, dogs, and horses may be asymptomatic carriers or exhibit *Salmonella* gastroenteritis (Wray and Wray 2000).

Pigeons colonizing two water towers in Missouri, USA, were considered the source of the *S*. Typhimurium that contaminated the water supply, causing 600 illnesses and four deaths (Geldreich 1998).

14.7 WATER AS A VEHICLE

There are five critical elements in the transmission of infectious agents through water: (i) the source of the infectious agent, (ii) specific water-related modes of transmission, (iii) attributes of the organism that allow it to survive and possibly multiply and to move into and within the aquatic environment, (iv) the infectious dose and virulence factors of the organism, and (v) host susceptibility factors (Moe 2002). The transmission of salmonellae through water can be described using the five stated elements.

The microbial flora of sewage is predominantly from faecal wastes, including pathogens shed from individuals in the community. Urban storm runoff, street flushings, automatic car washing operations, and the processing of garden produce in markets, homes, and restaurants contribute to the microbial flora.

Solid waste, generally referred to as garbage, contains a multitude of materials, including faecal material. Much of the faecal material in urban areas is derived from disposable nappies, pet litter material, and faeces of rodents foraging for

food in these waste collections. Poor placement of landfill sites may result in the migration of leachates into nearby surface waters and groundwater resources.

14.7.1 Natural waters

14.7.1.1 Surface waters

Natural waters are replenished through rain. The more dust encountered, the greater the risk of bacterial contamination. In remote areas where human and farm animal populations are sparse, most organisms in water originate from soil with little evidence of contamination. As these waters travel down a watershed, contact with agricultural and industrial activities increases, and the river becomes laden with a variety of domestic and industrial wastes. The greater the magnitude of faecal pollution, the greater the chance that some bacterial pathogen may be present.

14.7.1.2 Groundwater

Groundwater resources are the major source of water supply for many communities, farms, and individual families worldwide. The quality of water flowing from springs depends mainly on their source and surroundings.

14.7.1.3 Cisterns

This source of water is generally rainfall from some catchment surface, which often is a residential roof or paved hillside down which water drains into a storage tank. Bacteriological quality is a reflection not only of rainwater and dust particles, but also of faecal contamination from birds perching on the catchment surface. Many wild bird species, particularly pigeons and seagulls, are known to harbour salmonellae.

14.7.1.4 Estuarine areas

These are the areas where fresh water mixes with the saltwater environment, either through direct discharge to the sea or by tidal flooding of freshwater pools near the ocean. Water quality protection is particularly important for shellfish cultivation.

14.7.1.5 Coastal waters

Major sources of pollution are stormwater runoff along the beach areas, release of sewage offshore, sanitary wastes from ships in harbour, and improper disposal of rubbish. Seagulls are scavengers that frequent open rubbish dumps, eat contaminated food wastes, and contribute their faecal droppings to coastal lakes. Fresh seawater brings about a fairly rapid decline in faecal bacteria, salmonellae,

and viruses. The main inactivating agent is solar radiation at wavelengths less than 400 nm.

14.7.2 Food processing effluent

Beet processing and sugar cane production have a drastic impact on the bacterial flora and the self-purification capacity of receiving waters. Most troubling is the persistence of *Salmonella* under these conditions (Geldreich 1972).

14.7.3 Animal effluent

The use of animal excreta on farmland presents potential health hazards to domestic animals and humans (Wray 1975).

Animal feedlot operations that require the confinement of cattle in small areas create faecal waste removal equal to domestic waste discharges of small cities. In cattle feedlot operations, the density of cattle per square kilometre may approach 4000 animals (approximately 39 per hectare). The closeness of farm animals in confined feeding operations invites the spread of disease, such as salmonellosis, in a healthy herd or flock. Under such restrictions, removal of faecal wastes is a major disposal operation.

If the animal waste is not discharged to a lagoon or landfill, the stormwater runoff over the animal feedlots will bring massive loads of faecal pollution to the drainage basin. Poultry farm faecal wastes, perhaps with associated salmonellae, may contribute similar problems.

14.7.4 Other effluents

In major river systems receiving discharges of meat processing wastes, raw sewage, and effluents from ineffective sewage treatment plants, the density of *Salmonella* spp. may be substantial (Geldreich 1998).

14.8 DETECTION IN WATER

The methods for the detection and isolation of salmonellae from environmental fresh waters, drinking-water, wastewater, and sludge are both diverse and complicated. Various issues and techniques are discussed extensively by Toranzos *et al.* (2002) and Cooper and Danielson (2002).

It has been suggested that salmonellae may occasionally be present in water in the viable but non-culturable forms. In this instance, *Salmonella* may not be detected using conventional culture techniques. Furthermore, any molecular

technique used to detect salmonellae must be able to detect viable organisms if the results are to have any public health relevance.

14.9 EMERGING PROBLEMS

For the last two decades, *Salmonella enterica* serovar Enteritidis, particularly PT4, has caused a growing worldwide pandemic (except in Australia) (Tauxe 1999). This pandemic makes *S*. Enteritidis the most common of non-typhoid serovars of *Salmonella* in many countries and has lent new impetus to food safety efforts everywhere. In the last couple of years, the incidence of *S*. Enteritidis PT4 has declined mainly due to mass vaccination of poultry flocks. However, there has been a steady rise in the incidence of other *S*. Enteritidis phage types, such as PT6 and PT6a. This is of growing concern, as these phage types have acquired resistance to some antibiotics. The majority of *S*. Enteritidis PT4 strains remain sensitive to antibiotics.

In contrast to previous epidemic multiresistant phage types of *S*. Typhimurium, such as DT29, DT193, DT204, and DT204c, which for the most part are confined to cattle, multiresistant DT104 has become common in poultry (particularly turkeys), pigs, and sheep. Human infection with multiresistant DT104 has been associated with consumption of chicken, beef, pork sausages, and meat paste and, to a lesser extent, with occupational contact with infected cattle. *S*. Typhimurium DT104 has caused outbreaks of infection in food animals and humans in numerous countries (Threlfall 2000). There has even been an international outbreak traced to DT104-contaminated sesame seed product from Turkey (Anonymous 2001). Of particular concern has been the resistance of the organism to a wide range of therapeutic antimicrobial agents. Also, in some countries, there have been reports that this organism may cause more serious disease (Threlfall 2000).

Another *Salmonella* causing concern is the multiresistant *S*. Newport, first isolated from dairy cows in the USA. The numbers of these strains continue to increase.

14.10 ZOONOTIC SPECIES (NOT *SALMONELLA*)

Many other bacterial species, including *Shigella*, *Vibrio*, *Aeromonas*, and other members of the family Enterobacteriaceae known to cause human gastroenteritis, are not generally regarded as zoonotic organisms. However, waterborne transmission is a recognized feature of these bacteria.

14.10.1 *Yersinia enterocolitica*

The natural reservoirs of *Y. enterocolitica* include a variety of domestic and wild species. The prominent hosts are pigs, rodents, rabbits, sheep, goats, cattle, horses, cats, and dogs. It has also been found in turkeys, ducks, geese, pigeons, pheasants, and canaries. In some animals, it is a significant pathogen.

Yersinia are localized in the oropharangeal cavities and lumens of the gastrointestinal tract of animals. They are excreted in the faeces. Studies of pigs in slaughterhouses suggested that the tonsils and tongues contained *Y. enterocolitica.* Raw intestines of pigs (chitterlings) have been implicated as a source of human infection. *Yersinia* have also been detected in raw milk (Butler 1998).

Humans are accidental hosts for *Yersinia* bacteria, after they ingest contaminated animal products. An inoculum of 10^9 organisms is required to cause infection. *Yersinia enterocolitica* is a relatively infrequent cause of diarrhoea and abdominal pain in the USA, but is more common in northern Europe. Infections have been documented in other parts of the world.

Yersinia enterocolitica is a cold-tolerant organism, withstands freezing and thawing, and can survive in extended periods in frozen conditions. It can multiply at temperatures as low as -5 ºC, is capable of growth at temperatures up to 44 ºC (optimum range of 22–28 ºC), tolerates a pH range 4.6–9 (optimum 7–8), and can grow in the presence of 5% sodium chloride.

Many of the concerns described for the other animal species with regard to salmonellosis are equally relevant to the control of yersiniosis.

14.11 SUMMARY AND CONCLUSION

Salmonellae are ubiquitous. A variety of issues — such as the intensive rearing of food animals; contamination of pastures by animals; the production of animals, vegetables, and fruits in regions with poor water quality and handling practices; disposal of pathogen-contaminated waste; production of enormous amounts of animal and industrial waste; the movement of food to all parts of the world — may contribute to contamination of the environment, including all water sources, with salmonellae. As well as control measures to ensure delivery of microbiologically safe water and pathogen-free food, national and international surveillance schemes and specialized typing laboratory facilities must be established and maintained to monitor and identify trends in infectious enteric disease.

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15

Prospects of waterborne viral zoonoses

D.O. Cliver and C.L. Moe

15.1 INTRODUCTION

Viruses are transmitted as particles much smaller than bacterial cells (diameters 25–100 nm). A virus particle comprises a small amount of nucleic acid (RNA or DNA, either single- or double-stranded) coated with protein (Flint *et al*. 2000). Some viruses also have an outer, lipid-containing envelope, but this is rare among waterborne viruses. The nucleic acid contains all of the information required to instruct a host cell to produce progeny virus in a factory-like operation. The protein coat protects the viral nucleic acid during transmission through the environment and the digestive tract until susceptible cells are reached; it must interact specifically with the receptors on the surface of a

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susceptible cell; and, because the coat is protein, it also functions as the antigen against which antiviral immunity develops (Nuanualsuwan and Cliver 2003).

15.1.1 Virus specificity

Much public health and animal health policy is based on the host specificity of viruses. It is assumed that each virus has a distinct and limited range of host species that it can infect, with some possible exceptions (Mahy and Brown 2000; Enriquez *et al*. 2001). Beyond species specificity, viruses are restricted as to which tissues of the host's body they can infect; this is called *tropism*, and it affects both the mode of transmission (route of entry of the virus into the host) and the disease that the virus infection may cause. Most waterborne viruses are thought to be transmitted by a faecal–oral cycle, so the virus is shed via the intestines and infects upon ingestion. This requires a tropism that includes the lining of the gastrointestinal tract — most likely the small intestine. Viruses that infect via the intestine may cause gastroenteritis (e.g., noroviruses) but may also have *secondary tropisms* in other tissues. The enteroviruses can infect the central nervous system and cause poliomyelitis or meningitis, whereas the hepatitis viruses infect the liver.

It appears that both host specificity and tropism are mediated in the first instance by the ability of a virus to attach to receptors on the surface of a cell. The interaction of the virus with the cell receptor is required in order for the viral particle to be engulfed and uncoated, so that the viral nucleic acid can enter the cell's interior and direct the cell to begin producing progeny virus. If the virus cannot interact specifically with receptors on the cell, it will not attach, and no infection will result. Under experimental conditions, viral nucleic acid can be delivered inside of a cell that lacks receptors, and the cell will produce progeny virus, which suggests that, in many instances, the cell apparatus that produces the virus is not specific. There may be exceptions.

15.1.2 *In vitro* infectivity

A great deal of virus research has been done using cultured animal cells that partially reflect the host specificity, but not the tropisms, of viruses. Many of the cell lines used with human enteroviruses were originally derived from the kidneys of various monkey species. These monkey species are probably susceptible to most of the human enteroviruses *in vivo*, but their kidneys would not be infected. Monkey kidney cell cultures can be infected by enteroviruses of monkeys, humans, and cattle, and the interaction of the virus with the cells' receptors is an indication of the viruses' infectivity. However, many important enteric, waterborne viruses of humans — notably the entire norovirus and

sapovirus genera — will not infect cultured cells from any source tested to date. It is also probable that monkeys are not infected by bovine enteroviruses *in vivo*. Thus, it is not clear that *in vitro* infectivity is relevant to the *in vivo* host ranges of viruses.

15.2 WATERBORNE ZOONOSES

The present subject concerns waterborne zoonotic agents. To be included, a virus would have to have a reservoir in a non-human animal species and be transmissible via water to people. Although no such transmission appears to have been reported to the US Centers for Disease Control and Prevention, it is possible to consider the circumstances under which such transmission might occur, as well as how the event might be detected. Waterborne viral zoonoses may be more likely to occur in areas where animal contamination of water supplies is common and water supplies are unprotected and untreated. However, recognition and investigation of endemic or epidemic waterborne viral zoonoses are challenging because of inadequate diagnostic techniques for many viral infections and limited methods to detect viruses in water.

15.2.1 Virus replication

Virus replication takes place in appropriate living host cells. Unlike bacteria and other cells, which multiply by duplicating their parts and then splitting into "daughter cells," virus parts are produced and assembled into progeny virus in a factory-like system in the host cell (Flint *et al*. 2000). The nucleotide sequence of the viral nucleic acid is passed in the nucleic acid of the progeny virus; the nucleotide sequence also specifies the amino acid sequence of the viral coat protein and other virus-specific proteins.

The most commonly waterborne and foodborne viruses belong to the picornavirus and calicivirus families, which contain single-stranded RNA. Early in the infection, the RNA serves as messenger and is translated into protein, both protein for the viral coat and enzymes that are essential to virus replication. One of these enzymes directs production of a complementary RNA strand on the original. This negative-sense strand then serves as the template for synthesis of positive-sense RNA that will be incorporated into the progeny virus. The two stages of RNA-dependent RNA synthesis are said to be much more error-prone than the host cell's system, in which DNA is synthesized on a DNA template and later specifies the nucleotide sequence of RNA transcribed from the DNA. Cells have special "chaperones" to minimize errors in nucleic acid synthesis and transcription, but these do not participate in RNA-dependent RNA synthesis.

15.2.2 Genetic variation

Transcription errors in viral RNA synthesis provide frequent opportunities for genetic variation. The variation may yield RNA that is not functional, whereby the progeny virus is not infectious. The virus might conceivably change host specificity or even tissue tropism. Expression of such genetic change would often come only after the altered virus had entered another host cell, perhaps in another host organism. The ability of the progeny virus to start a new infectious cycle would depend on the ability of its coat protein to attach to the receptors of another cell and induce engulfment. Therefore, a change of host specificity of a virus would result only if the alteration in the RNA produced a corresponding alteration in the receptor affinity of the coat protein. Because the coat protein is synthesized separately, not necessarily on the RNA molecule that is eventually incorporated into the progeny virus particle, the probability of a sustainable transition is small. Nevertheless, infected cells produce hundreds or thousands of progeny virus particles each, and infected animal hosts produce billions of progeny viral particles in the course of an infection, so the possibility of a durable genetic alteration is not negligible. Experience shows that such events are rare, but it may also be that an entire family of viruses, such as the picornaviruses, derive from a single progenitor whose progeny branched out further and further as regards host range, tropism, and antigenic specificity. No fossil record exists, but there has presumably been ample time for trial-and-error variation and adaptation among this broad family.

15.2.3 Criteria

To function as a waterborne zoonosis, a virus would need to carry, on its coat protein, the ability to attach to receptors of more than one species. The animal reservoir species would need to shed the virus in a way (usually via faeces) that would lead to water contamination, and a human would need to ingest the contaminated water. It is also essential that the virus be robust enough to retain infectivity in transit in the environment between hosts — particularly in the water environment. Such durability seems to be a function of the coat protein, rather than the nucleic acid, and the durability needed for transmission via water seems to be more prevalent among enteric viruses than in viruses transmitted by the respiratory or other routes. Nevertheless, if millions of infectious doses are shed into water, inactivation of the majority of these will not preclude transmission via water. This might happen with significant frequency around the world without being noticed, so indirect criteria may be needed to determine the incidence of such indirect transmission.

The criteria for determining whether a virus can function as a waterborne zoonosis can be summarized as follows:

(1) *Animal reservoir*: Does the agent regularly infect at least one animal species, independent of exposure to humans?
(2) *Transmission to humans*: Are humans who are in contact with the alleged animal reservoir more frequently infected with this virus than people who are not?
(3) *Shedding*: Is the candidate virus shed by the reservoir animal species in ways that might lead to contamination of water?
(4) *Stability*: Is the candidate virus stable enough in the water vehicle to permit transmission by this route?

15.3 CANDIDATE AGENTS

No confirmed examples of waterborne viral zoonoses have been reported. However, there are a number of viruses that are potentially transmissible between species, and water may serve as a vehicle for some of these on occasion.

15.3.1 Apparently human reservoir — possible animal carriage

Among the human enteroviruses, the coxsackieviruses were first detected by their ability to infect injected suckling mice. One of these viruses, coxsackievirus B5, has been found to be closely related to the virus that causes swine vesicular disease. Coxsackievirus B5 infects swine experimentally (Monlux *et al*. 1975), as well as explant cultures of swine intestine *in vitro* (Heinz *et al*. 1987; Heinz and Cliver 1988). Human infections with swine vesicular disease virus have occurred (Brown *et al*. 1976).

A virus that came to prominence in 2003 is the agent of severe acute respiratory syndrome (SARS). The apparent cause is a new member of the coronavirus group (Kuiken *et al*. 2003). Transmission is most often from person to person over short distances, via aerosols, but a large cluster of cases in a high-rise apartment complex in Hong Kong may have been due to spreading in aerosols from sewage (WHO 2003a). Another area of investigation of SARS is the quest for animal reservoirs (WHO 2003b). Some captured feral animals that were intended to be eaten by people in Guangdong Province, China, have been reported to be infected with the SARS virus, but confirming these findings has proven difficult (Normile and Enserink 2003).

15.3.2 Bovine reservoir — possible transmission to humans

Cattle are hosts to various serological types of enteroviruses. From the earliest investigations, these agents have been known to infect primate cells *in vitro*. However, they do not generally infect cultured human cells. When subjected to reverse transcription–polymerase chain reaction with the primers that react with all human enteroviruses, they produce an apparently identical amplicon. The bovine enteroviruses occur in surface waters and have been used to identify sources of faecal contamination (Ley *et al*. 2002). They are not known to infect humans, but undetected occurrences are possible. They are not associated with severe illness in cattle — certainly nothing resembling the severe central nervous system syndromes (poliomyelitis, meningitis, etc.) that human enteroviruses cause in people.

There are many animal rotaviruses, and both bovine and porcine rotaviruses have been detected in drinking-water (Gratacap-Cavallier *et al*. 2000). Reports of bovine–human reassortant strains of rotavirus (viruses that contain gene segments from both human and animal strains) in infants in Bangladesh (Ward *et al*. 1996) suggest that co-infection with both human and bovine rotavirus must have occurred in a human or animal host. In areas where humans and cows live in close proximity, it seems plausible that faecally contaminated water could play a role in the transmission of bovine rotaviruses to humans and forming bovine–human reassortant strains. Efforts to develop rotavirus vaccines for humans have included human challenge studies with bovine rotavirus strains and bovine–human reassortant rotavirus strains (Vesikari 1994). These studies demonstrate that it is possible for humans to become infected with bovine and bovine–human rotaviruses. However, these viruses do not multiply effectively in the human host and tend to cause short, non-invasive infections (Vesikari 1994).

The prions that cause bovine spongiform encephalopathy (BSE, “mad cow disease”) are smaller than viruses and contain no nucleic acid. They are the apparent cause of variant Creutzfeldt-Jakob disease (vCJD) in some of the humans who have ingested them (Schonberger 1998). The vehicle in transmission of BSE to humans as vCJD has been thought to be beef products containing infectious prions (Brown 2001). Transmission via water has not been ruled out, and rigorous regulations have been imposed on BSE carcass disposal, including efforts to protect groundwater from potential contamination.

15.3.3 Swine reservoir — possible transmission to humans

The porcine digestive tract is reasonably similar to that of humans, and swine have their own set of enteroviruses. These generally do not infect primate cells

in vitro. However, as noted above, the swine vesicular disease virus, which is closely related to human coxsackievirus B5, has apparently infected humans and produced illness (Brown *et al*. 1976). Of four such infections, three probably resulted from direct exposure to infected animals and the other from exposure to the virus in the laboratory; two of the illnesses were severe, with one hospitalization.

Another concern is the presence in swine of a close relative of the human hepatitis E virus (HEV). It is highly prevalent on Taiwan (Hsieh *et al*. 1999), is widespread in the USA (Huang *et al*. 2002a), and is probably present in swine in much of the world. The swine HEV shows a genetic organization like that of human HEV, and isolates of human and swine HEV share 92% nucleotide identity (Emerson and Purcell 2003) and >97% amino acid identity in open reading frames 1 and 2 (Meng *et al*. 1998b). Also, the two viruses cross-react serologically to a considerable extent (Meng *et al*. 1997). Evidently, neither agent replicates well in cultured human or swine cells in the laboratory. The swine HEV has not been associated with overt illness in infected swine. It is transmitted from pig to pig by contact and is shed in faeces, but it has not been shown to infect pigs by peroral inoculation. Intravenous infection of swine with both the swine HEV and some strains of human HEV has been reported (Meng *et al*. 1998a). People may be at risk of infection by contact with swine, especially where sanitation is poor. Serological surveys of swine veterinarians in the USA have indicated a higher prevalence of anti-HEV than in the general population, but the prevalence of antibody in the veterinarians was seemingly not related to their degree of exposure to swine (Meng *et al*. 2002). Antibody prevalence in farm workers was apparently related to exposure to swine (Withers *et al.* 2002). Swine HEV may represent a risk to human health in certain circumstances, but transmission via water seems unlikely, given that the agent does not infect swine perorally. Recent reports from Japan, discussed below, indicate that the HEV that occurs in wildlife there is transmissible to humans who eat raw flesh (Tei *et al*. 2003). Anti-HEV has been reported in rats, sheep, cattle, and chickens, and an HEV-like virus was recently detected in chickens. However, it is not clear at this time if these animal HEV strains are similar to human strains (Emerson and Purcell 2003).

Swine and bovine caliciviruses have been isolated from stool samples collected from farms in Europe and Japan (van der Poel *et al*. 2000). These viruses are genetically similar to human caliciviruses, and they may have evolved from a common ancestor. However, animal-to-human transmission of these enteric caliciviruses has not been demonstrated.

Pigs are believed to serve as intermediate hosts for the adaptation of avian influenza viruses to humans. Genetic reassortant strains of human and animal influenza viruses have been detected in swine and in humans, and these novel

strains have the potential to cause pandemics (Olsen *et al*. 2002). There is also historic evidence that influenza of swine origin has been transmitted to humans (Centers for Disease Control and Prevention 1988). However, there are no data to suggest that water has been involved in the transmission of these viruses from swine to humans. Several studies suggest that the degree of human contact with swine is the primary risk factor for zoonotic transmission of swine and reassortant influenza viruses (Olsen *et al*. 2002).

15.3.4 Poultry reservoir — possible transmission to humans

Poultry have been a continuing concern as a source of influenza virus that is transmissible to humans, particularly in Hong Kong and mainland China. However, the water vehicle does not seem to have been implicated in these incidents. Avian enteric coronaviruses are perhaps not threats to human health but may be useful surrogates for experiments with transmission of the SARS virus via water.

A further concern is the recent report of an avian HEV in the USA (Haqshenas *et al*. 2001) and apparently elsewhere (Haqshenas *et al*. 2002). The agent is evidently widespread in chicken flocks in the USA and appears to cause enlargement of the liver and spleen (Huang *et al*. 2002b). Whether it is transmissible to humans or swine remains to be seen.

15.3.5 Wild animal reservoir — possible transmission to humans

Pinniped caliciviruses, notably a group that infects California sea lions (*Zalophus californianus*) and causes vesicular disease, have been known for years. Antibodies in sea lions have been found to neutralize the Tillamook calicivirus that infects calves in Oregon (Barlough *et al*. 1987). San Miguel sea lion virus type 13 produced severe vesicular disease when inoculated into weaned pigs, and the infection spread to uninoculated pigs in the same pen (Berry *et al*. 1990). The agents are apparently transmissible among species and probably via water, but waterborne human infections have not been demonstrated.

Recently, four human cases of hepatitis E in Japan have been attributed to eating raw venison from wild-killed Sika deer (Tei *et al*. 2003). The HEV genome from a frozen sample of the venison essentially matched those from the patients. The level of contamination of the venison was estimated at 10^5 RNA copies per gram. Those who were ill reported eating about 100 g each, whereas another person who ate less remained well. Subsequently, there was a report that

two men in another prefecture had contracted hepatitis E from eating wild boar meat that had not been cooked.

Prions of chronic wasting disease (CWD) are probably transmitted by the faecal–oral route among North American cervids (Williams and Miller 2002). If CWD prions are shed in the faeces of infected animals, these prions are likely to occur in water in enzootic areas. Transmissibility of CWD to humans has not been demonstrated but is under study.

15.3.6 Rodent reservoir — possible transmission to humans

There have been reports of prevalent HEV infections in wild rats (but not mice) in Japan (Hirano *et al.* 2003), the USA (Kabrane-Lazizi *et al.* 1999; Smith *et al.* 2002), and Nepal (He *et al.* 2002), which is a hyperendemic area. Rat infections were detected in association with an outbreak of hepatitis E in Russia (Karetnyi *et al*. 1993), but elsewhere the relationship between rat infections and human disease is far from clear-cut.

Hepatitis E is said to be transmitted by a faecal–oral cycle, which is also true of hepatitis A; both are more prevalent in the poorer countries of the world, where hygiene is deficient. Still, hepatitis A typically infects children early in life in those countries, so that essentially all are immune by the age of 5 years. The same is clearly not true of hepatitis E, which causes outbreaks (in developing countries), affecting principally young adults. If both rats and humans are sources of HEV, one would expect infections to occur earlier in life, rather than later, in these countries.

Antibody against HEV is common in rats in some areas, which is a clear sign that infection has occurred. The Russian study reported the presence of the virus in rat faeces (Karetnyi *et al*. 1993), but other infections seem to have been demonstrated by testing blood. Those who are studying hepatitis E in swine say that they have been unable to infect the animals perorally, and the experimental infections in laboratory rats are said to have been accomplished by intravenous injection (Maneerat *et al*. 1996). Thus, the faecal–oral mode of transmission from these candidate reservoirs to humans awaits demonstration.

Antibody to HEV is more prevalent in humans exposed to apparently infected rats or swine in the USA, but human illnesses resulting from the infections are generally not recorded (Kabrane-Lazizi *et al*. 1999; Smith *et al*. 2002). This suggests that the animal version of HEV (at least in the USA) might fortuitously be non-pathogenic in humans.

15.4 CONCLUSIONS

Several viruses meet some of the criteria for a waterborne zoonotic virus outlined in section 15.2.3:

- **Criteria 1 and 3:** There are clearly animal and human viruses that are excreted into water. Both animal and human viruses have been detected in drinking-water, ambient waters and sewage.
- **Criterion 2:** There is some evidence that humans in contact with some animal reservoirs may be more frequently infected with certain viruses (HEV and swine handlers and swine veterinarians). This second criterion can be difficult to judge based on serological evidence, because antibodies made against human viruses can cross-react with closely related animal viruses. Animal analogues of the most frequently observed waterborne human viruses, members of the picornavirus and calicivirus families, are well known. Generally, viruses are believed to be relatively host-specific; however, given the high error rate in transcription of the RNA of such viruses, the opportunities for host range mutations are great. To be effective, such a mutation would have to be manifest as a change in both the nucleotide sequence and the amino acid sequence that governed the receptor affinity of the virus. Since the virus coat protein does not need to have been translated from the same RNA molecule that it later incorporates as progeny virus, this combination need not happen. All the same, enormous numbers of viruses are produced by one host in the course of an infection, and animals all over the world have been infected with viruses through endless generations, so the opportunities for such events are plentiful. There are increasing examples of cross-species transmission of animal viruses to humans (e.g., avian influenza virus, monkeypox virus, West Nile virus, equine morbillivirus, and possibly Ebola virus and SARS). Reassortant viruses (rotaviruses and influenza viruses) provide evidence that co-infections of animal and human viruses do occur and result in new virus strains that are infectious to humans.
- **Criterion 4:** There are data indicating that many human enteric viruses are quite stable in water and also in soil, so it is possible that viruses or sewage deposited on soil can move into water during rainfall and floods. There are few data on the stability of animal viruses in water. Given the genetic similarity between many human and animal viruses, it seems likely that most animal enteric viruses would also be stable in water, wastewater and soil.

To date, there is no documented evidence for humans to have become infected from animal viruses present in water. Water is one vehicle that is commonly contaminated by both human and animal faeces and is ingested by both humans and animals. Therefore, it is plausible that waterborne transmission of animal viruses and reassortant viruses can occur. Lack of documentation of these events may be due to the relatively poor diagnostic methods for viral infection and the difficulty in detecting viruses in water. Human enteric viral infections are rarely confirmed by laboratory testing except in outbreak investigations. Detection of viruses in animal faeces has been limited to a few research studies, and sequencing and comparing human and animal enteric virus isolates have been done only relatively recently. Increasing concern about waterborne zoonoses, documentation of animals that are infected with enteric viruses closely related to human strains, and growing awareness of cross-species transmission of animal viruses should promote more investigations in this area, which will provide better information on the true risks of waterborne viral zoonoses. Prions of CWD seem more likely than those of BSE to occur in water, but only BSE prions are yet known to infect humans perorally; more research is needed to examine this potential risk.

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16

Waterborne zoonotic protozoa

R. Fayer

16.1 INTRODUCTION

Pathogens originating in the faeces of humans or other animals, including microsporidia, amoebae, ciliates, flagellates, and apicomplexans, have been found in surface waters worldwide. Transport and survival of these pathogens to estuarine and marine waters, although possible and even likely, have not been well studied or documented.

The present chapter contains synopses of major waterborne zoonotic protozoa. Life cycles, prevalence, distribution, disease, treatment, and other factors relative to environmental contamination are discussed. Pathogens of concern include the microsporidian species of *Encephalitozoon* and *Enterocytozoon*, the amoeba *Entamoeba histolytica*, the flagellate *Giardia intestinalis*, the apicomplexans *Toxoplasma gondii*, and species of

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Cryptosporidium. Other waterborne protozoa have been omitted from this review for a variety of reasons, some because they are known not to be zoonotic, others because they are not known to be zoonotic.

The amoebae *Naegleria* and *Acanthamoeba* are free-living organisms found in soils and moist or aquatic environments (Marshall *et al.* 1997). Rainfall and runoff can transport these organisms from soil to aquatic environments. There are six species of *Naegleria*, but *Naegleria fowleri* is the primary human pathogen causing primary amoebic meningoencephalitis, a disease that is almost always fatal. *Acanthamoeba* species produce granulomatous amoebic encephalitis and other diseases, such as keratitis and pneumonitis. Both are recognized as opportunistic pathogens, but neither is considered zoonotic.

The ciliated protozoan *Balantidium coli* is an intestinal pathogen of humans and other primates, causing diarrhoea and producing undermining lesions similar to those caused by *Entamoeba histolytica* (Levine 1973). It has been found in a variety of mammals, including rhesus monkeys, dogs, pigs, rats, and possibly the zebu, water buffalo, and dromedary (Levine 1973). Care must be taken not to confuse it with *Buxtonella*. Although there may be a potential for animal-to-human transmission, waterborne transmission has not been proven, and data on this parasite's ability to survive in a freshwater or marine environment are lacking.

Although many cases of cryptosporidiosis related to water in swimming pools, water parks, fountains, or other recreational facilities have been reported, the most likely sources of infectious agents in these public and commercial facilities were humans. Therefore, transmission related to such facilities is not considered zoonotic and is not reviewed in this chapter.

Species of *Cyclospora* have been found in moles, rodents, snakes, non-human primates, and humans. However, *Cyclospora cayetanensis* is the only species known to infect humans. Organisms similar in appearance to *Cyclospora* have been found in persons with diarrhoea worldwide (Marshall *et al.* 1997). Like other apicomplexan parasites, the oocyst is the infective stage, but our knowledge of the life cycle is incomplete; despite epidemiological surveys and attempts to develop a laboratory animal model, no non-human hosts of this species are known.

16.2 MICROSPORIDIA

The phylum Microspora contains a diverse group of single-celled, obligate intracellular pathogens characterized by having a spore stage with a unique organelle — the polar tube. Although microsporidians have long been identified as protozoa, recent molecular studies identify them as fungi (Hirt *et al.* 1999; Weiss *et al.* 1999; Keeling *et al.* 2000; Van de Peer *et al.* 2000). Microsporidia

are classified by the ultrastructure of the spore stage, including its size, morphology, and the number of coils of the polar tube, the host range, and the life cycle stages. Molecular analysis of rRNA and other genes has begun to impact the identification and naming of species. Most of the over 1000 species of Microspora infect arthropods and fish, but recently 14 species have been identified in humans (primarily immunocompromised persons) (Weiss 2001). Even more recently, some of those species infecting humans have been identified in farm animals, wildlife, and birds. The spore stage normally infects via the oral route.

The species found infecting humans include *Enterocytozoon bieneusi* (the most prevalent), *Encephalitozoon intestinalis* (the second most prevalent), *Encephalitozoon hellem*, *Encephalitozoon cuniculi*, *Pleistophora* sp., *Trachipleistophora hominis*, *Trachipleistophora anthropothera*, *Nosema ocularum*, *Brachiola vesicularum*, *Brachiola algerae*, *Brachiola connori*, *Vittaforma cornea*, *Microsporidium africanus*, and *Microsporidium* sp.

16.2.1 Biology, life cycle, and transmission

Infection begins when the polar tube within the environmental spore stage everts with explosive force and propels sporoplasm into a host cell, where it initiates the proliferative phase of development, forming multinucleate stages by a variety of patterns. A sporogonic phase follows in which further nuclear division gives rise to spore forms that pass from the body into the environment, usually in urine or faeces.

16.2.2 Prevalence and distribution

Accumulating data from prevalence studies suggest that microsporidiosis in humans is a common but self-limited or asymptomatic infection in healthy persons and that microsporidia are common enteric pathogens in immunocompromised patients with human immunodeficiency virus (HIV) infection (Weiss 2001). Prevalence studies indicate infection rates of 30–70%, depending on the population studied and the diagnostic techniques used (Weiss 2001).

16.2.3 Microsporidiosis: disease and treatment

Several genera have been associated with human disease, including *Encephalitozoon*, *Enterocytozoon*, *Vittaforma*, *Pleistophora*, *Trachipleistophora*, *Brachiola*, and *Microsporidium* (used to designate microsporidia of uncertain taxa; Weiss 2001). *Encephalitozoon hellem* has been associated with keratoconjunctivitis, sinusitis, respiratory disease, prostatic

abscesses, and disseminated infection. *Encephalitozoon cuniculi* has been associated with encephalitis, hepatitis, and disseminated disease. *Encephalitozoon intestinalis* and *Enterocytozoon bieneusi* have been associated primarily with diarrhoea, but have been found in other extraintestinal sites. *Enterocytozoon bieneusi* has been associated with 30–70% of the cases of acquired immunodeficiency syndrome (AIDS)-related diarrhoea and wasting and is a cause of self-limiting travellers' diarrhoea in immunocompetent persons (Wasson and Peper 2000). Oral albendazole and topical fumagillin have been used to effectively treat cases of microsporidiosis caused by microsporidia other than *Enterocytozoon bieneusi.*

16.2.4 Detection of spores

Spores are the main diagnostic stage. Spores of most microsporidian species infecting humans are small (1–3 μm) and can be difficult to differentiate from similar-sized particle in faeces and environmental debris using the light microscope. However, staining methods, including Uvitex 2B or Calcofluor white for fluorescence microscopy and trichrome or chromotrope modified stains for brightfield microscopy of faeces and other body fluids, have facilitated clinical diagnosis and epidemiological studies. Electron microscopy enables one to distinguish genera but is not applicable to situations in which few spores might be present. The application of monoclonal antibodies has facilitated detection, and molecular techniques such as the polymerase chain reaction (PCR) have greatly improved detection and identification of spores.

16.2.5 Spores in water

Spores of *Encephalitozoon intestinalis*, *Enterocytozoon bieneusi*, and *Vittaforma corneae* have been found in surface waters (Sparfel *et al.* 1997; Dowd *et al.* 1998; Fournier *et al.* 2000; Thurston-Enriquez *et al.* 2002). For HIV-infected persons in Massachusetts and Texas, USA, risk factors for acquiring intestinal microsporidiosis were identified as swimming in lakes, rivers, and ponds and drinking unfiltered water (Watson *et al.* 1996). Although an outbreak of intestinal microsporidiosis in France appeared related to the municipal water system (Cotte *et al.* 1999), neither the source nor the route of transmission could be confirmed by detection of organisms. Culture-derived spores of *Encephalitozoon cuniculi*, *E. hellem*, and *E. intestinalis* were stored in water at 10, 15, 20, 25, and 30 °C and tested for infectivity in monolayer cultures of MDBK cells (Li *et al.* 2003). At 10 °C, spores of *E. intestinalis* were infective at 12 months, whereas spores of *E. hellem* and *E. cuniculi* were infective for 9 and 3 months, respectively. At 15 °C, spores of these three species were infective for 10, 6, and 2 months. At 20 °C, spores were infective for 7, 5, and 1 month,

respectively. At 25 °C, spores of *E. intestinalis* and *E. hellem* were infective for 3 months, whereas spores of *E. cuniculi* were infective for only 3 weeks. At 30 °C, spores of *E. intestinalis* and *E. hellem* were infective for 3 weeks and 1 month, respectively, whereas spores of *E. cuniculi* were infective for only 1 week. A study on survival of microsporidia in seawater is in progress in our laboratory. Initial data indicate that spores of *E. cuniculi*, *E. hellem*, and *E. intestinalis* remain infectious for several weeks in seawater at salinities up to 30‰ and at temperatures of 10 and 20 °C.

16.2.6 Possible environmental sources

Enterocytozoon bieneusi, the most frequently found microsporidian infecting humans, has been identified in pigs, cattle, dogs, monkeys, a cat, and chickens. *Encephalitozoon intestinalis* has been found in faeces from a donkey, goat, pig, cow, and dog. *Encephalitozoon hellem* has been found to infect many psittacine birds, an ostrich, hummingbirds, and chickens. *Encephalitozoon cuniculi* has many mammalian hosts, including rabbits, carnivores, rodents, ruminants, and primates (Wasson and Peper 2000).

Faeces from over 500 beavers, foxes, muskrat, otters, and racoons were stained with Calcofluor white and examined by fluorescence microscopy, and 465 specimens were examined by using a two-step nested PCR protocol (Sulaiman *et al.* 2003). Ultimately, 59 samples were sequenced, and 15 genotypes of *E. bieneusi* were identified. Of these, 13 genotypes had not been reported before. Most were found in multiple species of wildlife. Some isolates from muskrats and racoons formed two distinct groups; the others identified with all the previously described *E. bieneusi* genotypes from human and non-human sources, indicating that wildlife can serve as reservoirs of human pathogenic *E. bieneusi*.

16.3 AMOEBAE

This assemblage of protozoa is grouped together not because its members are clearly related but because they reproduce asexually, they are often naked cells in the trophic stage, and they move with the aid of so-called pseudopodia or by protoplasmic flow. Lobose amoebae (those with pseudopods that extend from a broad hyaline lobe) include many free-living species, including *Naegleria* and *Acanthamoeba* species, as well as those of the genus *Entamoeba* that live in invertebrate and vertebrate animals.

16.3.1 *Entamoeba histolytica*

Entamoeba histolytica was once thought to be a complex of a pathogenic invasive form and a non-pathogenic non-invasive form that were morphologically indistinguishable. Based on genetic, biochemical, and immunological studies (Clark and Diamond 1992), the pathogenic form retained the name *E. histolytica*, and the non-pathogenic form was named *E. dispar*.

16.3.1.1 Biology, life cycle, and transmission

The life cycle consists of a series of stages that develop after the cyst, the infective stage acquired from the environment, is ingested. In the intestine, when the pH becomes neutral or alkaline, small trophozoites emerge. These develop into trophozoites that establish themselves in the lumen of the large intestine. Cysts form only in the intestinal tract, and one, two, or four nucleated cysts pass from the body with faeces. Transmission of cysts via the faecal–oral route is by direct contact or through contaminated water and food. Waterborne transmission is common in developing countries, where much of the drinking-water is contaminated with faeces but is not treated (Marshall *et al.* 1997). Many non-human primates, dogs, cats, pigs, rats, and possibly cattle are potential reservoirs of infection (Levine 1973). Rats, mice, guinea-pigs, and rabbits have been infected experimentally (Levine 1973).

16.3.1.2 Prevalence and distribution

Human infections with *E. histolytica* have been reported worldwide. An estimated 12% of the world's population is infected, and about 10% of those have clinical symptoms (Marshall *et al.* 1997). Except for malaria and schistosomiasis, amoebiasis from *E. histolytica* causes more deaths than any other parasite (Marshall *et al.* 1997). Many reports on the local or regional prevalence of human infections with *E. histolytica* worldwide (especially in non-industrialized countries) have suggested that sources of infection include surface water, drinking-water, and seawater (e.g., Germani *et al.* 1994; DeLuca *et al.* 1997; Saidi *et al.* 1997; Torres *et al.* 1997; Roche and Benito 1999; Chen *et al.* 2001; Blessmann *et al.* 2002). However, cysts have rarely been recovered from drinking-water supplies or natural surface waters (Feachem *et al.* 1983). Histological specimens from some persons who died during an outbreak that affected 1409 people and resulted in 98 deaths from community drinking-water at the time of the 1933 Chicago World's Fair and from over 30 persons who died in 1953 in South Bend, Indiana, have recently been re-examined (ProMED-mail 1996), and the findings suggested that *E. dispar*, not *E. histolytica*, was present and that another pathogen was responsible for the morbidity and mortality. Little is known of the ability of *E. histolytica* to survive in seawater.

Kheissin and Dmitieva (1935) reported that "salts dissolved in water at minimal concentrations do not harm cysts and thus make possible cyst distribution in water, and even seawater."

16.3.1.3 Amoebiasis

The incubation period from time of exposure to development of symptoms ranges from a few days to months. Infections can range from asymptomatic to disseminated and fatal. Four major intestinal presentations include asymptomatic colonization; acute colitis with abdominal pain and bloody stools; fulminant colitis with diffuse abdominal pain, bloody diarrhoea, and fever; and amoeboma, an asymptomatic lesion or a mass accompanied by dysentery. After recovery from infection, reinfection with invasive colitis or amoebic abscess is unusual (Marshall *et al.* 1997). Because *E. histolytica* can invade tissues, extraintestinal sites such as the liver are sometimes affected. Marshall *et al.* (1997) list iodoquinol, diloxanide, metronidazole chloroquine, and dehydroemetine as potentially effective medications.

16.4 FLAGELLATES

Flagellates are not a strict taxonomic group, but they provide a convenient grouping based on the presence of flagellae. Under the microscope, the trophozoite stage of the diplomonad subgroup appears as a pear-shaped miniature face with two nuclei that appear as eyes. They are actually double animals, each with 1–4 flagellae. Some are free-living in water. Most live in the intestinal tract of animals, where they reproduce by binary fission and form cysts excreted by the host into the environment and then transmitted to other susceptible hosts. One genus of concern is a widespread, frequently encountered zoonotic pathogen — *Giardia*.

16.4.1 *Giardia*

Giardia lamblia, *Giardia intestinalis*, and *Giardia duodenalis* are synonyms for the same species. Usage appears to vary among taxonomists and others who prefer one name over the others. For example, persons in the medical community tend to prefer *G. lamblia*.

16.4.1.1 Biology, life cycle, and transmission

There are five species in the genus *Giardia* based on morphology of the trophozoite stage, but application of molecular tools has brought a new perspective to the taxonomy of this genus (Thompson 2000, 2002). *Giardia*

duodenalis infects humans as well as a wide range of domestic and wild mammals. *Giardia duodenalis* genotypes in Assemblages A and B infect humans and a number of other animal species and might actually represent unique species. Assemblage A has been found in humans, livestock, cats, dogs, and white-tailed deer (O'Handley *et al.* 2000; Thompson 2000; Trout *et al.*, in press), and mice and gerbils have been experimentally infected. Assemblage B has been found in humans, beavers, dogs, and rats (Thompson 2000). Thus, the potential certainly exists for transmission between humans and animals, as well as for contamination of surface waters with cysts infectious for a range of host species. Although Assemblages A and B have been found in both humans and other animals, other genotypes of *G. duodenalis* have not been found in humans.

The life cycle of all *Giardia* is simple and clearly defined (Erlandsen *et al.* 2002). Transmission of cysts via the faecal–oral route is by direct contact or through contaminated water and food. After cysts are ingested by a susceptible host and pass to the intestine, the trophozoite, protected within the cyst, excysts and attaches to the luminal surface of the small intestine by an adhesive disc, where it reproduces by binary fission. In response perhaps to bile concentration in the intestine, trophozoites release from the intestinal surface and encyst by forming a protective cyst wall around themselves. Cysts are excreted in the faeces, ready to infect another host. Young ruminants are highly susceptible to infection, and data collected in our laboratory (unpublished) indicate that calves can shed cysts for more than 2 months. Cyst shedding can also be intermittent, and several days can pass between positive faecal samples. Thus, a single negative sample does not indicate that an active infection is not present.

Waterborne outbreaks have been documented in Europe and North America (Craun 1984; Jephcott *et al.* 1986). Foodborne *Giardia* has been identified in several outbreaks, most likely when an infected food handler contaminates freshly prepared food (Adam 1991). Person-to-person spread has been well documented in day care facilities, schools, and residential institutions. Transmission from animals to humans or vice versa has not been as well documented.

16.4.1.2 Prevalence and distribution

Giardia, reported as the most common cause of protozoan diarrhoeal illness worldwide (Farthing 1989; Adam 1991), has also been the most frequently diagnosed gastrointestinal illness in US public health laboratories. Between 1971 and 1994, more than 25 000 cases of giardiasis were recorded in the USA (Craun 1986; Anonymous 1993, 1996). Giardiasis has been reported in both tropical and temperate climates, with prevalences of 2–5% in industrialized countries and 20–30% in developing countries. Infection is especially common in children in developing countries. For example, faecal examination revealed a

prevalence of 20% in Zimbabwe and Bangladesh, whereas seroprevalence in Peruvian children was 40% by 6 months of age (Adam 1991). Some rural Guatemalan children followed from birth to 3 years of age all acquired *Giardia* infections; many were recurrent (Farthing *et al.* 1986a, 1986b).

16.4.1.3 Giardiasis

Giardiasis can be asymptomatic, acute, or chronic. Asymptomatic individuals excrete infectious cysts while exhibiting no signs of disease; the underlying host–parasite interaction that makes this possible is not understood (Farthing 1989). Most cases of acute giardiasis resolve within 2–4 weeks after symptoms appear. The primary symptom is watery diarrhoea. Other symptoms can include nausea, vomiting, bloating, and abdominal discomfort. Approximately 30% of acute cases become chronic. Persistent diarrhoea, often associated with steatorrhoea (fatty stools), can continue for weeks or months. Malabsorption of nutrients, particularly lipids, can result in weight loss. Severity of giardiasis varies with the individual, particularly with the individual's general health or immune status. Infants and young children, as well as non-immune travellers to highly endemic areas, are at greatest risk of acquiring infection (Farthing 1984; Farthing *et al.* 1986a). Immune deficiency appears to predispose individuals to infection and persistent symptoms (Webster 1980). Chronic giardiasis is prevalent in HIV-positive and HIV-negative male homosexuals (McGowan and Weller 1990).

For treatment of giardiasis, nitroimidazole derivatives are frequently the drugs of choice. Furizolidone, quinacrine, and praziquantel (Reynoldson 2002) as well as paromomysin have been effective. No drug is effective 100% of the time, and resistance to nitroimidazoles, furizolidone, and quinacrine has been reported (Upcroft *et al.* 1990, 1996a, 1996b; Borst and Ouelette 1995).

16.4.1.4 Giardia cysts in water and their survival

Giardia cysts have often been detected in surface water and groundwater (LeChevallier and Norton 1995; Hancock *et al.* 1998), in drinking-water (Hashimoto *et al.* 2002), in estuarine waters (personal observations), and in marine waters (Johnson *et al.* 1995; Lipp *et al.* 2001) worldwide.

Giardia cysts have routinely been detected in human wastewater (Sykora *et al.* 1988, 1991). Although treatment in wastewater treatment plants reduces the numbers of cysts, it does not eliminate all of them (Rose *et al.* 2001).

Giardia was recovered from oysters in marine waters in the Netherlands (Schets *et al.* 2003). *Giardia* (Assemblage B) was recovered from shellfish from various European sites (Gomez-Couso *et al.* 2003).

Most *Giardia* cyst survival studies have been performed with *G. muris*, probably because this species is easily propagated in laboratory rodents. *Giardia muris* cysts survived in freshwater lakes and rivers for 56–84 days at winter water temperatures (0.7–3.2 °C, average) and for less than 56 days at warmer temperatures (6.6–23.0 °C) (deRegnier *et al.* 1989). Cysts of a bovine isolate of *G. duodenalis* in deionized water remained infective for 8 weeks at 4 °C (Fayer and Trout, in press). Those held at room temperature (20.8–24.7 °C) in tap water were no longer viable by 14 days (deRegnier *et al.* 1989). Cysts suspended in seawater survived up to 77 h in the dark (Johnson *et al.* 1997). In general, exposure to sunlight or higher salinity (35‰ versus 28‰) also decreased cyst survival times (Johnson *et al.* 1997). Survival times reflect the maximum time a few cysts remain infectious, although most are no longer viable at such times.

16.4.1.5 Giardia in the marine environment: Evidence from sea mammal infections

Giardia cysts were found in faeces of ringed seals (*Phoca hispida*) from the Western Arctic and harp seals (*Phoca groenlandica*), grey seals (*Halichoerus grypus*), and harbour seals (*Phoca vitulina*) from the St. Lawrence estuary in eastern Canada (Olson *et al.* 1997; Measures and Olson 1999). *Giardia* trophozoites were also found on the mucosal surface of the small intestine of phocids. All *Giardia* isolated belonged to the zoonotic Assemblage A genotype. Immunoglobulins (IgG) specific for *Giardia* were found in the serum and milk of seals. Weanling harp seals were experimentally infected with *G. duodenalis* Assemblage A from sheep (Olson *et al.* 2003). This is the first report of indirect, waterborne transmission of *Giardia* in phocids via faecal contamination of the tank salt water. While Pacific harbour seals (*Phoca vitulina*) and northern elephant seals (*Mirounga angustirostris*) from the coast of California tested negative for cysts, *Giardia* was detected in one of three California sea lions (*Zalophus californianus*) (Deng *et al.* 2000).

The origin of naturally acquired *Giardia* infections in marine mammals is unknown but likely results from faecal pollution originating from human activities. Seals from the St. Lawrence estuary, which receives sewage and agricultural runoff, were infected with a zoonotic genotype (Olson *et al.* 2003).

16.4.1.6 Reducing environmental contamination

The *Giardia* cyst, from human waste and wastewater treatment facilities, companion animals, livestock, and wildlife, is the source of all infections acquired from the environment. Efforts to control contamination require a broad approach, including reducing the number of cysts from wastewater treatment facilities, enforcing proper septic tank installation and maintenance, enforcing

dumping regulations for commercial and recreational boats, reducing free-ranging cat and dog populations, reducing runoff from animal agriculture facilities, and managing wildlife populations in areas adjacent to surface water used for human consumption.

16.4.1.7 Regulations

See section 16.5.2.9.

16.5 THE PHYLUM APICOMPLEXA

This phylum includes many globally important pathogens. Some infect only humans, some infect only other animals, and some are zoonotic. The most widely recognized genera include *Toxoplasma*, *Cryptosporidium*, *Cyclospora*, *Isospora*, *Eimeria*, and *Plasmodium.* All have life cycles involving one or two hosts and a cycle of asexual and sexual internal stages. The asexual sporozoite and merozoite stages are motile and possess a complex of organelles at the anterior end used for invasion of host cells. Collectively, these organelles are referred to as the apical complex.

16.5.1 *Toxoplasma*

In this genus, there is a single species, *Toxoplasma gondii*, named for the North African rodent in which it was first found (i.e., the gondi).

16.5.1.1 Biology and life cycle

Toxoplasma gondii is an obligate intracellular parasite that infects over 350 vertebrate species. However, only domesticated and wild felids serve as final hosts, with asexual and sexual stages in the intestine eventually producing oocysts that are excreted in the faeces. Oocysts of *T. gondii* are subspherical bodies measuring approximately 10 × 12 µm with a tough outer wall that resists chemical disinfection and environmental stresses. Upon excretion, they are non-infectious, containing a nucleus and undifferentiated cytoplasm. With exposure to air, adequate moisture, and non-freezing temperatures, they undergo a process of nuclear and cytoplasmic differentiation called sporulation until they contain two sporocysts, each containing four infectious sporozoites. At 15 °C, sporulation is complete in 2–5 days; at 11 °C, it requires 21 days; and at 4 °C, sporulation does not occur (Dubey *et al.* 1970). After sporulated oocysts have been ingested by a susceptible host and pass to the intestine, openings appear in the oocyst and sporocyst walls, through which sporozoites excyst and enter cells in the small intestine. In vertebrates other than felids, sporozoites penetrate the intestine, are

carried to various organs throughout the body, and multiply rapidly (tachyzoite stage), causing tissue damage, inflammation, and disease. For humans or other animals that acquire toxoplasmosis during pregnancy, the fetus is at risk of transplacental infection. In immunocompetent hosts that survive acute infection, cysts develop around slowly multiplying forms (bradyzoites) in organs throughout the body, often in the central nervous system. If immune competence becomes impaired by disease or medication, bradyzoites can leave the cyst and transform into tachyzoites, causing a recrudescence of disease.

16.5.1.2 Distribution and prevalence

Toxoplasma has been found worldwide. Prevalence varies with location, sample size, and detection techniques. Based on serological surveys, it ranges from 0% in Eskimos (Inuit) in Alaska and in residents of New Guinea, where cats are absent, to 100% in Easter Island residents (Dubey and Beattie 1988). An estimated 30% of adults in the USA and the United Kingdom tested seropositive, whereas an estimated 50–80% of adults in continental Europe were seropositive. Prevalence appears higher in less industrialized countries, in warmer climates, in low-lying areas, in adults, and in persons with greatest contact with soil and animals.

16.5.1.3 Toxoplasmosis

Infection with *T. gondii* ranges from mild to severe, from flu-like illness to specific organ impairment affecting virtually any organ of the body. Toxoplasmosis can be fatal for the fetus and immunocompromised humans and other animals.

Despite its wide host range and worldwide distribution, *T. gondii* has low genetic diversity. Humans have three clonal lines that correlate with *T. gondii* genotypes (Howe and Sibley 1995). Type I predominates in congenital infections, and Type I or Type I-like strains are associated with ocular toxoplasmosis in immunocompetent adults (Grigg and Boothroyd 2001; Grigg *et al.* 2001). Isolates, mostly from human cases, have been highly virulent for outbred laboratory mice. Type II appears to predominate in infections of immunocompromised patients.

16.5.1.4 Transmission

Oocysts excreted by felids can be transmitted to virtually all non-immune vertebrates by ingestion of contaminated food or water or by direct exposure to faeces. Tachyzoites can be transmitted from a pregnant female through the placenta to the fetus, by blood transfusion, or by organ transplantation. Bradyzoites in cysts are transmitted to carnivores that eat infected organs or muscle. When persons or animals harbour cysts or receive organ transplants containing cysts and subsequently lose immune competency, the cyst wall appears

to break down, and bradyzoites develop into tachyzoites that invade adjacent tissue.

16.5.1.5 Environmental source and prevalence

Wild and domestic felids, the source for all environmental contamination with *T. gondii*, have a high prevalence of exposure to this parasite. In trapped lynx and bobcats in Canada, antibodies to *T. gondii* were detected in 44% and 40% of the animals, respectively (Labelle *et al.* 2001). Oocysts of *T. gondii* have been found at sites throughout the environment where lynx and bobcats were trapped. Examination of sera from 865 captive neotropical felids from 20 states in Brazil revealed antibodies to *T. gondii* in 54.6% of cats, 45.9% of jaguarundis, 57.7% of ocelots, 51.9% of oncillas, 55.5% of margays, 12.5% of Pampas-cats, 75% of Geoffroys-cats, 63.2% of jaguars, and 48.2% of pumas (Silva *et al.* 2001). Based on 19 serological surveys of domestic cats conducted in 16 states in the USA from 1957 to 1986, 25.3% of 4871 cats had antibodies to *T. gondii* (Dubey and Beattie 1988). An even higher seroprevalence was found in Rhode Island, where 42% of 200 cats had antibodies to *T. gondii* (Defeo *et al.* 2002), and in Ohio, where 48.4% of 275 cats had antibodies to *T. gondii* (Dubey *et al.* 2002). In the latter survey, 62% of 78 outdoor cats had antibodies to *T. gondii*, suggesting widespread contamination of the rural environment with oocysts.

16.5.1.6 Oocyst survival under environmental conditions

Aqueous oocyst suspensions stored in covered petri dishes at 4 °C in the laboratory survived for over 410 days; others stored outdoors in direct sunlight at a mean temperature of 20 °C (extremes of 6–39 °C) survived 306 days; and still others stored outdoors in the shade at a mean temperature of 19.5 °C (extremes of 5.5–35.5 °C) survived to 410 days (Yilmaz and Hopkins 1972). Oocysts in cat faeces buried in soil, simulating natural disposal by cats, remained infectious for 1 year in shaded, moist, and dry sites in Costa Rica and for 18 months at a site in Kansas, USA (Frenkel *et al.* 1975). From 75 to 80% of unsporulated (non-infectious) oocysts suspended for 3 days in 15‰ and 32‰ artificial seawater at 24 °C became sporulated and were infectious for mice (Lindsay *et al.*, in press). Sporulated oocysts stored in 15‰ artificial seawater at 4 °C or room temperature were still viable after 28 days.

16.5.1.7 Waterborne disease

Epidemiological evidence suggested that 39 of 98 US Army soldiers acquired toxoplasmosis from drinking water collected at two sites in a jungle stream in Panama (Benenson *et al.* 1982). An outbreak involving up to 7700 people was associated with a municipal water source in Victoria, British Columbia, Canada

(Bowie *et al.* 1997). It was suspected that a surface water reservoir became contaminated with oocysts from domestic cats or cougars (Isaac-Renton *et al.* 1998). An investigation of the Victoria watershed a year later found that deer mice in the riparian environment of the watershed had antibodies to *T. gondii*, suggesting that oocysts were present near the water's edge (Aramini *et al.* 1999). A reservoir supplying water to half the population of Santa Isabel do Ivai, Brazil, was contaminated with oocysts in cat faeces, and 176 people contracted toxoplasmosis. A high correlation was found between drinking unfiltered water and an 84% and 62% seropositivity to *T. gondii* in lower and middle socioeconomic populations, respectively, indicating the importance of waterborne transmission in this region (Bahia-Oliveira *et al.* 2003).

16.5.1.8 Detection of oocysts in water

No studies have been specifically designed to test the efficacy of various recovery methods for *T. gondii* oocysts from water sources. Using the US Environmental Protection Agency's (EPA) method for detection of *Cryptosporidium* oocysts by cartridge filtration, *T. gondii* oocysts were recovered from large volumes of drinking-water, and their presence was confirmed by mouse bioassay (Isaac-Renton *et al.* 1998). Using demineralized or tap water seeded with 10^5 and 10^4 purified oocysts, recovery by centrifugation at 2565 × *g* ranged from 35.8 to 82.5%, and recovery by flocculation with aluminium or iron sulfate ranged from 35.9% ± 12.3 to 100.3% ± 26.9 (Kourenti *et al.* 2003). The lack of data on the prevalence of *T. gondii* in surface water is also due to the lack of a rapid and sensitive method to detect the oocyst stage in this environment. Bioassays using animals or cell culture are unavailable in many locations and expensive, and it take days or weeks to obtain results. The application of PCR to detect *T. gondii* nucleic acid has recently been reported (Schwab and McDevitt 2003).

16.5.1.9 Sea mammal infections: evidence of T. gondii in the marine environment

The population of the southern sea otter (*Enhydra lutris nereis*) along California's Pacific coast shoreline expanded from about 50 animals in the early 1900s to about 2500 animals in the 1990s. However, the slow rate of recovery, possibly related to high mortality, prompted a survey beginning in 1992. In California, after a rainfall event, storm drains, ditches, and culvert pipes carry untreated surface water runoff or irrigation water from lawns, streets, and open land to coastal streams or directly to the coast. Oocysts in cat faeces and other faecal-borne pathogens can be transported in these waters to the ocean, where many of the marine species on which otters feed could potentially concentrate *T. gondii* oocysts and other pathogens from such contaminated water. Examination of

environmental, serological, and other data for over 200 sea otters revealed that 42% of live otters and 62% of dead otters had been exposed to *Toxoplasma* and suggested that land-based freshwater runoff was a source of the parasite (Miller *et al.* 2002a, 2002b). Although exposure to *Toxoplasma* has been found in a variety of marine mammals in coastal areas worldwide, no antibodies against *T. gondii* were found in harp (*Phoca groenlandica*), ringed (*Phoca hispida*), and hooded (*Cystophora cristata*) seals or minke whales (*Balaenoptera acutorostrata*) in the North Atlantic Ocean far from human habitation and potential runoff.

16.5.1.10 Reducing or preventing environmental contamination

Reduction in the number of free-ranging and feral domestic cats can reduce the sources of oocysts that enter surface waters through sewage outfalls and land-based surface runoff from paved surfaces, residential areas, agricultural settings, and wildlife habitats. It is estimated that 40 million cats are owned in the USA. Most of these cats spend some or all of their time outdoors. Unowned cats are estimated to number 40–60 million. These large populations of free-ranging and feral cats could sustain a sylvatic *Toxoplasma* cycle that produces large numbers of oocysts that find their way into surface waters and ultimately impact even the marine environment. Actions needed to reduce the introduction of new cats into the wild include mandatory licensing and tagging of cats, mandatory spaying or neutering of new pets, laws requiring owners to restrict pets to their property, laws prohibiting abandonment and feeding of stray cats, and posting of signs in public areas indicating that feeding stray cats in designated wildlife areas is illegal.

16.5.1.11 Regulations

No specific regulations exist for treatment of water to remove or disinfect *Toxoplasma*.

16.5.2 *Cryptosporidium*

The genus *Cryptosporidium* consists of 14 species, but is in a state of rapid taxonomic change. *Cryptosporidium hominis* (syn. *Cryptosporidium parvum* genotype 1) is the most prevalent species found in humans and is transmitted from humans to humans. *Cryptosporidium parvum* (previously referred to as *C. parvum* bovine genotype or genotype 2) has been reported in many mammalian species and is the second most reported species in humans. Species originally described in animal hosts have been reported primarily infecting immunocompromised humans, but some immunologically healthy persons as well. These include *C. meleagridis*, originally found in turkeys, *C. canis* from dogs, *C. felis* from cats, and *C. muris* from mice.

16.5.2.1 Biology, life cycle, and transmission

Molecular and morphological data are based on the oocyst stage. For most species, oocysts measure 4–6 μm, appear nearly spherical, and have obscure internal structures. This stage is also of primary importance for the dispersal, survival, and infectivity of the parasite. Following ingestion by a susceptible host, sporozoites, released from within the oocyst, invade intestinal epithelial cells and initiate development of asexual and sexual stages. All stages are intracellular at the apical surface and protrude into the lumen of the intestine (Fayer *et al.* 1997). Oocysts sporulate *in situ* to produce sporozoites and then pass out of the body mixed with faeces. Some oocysts develop thin walls and are thought to release sporozoites internally, initiating a cycle of autoinfection. For immunocompetent humans, it takes 4–22 days from ingestion of oocysts to completion of the life cycle and passage of new oocysts. Oocyst excretion lasts 1–20 days. Oocysts are transmitted by the faecal–oral route. Potential routes of transmission include person to person through direct or indirect contact, animal to animal, animal to human, human to animal, waterborne from humans or animals through drinking-water or recreational water, and foodborne from contaminated water used in food production and preparation or from food handlers.

Newborn ruminants are highly susceptible to infection with *C. parvum* and can excrete as many as 10^7 oocysts per gram of faeces (Fayer *et al.* 1997). To determine how many oocysts of a *C. parvum* bovine isolate were required for seronegative healthy persons to become infected, 29 volunteers each ingested a single dose of oocysts (Dupont *et al.* 1995). Of five persons who ingested 30 oocysts, one became infected; of seven persons who ingested 1000 or more oocysts, all became infected. The ID_{50} was calculated to be 132 oocysts. Additional data resulted in recalculation of the ID_{50} at 87 oocysts, and additional isolates of *C. parvum* were found to have ID_{50} values for human volunteers ranging from 9 to 1042 oocysts for TAMU and UCP isolates, respectively (Okhuysen *et al*. 1999).

16.5.2.2 Prevalence and distribution

Human infection with *Cryptosporidium*, first reported in two cases in 1976 and a further 11 cases over the next 6 years, has now been reported from over 90 countries on six continents. Specific locations and prevalence have been reviewed by Ungar (1990). Based on US public health records, an estimated 2% of stools tested by health care providers were positive for *Cryptosporidium* (Mead *et al.* 1999). At approximately 15 million annual visits for diarrhoea, an

estimated 300 000 persons acquire cryptosporidiosis annually, 45 times more persons than estimates based on FoodNet surveillance (Mead *et al.* 1999). Surveys from developing countries indicate a higher prevalence of infection than in industrialized countries, where sanitation is better and clean drinking-water is more readily available (Ungar 1990). However, within these populations are groups at greater risk of infection, including children, malnourished persons, and a range of immunocompromised individuals, such as transplant recipients, cancer patients, and patients with immunosuppressive infectious diseases, including AIDS patients.

16.5.2.3 Cryptosporidiosis

Cryptosporidiosis has been reported worldwide. *Cryptosporidium hominis* (formerly *Cryptosporidium parvum* genotype 1) is widespread in humans, and *C. parvum* (genotype 2) is widespread in humans and other mammals. The clinical course and severity of the illness from both species can vary from person to person, depending primarily on the person's immune status. Infections can range from asymptomatic to prolonged watery diarrhoea to extraintestinal organ involvement. Diarrhoea, the most common symptom, is followed by abdominal cramps, anorexia, nausea, vomiting, fever, fatigue, weakness, and respiratory problems (Ungar 1990). In a group of healthy adults, symptoms lasted 2–26 days. Persons with HIV infection, those receiving immunosuppressive medication, cancer patients, hypo- or agammaglobulinaemic patients, malnourished persons, children, and those with viral infections are at elevated risk. Autopsies of immunocompromised persons with diarrhoea have demonstrated the presence of *Cryptosporidium* throughout the gastrointestinal tract from the oesophagus and stomach to the rectum, as well as the liver, gall-bladder, pancreas, and respiratory tree. Withdrawal of immunosuppressive therapy and treatment with anti-HIV drugs have reduced the severity of the disease. Despite a decade of testing, there are no specific drugs approved for treatment of cryptosporidiosis.

16.5.2.4 Oocyst survival

Depending on ambient conditions, some oocysts of *C. parvum* can remain viable for many months. When oocysts removed from debris were stored in water at 20 °C for 6 months, many were still infectious for suckling mice, whereas others held at 25 and 30 °C remained infectious for 3 months (Fayer *et al.* 1998a). When an aqueous suspension of oocysts was heated from 9 to 55 °C over 20 min, infectivity for suckling mice was lost (Anderson 1985). Oocysts held in water at 59.7 °C for 5 min had very low infectivity (Fayer 1994), and others held at 71.7 °C for only 5 s were killed (Harp *et al.* 1996). Some oocysts held at

-5 °C for up to 2 months, others held at -10 °C for up to 1 week, and still others held at -20 °C for up to 8 h, but not those held at -20 °C for 24 h, were infectious for mice (Fayer and Nerad 1996; Fayer *et al.* 1998a). Oocysts of *C. parvum* stored at 10 °C in salinities up to 30‰ in artificial seawater remained infectious for mice for 12 weeks (Fayer *et al.* 1998b). Those stored at 20 °C remained infectious for 4, 8, and 12 weeks at 30, 20, and 10‰, respectively (Fayer *et al.* 1998b). Similar studies found that some oocysts held in artificial seawater at a salinity of 35‰ for 40 days at 18 °C and others held for 12 months at 8 °C remained infectious for mice (Freire-Santos *et al.* 1999; Tamburrini and Pozio 1999). In contrast to the aforementioned studies, oocysts that had been stored in 2.5% potassium dichromate at 4 °C and were 4 months old when tested for viability (by *in vitro* excystation) after exposure to natural seawater from various locations around Honolulu, Hawaii, USA, did not survive for more than a few days (Johnson *et al.* 1997). Both the long storage time and method of determining viability could have contributed to these findings. Oocysts do not survive long in dry environments: 97% were killed after 2 h of desiccation, and 100% were killed after 4 h (Anderson 1986; Robertson *et al.* 1992).

16.5.2.5 Dispersal of oocysts

Effluent from wastewater treatment plants that empty into rivers and the marine environment in England, Italy, Hawaii, the US mainland, Scotland, and elsewhere has been found to contain *Cryptosporidium* oocysts (Madore *et al.* 1987; Johnson *et al.* 1995; Carraro *et al.* 2000; Robertson *et al.* 2000). Movement of oocysts from faeces on land surfaces to surface water and groundwater has received little investigation (Anguish and Ghiorse 1997). Under highly controlled conditions, irrigation applied to faeces–soil mixtures on a greenhouse soil tilting table was used to detect movement of *C. parvum* oocysts in a variety of soil types (Mawdsley *et al.* 1996). Oocysts moved within the soil for several weeks, in some cases for over 70 days. Most oocysts were found in the upper 2 cm of soil; some were recovered at a depth of 30 cm, but none at 70 cm. In nature, increased numbers of oocysts have been reported in association with surface waters after rainfall events compared with periods of drought (Fayer *et al.* 2002; Lemarchand and Lebaron 2003). In some cases, humans and other animals contribute directly to the mechanical dispersal of oocysts. When oocysts of an unknown species of *Cryptosporidium* were isolated from gulls, investigators postulated that birds could distribute oocysts over wide areas (Smith *et al.* 1993). After a single experimental dose, *C. parvum* oocysts passed through the gastrointestinal tract of Canada geese (*Branta canadensis*) and Peking (Mallard) ducks (*Anas platyrhynchos*) for nearly 1 week while retaining infectivity for mice (Graczyk *et al.* 1997). Subsequently, viable *C. parvum* oocysts were recovered from faeces in fields where Canada geese rested

along their migration route (Graczyk *et al*. 1998). Other studies suggested that cockroaches, filth flies, dung beetles, and rotifers could ingest and possibly transport oocysts to new locations (Fayer *et al.* 2000). Ultimately, to initiate infection, oocysts must be ingested with food, ingested with water, or transmitted by close personal contact with infected people, animals, or contaminated surfaces or recreational water.

16.5.2.6 Waterborne disease

From 1984 to 1999, 69 outbreaks of waterborne cryptosporidiosis were reported (see review by Fayer *et al*. 2000). The first reported waterborne outbreak of cryptosporidiosis was in the summer of 1984 in Braun Station, a suburb of San Antonio, Texas, USA. Diarrhoea was the major symptom. A telephone survey of 100 homes identified 2000 sick persons out of approximately 5900 persons interviewed. Potable, unfiltered artesian well water contaminated with faecal coliforms supplied all 1791 homes. Dye introduced into the community sewage system was traced to the well water.

In 1987, an outbreak of gastroenteritis among college students affected about 13 000 of the 64 900 residents in Carroll County, Georgia, USA. Oocysts were detected in water from the water treatment plant, from dead end water mains, and from streams above the plant. Dye added to a sewage overflow was traced to the plant. In the plant, several failures were found, including mechanical agitators removed from the flocculation basins, impaired filtration, and filters that were not being backwashed.

In 1993, an estimated 403 000 of approximately 1 610 000 people in the Milwaukee, Wisconsin, area experienced the largest recorded waterborne disease outbreak in US history. After the health department was notified of gastrointestinal illness causing high absenteeism of hospital employees, students, and teachers, an epidemiological investigation began. Within 4 days, oocysts were found in patients' stools. Treated water from one of the two water plants was recognized as highly turbid, the plant was closed, and a boil water advisory was issued. Oocysts were found in ice made before and during the outbreak. It appeared that oocysts from Lake Michigan water were taken into the southern treatment plant. It is possible that polyaluminium chloride or alum coagulant failed to reduce the high turbidity and recycled filter backwash water contributed to high numbers of oocysts in the finished water. Although heavy rainfall, cattle manure in the watershed, abattoir waste, and sewage overflow were considered potential sources, oocysts from four infected persons failed to infect animals and were identified genetically to be of human origin, suggesting that the probable source was sewage overflow.

Cattle and sheep have repeatedly been implicated as sources of waterborne outbreaks. However, these animals have not been conclusively identified (by genotyping) as the source of any waterborne outbreak within the USA. The only waterborne outbreak in North America in which oocysts of the bovine genotype have been identified was in Cranbrook, Canada.

Most epidemiological investigations have detected a combination of causes for waterborne outbreaks, including contaminated source water, high turbidity, and failures at the treatment plant.

16.5.2.7 Detection of oocysts in water

The presence of *Cryptosporidium* oocysts in surface waters and groundwater has been determined by direct observation and molecular techniques (Rose *et al*. 1997) and has been implied by epidemiological data on numerous occasions (Fayer *et al.* 2000). The presence of oocysts in tidal or marine waters is not well documented. It is implied primarily by recovery from shellfish that filter these waters (Fayer *et al.* 1999, 2003). However, oocysts have been recovered directly from marine waters at the outfall that discharges primary sewage effluent 3 km offshore and at Waikiki Beach on the coast of Honolulu, Hawaii, USA (Johnson *et al.* 1995) and from the Tech River watershed on the western Mediterranean coast of France (Lemarchand and Lebaron 2003).

16.5.2.8 Reducing or preventing environmental contamination

The oocyst, in faeces from infected humans and other animals, is the source of all environmental contamination. Contamination of watersheds is significantly affected by human activities on land, including sewage discharge, direct deposit of faeces, leaking septic tanks, animal agriculture, and pet ownership. Data indicating that over 150 species of wild mammals have been identified with cryptosporidiosis (Fayer *et al*. 2000) suggest that sylvatic cycles of transmission are also likely to contribute to widespread contamination. To reduce oocyst contamination, there need to be higher standards for municipal wastewater treatment facilities, enforcement of proper septic tank usage, reduction of runoff from animal agriculture sites, and education and regulations to reduce the quantity of pet faeces in public places that eventually are carried to rivers and coastal areas via storm sewers.

16.5.2.9 Regulations

Each country differs in its regulations or lack thereof for providing safe drinking-water to its population. In the USA, the *Clean Water Act*, regulating point and non-point discharges of coliform bacteria into receiving waters in an attempt to improve the safety of drinking-water, did not account for the fact that

some discharges might contain low levels of bacteria but high levels of organisms such as *Cryptosporidium*, which were much more resistant to disinfectants used for bacteria (Rose *et al.* 1997). The US EPA's Surface Water Treatment Rule under the *Safe Drinking Water Act* required a specified series of disinfection treatments of surface water supplies and groundwater directly impacted by surface water, including a minimum treatment level of 3 $\log_{10}$ for *Giardia* and 4 $\log_{10}$ for viruses. Although helpful in providing some protection against *Cryptosporidium*, the rule required modifications for various reasons. An Information Collection Rule (14 May 1996) was developed to support future regulations and was intended to provide the US EPA with information on chemical by-products that form when disinfectants used for microbial control react with chemicals already present in source water and on disease-causing microorganisms including *Cryptosporidium*, as well as engineering data to control these contaminants. An Interim Enhanced Surface Water Treatment Rule (ESWTR) included treatment requirements for waterborne pathogens such as *Cryptosporidium*. In addition, systems had to continue to meet existing requirements for *Giardia* and viruses. Specifically, the rule included a maximum contaminant level goal of zero for *Cryptosporidium*, a 2-$\log_{10}$ removal requirement for *Cryptosporidium* for all systems that filter, various turbidity performance standards, watershed control requirements for unfiltered public water systems, cover requirements for new reservoirs, and sanitary surveys for all surface water systems. Long Term 1 and Long Term 2 ESWTRs have provided further guidance for implementing treatment.

16.6 SUMMARY AND CONCLUSIONS

Although water has long been thought to serve as a vehicle for transmission of zoonotic protozoa, the protozoa rarely have been recovered or, when recovered, have been found in relatively small numbers in drinking-water supplies or natural surface waters. Therefore, despite a high prevalence of human infection with some organisms, determination of waterborne transmission has been problematic, and the role of water in the transmission of zoonotic protozoan diseases has been under-recognized. The lack of data on the prevalence of zoonotic protozoa in surface water has been due in part to the lack of rapid and sensitive methods to recover and detect the exogenous stages in this environment. Bioassays using animals or cell culture are unavailable in many locations and expensive, and it takes days or weeks to obtain results. The application of molecular techniques for identification of species and genotypes in humans and other animals as well as for the detection of low numbers of these protozoa in aqueous environments has enabled scientists and health care

workers to find these organisms in surface waters, drinking-water, and seawater environments where they have rarely or never been found before. The ability to conduct source tracking and epidemiological studies relating these organisms to water will now provide a basis for planning prevention and control strategies.

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17

Cyclosporiasis

J.H. Cross and J.B. Sherchand

17.1 INTRODUCTION

Although there are a myriad of coccidian protozoan parasites in animals, there are relatively few that infect humans. Attention to these protozoans in humans has increased in recent years, since many are associated with acquired immunodeficiency syndrome (AIDS). During the 1980s, a new intestinal parasite was found in patients with persistent diarrhoea (Shlim *et al*. 1991). The organisms were called cyanobacteria (blue-green algae)-like bodies (CLB). Earlier, in Papua New Guinea, coccidian-like oocysts were found in three patients (Ashford 1979). Later, the oocysts were recognized as a coccidian, and the organisms were named *Cyclospora cayetanensis* (Ortega *et al*. 1993). Since these early reports, the parasite has been reported widely in humans.

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17.2 PARASITE

Only a few species in the genus *Cyclospora* have been reported from myrapods, insectivores, and rodents. When passed in human faeces, the *C. cayetanensis* oocyst is unsporulated; after 5–10 days, two sporocysts develop in the oocyst, each having two sporozoites. The oocyst is round and measures 8–10 μm in diameter. When observed by brightfield microscopy, the oocyst is a non-refractile sphere containing a cluster of refractile membranous globules. This is the morula stage. In electron microscopic studies, the sporozoites were found to possess a membrane-bound nucleus and micronemes characteristic of coccidians of the phylum Apicomplexa (Ortega *et al*. 1993). Further studies using phylogenetic analysis confirmed that *C. cayetanensis* was a coccidian related to *Eimeria* and closely related to *Isospora* (Relman *et al*. 1996).

17.3 LIFE CYCLE

The life cycle of *C. cayetanensis* is not completely known. No animal model is available, hindering the acquisition of detailed information on the parasite's life cycle. Its life cycle is assumed to be similar to that of other coccidians. When passed in the faeces, the organism is unsporulated, and the oocysts are considered non-infectious; after sporogony, the oocysts are considered infectious. Upon ingestion, the sporozoites enter the intestinal epithelial cells, where they multiply, producing merozoites; the merozoites then emerge and enter the other cells, producing more merozoites or microgametocytes and macrogametocytes. Microgametes develop and enter the macrogametocyte to form an oocyst, which passes in the faeces. The life cycle in nature is unknown, except that the oocysts have been found in water.

The complete development of the parasite in intestinal tissue has not been demonstrated. There are several reports describing sporozoites, trophozoites, schizonts, and merozoites by light and electron microscopy, but sexual stages were not seen (Bendall *et al*. 1993; Connor *et al*. 1993; Sun *et al*. 1996). Further studies are required to completely describe the parasitic stages of the life cycle.

17.4 DISEASE

The parasite invades the epithelial cells of the small intestine, especially the jejunum. Infection occurs in immunocompetent as well as immunosuppressed patients. Diarrhoea, reported in most cases, lasts as long as 7 weeks, with six or more stools per day. Other symptoms include anorexia, fatigue, cramping, vomiting, and malaise (Shlim *et al*. 1991). There may also be low-grade fever and malabsorption of D-xylose (Connor *et al*. 1999). Abdominal gas and

bloating have been reported, and weight loss has occurred with long-term infections.

The incubating (prepatent) period varies from 1 to 14 days, with an average of 7 days. It is difficult to differentiate cyclosporal diarrhoea from diarrhoea associated with other infectious agents. The symptoms, especially diarrhoea, may be prolonged in immunocompromised people. Sequelae, such as Guillain-Barré syndrome, reactive arthritis syndrome (Reiter syndrome), and acalculous cholecystitis, have been reported (Connor *et al*. 2001; Zar *et al*. 2001). No deaths have been associated with infection.

17.5 DIAGNOSIS

Many of the methods used for the diagnosis of cyclosporiasis are similar to those used for the diagnosis of cryptosporidiosis. *Cyclospora cayetanensis* oocysts are larger (8–10 µm) than those of *C. parvum* (4–6 µm) and are more easily visualized in stool specimens by light microscopy. High magnification (400X) and patience are required. Various concentration techniques, such as sugar flotation and formalin–ethyl acetate centrifugation, are useful. The oocysts of *C. cayetanensis* are autofluorescent and under fluorescent microscopy appear as blue or green circles, depending on the filters (365 or 450–490 nm). This is useful for screening stool specimens. Acid-fast stains have also been used, but the staining is variable, with some organisms being found unstained pink or red. Another method using safranin and microwave heating has been reported to be superior to acid-fast staining (Visvesvara *et al*. 1997). Polymerase chain reaction (PCR) techniques have been developed to detect *Cyclospora* (Yoder *et al*. 1996; Varma *et al*. 2003) and have been reported to distinguish between *Cyclospora* and a closely related *Eimeria* species (Jinneman *et al*. 1998).

17.6 TREATMENT

Although cyclosporiasis is self-limiting in 6–7 weeks, treatment with cotrimoxazole (trimethoprim [TMP] 160 mg, sulfamethoxazole [SMX] 800 mg twice daily for 7–10 days) is the drug of choice for adults (Hoge *et al*. 1995). Immunocompromised patients may require higher dosages and long-term maintenance. The dosage is reduced for children (TMP 5 mg, SMX 25 mg/kg of body weight twice daily for 7 days). Ciprofloxacin (500 mg twice daily for 7 days) has been effective in treating patients intolerant to SMX (Verdier *et al*. 2000). Cotrimoxazole may also be given prophylactically.

17.7 EPIDEMIOLOGY

The life cycle of *C. cayetanensis* remains unknown, and no natural or experimental animal host has been determined (Eberhard *et al*. 2000). A great deal of information on the biology of the parasite could be obtained if a host other than humans could be found. Essentially, the means of transmission of the parasite remains an enigma.

Cyclosporiasis has been reported from most parts of the world. However, most endemic areas are in less developed countries, with the greatest number of cases reported from Nepal, Peru, and Haiti. Infections are reported in both immunocompetent and immunocompromised patients. Although cyclosporiasis is reported in AIDS patients, it is not considered an AIDS-related parasitosis. Infections appear equally in males and females. Although all age groups can be infected, most infections in endemic countries are reported in children. In one report from Nepal, 15 of 180 children aged 2 months to 13 years with diarrhoea were passing oocysts determined by light microscopy (Cross *et al*. 1997). Infections are most common in underdeveloped countries with poor sanitation and inadequate water supplies. Infections reported from more developed countries are most often associated with travellers returning home after visiting an endemic area. Most cases in Nepal occur during the rainy season (May–October) (Hoge *et al*. 1995), and most cases in Peru occur during the winter months (Madico *et al*. 1997). In early studies in Nepal, reports of the parasitoses were from expatriates. In a more recent study, however, 25% of 6562 stools from Nepalese seen at various health facilities and stools collected in rural areas were positive for *C. cayetanensis* (Sherchand and Cross 2001). Not all had diarrhoea, however.

Water, presumably contaminated with human faeces, seems to be the main source of infections. There is no evidence of direct human-to-human transmission, and none should occur, since it requires several days for the organism to become infectious. Waterborne oocysts have been found in Nepal (Rabold *et al*. 1994), the USA, and elsewhere (Table 17.1). Sewage water has been incriminated in Nepal (Sherchand and Cross 2001). Water contaminated with faeces is used in farm irrigation systems for vegetables, and contaminated water from ponds and streams is used to keep the vegetables looking fresh in the market. In cities of Nepal, water supplies are contaminated through sewage seepage into water pipes. The parasite has also been reported in tap water from Hanoi, Vietnam (Cam *et al*. 2001).

Food sources have also been incriminated. Oocysts have been recovered from washings of vegetables. In Nepal, washings from cabbage, lettuce, and mustard greens were found with *C. cayetanensis* oocysts (Sherchand and Cross 2001). Food has been incriminated as a vehicle of transmission, especially in

epidemics in the USA and Canada, where raspberries imported from Guatemala were suspected as a source (Herwaldt 2000). It was speculated that application to plants of irrigation water and water mixed with insecticides and pesticides may have been the source of contamination. Fresh basil was also involved in several outbreaks (Lopez *et al*. 2001). *Cyclospora*-like organisms have also been recovered from faeces from chickens (Garcia-Lopez *et al*. 1996), dogs (Carolla *et al*. 2001), rodents, and monkeys (Sherchand and Cross 2001). PCR and restriction fragment length polymorphism (RFLP) studies suggest that the oocysts from animals in Nepal were those of *C. cayetanensis* (D.M. Chu, J.B. Sherchand, J.H. Cross, and P. Orlandi, unpublished data), but animal infections are considered spurious, because animals are known to be copraphagic.

Table 17.1. Reports of *Cyclospora cayetanensis* in water

Location	Type of water	References (based primarily on Ortega *et al*. 1998)
Chicago, USA	Water reservoir	Anon 1991; Huang *et al*. 1995
Nepal	Untreated water	Hoge *et al*. 1993
Chicago, USA	Lake water	Wurtz 1994
Nepal	River/municipal water	Rabold *et al*. 1994
Utah, USA	Farm water	Hale *et al*. 1994
New York, USA	Water cooler	Carter *et al*. 1996
Massachusetts, USA	Well water	Ooi *et al*. 1995
Peru	Wastewater	Sturbaum *et al*. 1998
Nepal	Sewage water	Sherchand and Cross 2001
Vietnam	Tap water	Cam *et al*. 2001

17.8 SUMMARY

Cyclospora cayetanensis is now known to be distributed worldwide, with most infections reported from Nepal, Peru, and Haiti. Endemic areas have poor and inadequate water supplies and poor sanitation. Drinking-water as well as water for vegetables, which in some cases is mixed with insecticides and pesticides and applied to growing plants, are usually polluted and contaminated with human and animal faeces. The parasites resist chlorination.

The parasitoses cannot be controlled or eliminated until endemic areas change their sanitary practices. More importantly, improvements must be made to ensure a safe and adequate water supply. Coagulation, sedimentation, and filtration are barriers against most waterborne diseases. Fruits and vegetables, especially leafy vegetables, in endemic areas should be carefully washed with

clean, uncontaminated water before consumption. Cooking of vegetables is more reliable for preventing cyclosporiasis.

There are many unknowns associated with *C. cayetanensis*. Further knowledge on the life cycle and the possibility of a natural reservoir host would be of great value.

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18

Major helminth zoonoses in water

T. Endo and Y. Morishima

18.1 INTRODUCTION

WHO (1996) reported that, worldwide, *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms (*Ancylostoma duodenale* and *Necator americanus*) infect 1.4, 1.0, and 1.3 billion people each year, respectively. It is unlikely that increasing urbanization in developing countries will result in a decreasing trend. Unlike the case with other infectious diseases, it is important to realize that the majority of individuals infected with parasites are healthy and will remain so. Thus, there is a great difference between infection and disease. This is simply because, with very few exceptions, helminth parasites do not replicate in the definitive human host. Thus, the public health community tends to recognize helminth infections as common but not a problem requiring high-priority attention. Nevertheless, although the mortality rate from intestinal helminths is thought to be low, the number of deaths is fairly high because of the high

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prevalence of infection in developing countries. About 60 000 deaths per annum occur for *A. lumbricoides* infection as a consequence of intestinal obstruction in young children. Similar figures have also been reported for both *T. trichiura* and hookworm infections due to massive dysentery syndrome and severe iron deficiency anaemia, respectively (WHO 1996).

There are myriads of emerging pathogens, including helminth parasites, some of which are transmitted by a variety of water sources. For a majority of helminths, only one host is required for a parasite to complete its life cycle. Some species of helminths are highly host-specific. Others are less discriminating and may have several satisfactory hosts, including humans. In many examples, humans are only incidental hosts, with domestic and wild animals serving as reservoirs of the parasites. Multiple host susceptibility to a parasite introduces the concept of zoonosis involving humans, another vertebrate, often arthropods or molluscs, the parasites, and the environment — all forming an ecological whole. Nature consists of an interaction of such living things, which are continuously or sometimes intermittently modified by alterations of the environment.

18.2 LIFE CYCLES

The life cycles of helminth parasites can be divided into two basic types: the direct cycle with only the definitive host, and 2) the indirect cycle with a definitive host and one or more intermediate hosts. Parasites with a direct life cycle usually have a free-living phase during which they develop to the infective stage. Those with an indirect life cycle have a free-living stage between some of the hosts.

The digenetic trematodes have an indirect life cycle exclusively involving molluscs as the first intermediate host; they usually require a second intermediate host that harbours the infective metacercariae stage, with the exception of several species in the genus *Schistosoma*, blood flukes. In nearly all instances, cestodes have an indirect life cycle, with a definitive host and one or two intermediate hosts in the life cycle. Nematodes have either a direct or an indirect life cycle; larvae pass through a series of four moults to become adults.

18.3 ROUTE OF TRANSMISSION

Although, in the case of intestinal parasites, the most common portal of invasion is through the mouth, exposure can occur from one or more of the following sources:

(1) contaminated soil;
(2) contaminated water;
(3) food containing the immature infective stage of the parasite;
(4) blood-sucking insects;
(5) contact with animals, including humans, harbouring the parasite; and
(6) self-contamination.

Soil polluted with human and animal excreta is commonly responsible for exposure in which a few important nematodes actively enter the body through the skin. Water may contain viable embryonated ova of nematodes and cyclophyllidian cestodes and the infective cercarial stage of *Schistosoma* species, as well as (oo)cysts of many parasitic protozoa. Freshwater fishes, crabs, and crayfishes are well documented sources of trematodes and cestodes. Blood-sucking insects that breed in the immediate environment of water resources, irrigation ditches, and other water bodies can transmit a large number of parasites that require development in insect hosts.

Contamination of a water system with sufficient quantities of embryonated ova or infective larvae could potentially result in outbreaks. However, this scenario would be difficult in community water sources, since the ova and larvae are relatively large and would be readily removed by standard processes for drinking-water treatment, including flocculation, sedimentation, and filtration, used by municipal water systems in many countries. However, post-treatment contamination or breakthrough of helminths in water systems with poor or less stringent treatment is highly possible.

18.4 NEMATODES

Many species of nematodes are free-living forms, found everywhere in fresh or salt water, in mud, or in soil. Others are plant nematodes. Myriads of species of nematodes are parasites of invertebrate and vertebrate animals. These parasitic nematodes have both a direct and an indirect life cycle. Several nematode species, such as hookworms, *Ascaris lumbricoides*, *Trichinella spiralis*, and filarial worms, are important human pathogens. Some of them are primarily or absolutely human parasites; others (*T. spiralis*) have a variety of mammalian hosts; and still others (*Strongyloides stercoralis*) have, at least temporarily, exclusively a parasitic phase in humans or exclusively a free-living phase. Filarial species require an arthropod as the intermediate host. In addition, there are many species of nematodes that are commonly parasitic in animals other than humans and incidentally parasitize humans, at times with disastrous consequences.

18.4.1 *Ascaris lumbricoides* and *A. suum*

The roundworm *Ascaris lumbricoides* is found worldwide. The largest nematode parasitizing the human intestine, an adult female may reach over 30 cm. It has a direct life cycle. Ova are found in insufficiently treated sewage fertilizer and in soils, where they embryonate upon exposure to air or oxygen in order to become infective. Under ideal conditions, this usually requires about 3 weeks. The ova may contaminate crops grown in soil or fertilized with sewage that has received non-lethal treatment. People acquire the infection through consuming such raw produce or contaminated drinking-water.

After being swallowed, the infective larvae escape from the ova, penetrate the wall of the intestine, reach the mesenteric lymphatics, and are carried through the right heart to the lungs before they develop into adult worms. The migration of the larvae through the lungs causes the blood vessels of the lungs to haemorrhage, and there is an inflammatory response accompanied by oedema. The resulting accumulation of fluids in the lungs results in "ascaris pneumonia," and this can be fatal in a heavy infection.

While humans are normally infected from another human source, infection with pig *Ascaris suum* does occur. Sanitary disposal of both human and animal excreta is the main method of preventing transmission. All faeces, when used as agricultural fertilizer, should be composted with vegetable refuse, since a temperature of 50 °C will kill the ova.

18.4.2 *Toxocara canis* and *T. cati*

Toxocara canis is a parasite of dogs that is found worldwide, while *T. cati* has been found in domestic and wild cats. The incidence is known to be high in dogs and cats, especially in young animals.

Larval toxocariasis in human hosts, caused by the second-stage larvae of *T. canis*, had been recognized in the 1950s in children (Wilder 1950; Beaver *et al*. 1952; Nichols 1956). Human toxocariasis results from the presence of larvae in the tissues after ingestion of embryonated ova through swallowing earth containing infective ova or consuming contaminated water. The most outstanding features of the disease in humans are eosinophilia, lasting several months, with a rise from a pre-exposure level of 3–6% to over 50%; and enlargement of the liver associated with hypergammaglobulinaemia, lasting a few months or weeks.

Larvae have been found in the central nervous system. Generalized convulsions have been reported in children with signs but without proof of invasion of the central nervous system. Fatal cases are rare but reported. The migration of the larvae leads to haemorrhage, and the resultant granulomatous

lesions can be widely scattered throughout the central nervous system. The parasite may transport viruses and other microorganisms, especially the virus of poliomyelitis, by destroying the blood–brain barrier in its migration (Woodruff 1968). Eye involvement in *Toxocara* infection is a common presentation in children 4–6 years of age, although a few incidents of eye infection have also been found in much older children and adolescents.

18.4.3 *Baylisascaris procyonis* and other non-human ascarids

Baylisascaris procyonis, known to be the racoon roundworm, is recognized as a cause of fatal or severe neurological disease. This ascarid nematode is an important zoonosis, producing damaging visceral, ocular, and neural larva migrans in humans, too. A small percentage of larvae enter the brain, where they produce marked traumatic damage and inflammation that often result in clinical central nervous system disease. *Baylisascaris procyonis* is receiving increased attention in North America, Europe, and Japan. Racoons are native to North and Central America but have been introduced elsewhere, taking *B. procyonis* with them. Racoons have established in major areas of Europe and Asia, following their escape or release decades ago. For example, it is estimated that more than 100 000 wild racoons occur in Germany, with a prevalence of *B. procyonis* infection of 71% (Gey 1998). The increase in racoons in Europe has been accompanied by *B. procyonis*-induced larva migrans in various species, including humans (Koch and Rapp 1981; Küchle *et al*. 1993). Over 20 000 racoons have been imported into Japan as pets since 1977; some of these may have escaped and/or been released and now inhabit wild areas. Infection of *B. procyonis* has already been confirmed in racoons in Japan (Miyashita 1993). Racoons can be well adapted to coexistence with human beings in both urban and rural areas. This eventually brings extensive opportunities for contact and infection of human beings with *B. procyonis*. Infectivity and pathogenicity of other members of *Baylisascaris* remain to be elucidated. Other non-human ascarid nematodes (*Lagochilascaris*, *Hexametra*, *Porrocaecum*, etc.) might also cause larva migrans (Goddart *et al*. 1985; Rosemberg *et al*. 1986).

18.4.4 *Trichuris trichiura*

Trichuris trichiura is one of the most common human nematodes, and apparently the same species lives in monkeys. Similar species may be found in many other animals, including pigs (*T. suis*). The ova pass from the body to the soil with faeces and within a few weeks develop into larvae that can remain viable for many months under moist conditions (reviewed by Bundy and Cooper 1989).

18.4.5 *Ancylostoma duodenale* and *Necator americanus*

Ancylostoma duodenale and *Necator americanus* are the most important hookworms of humans. Ova of *A. duodenale* embryonate in moist warm soil, and larvae hatch within 24–48 h. In about a week, they become filariform larvae that crawl to a high point of dirt, vegetation, or other moist substrate, ready to enter the host directly through the skin or through ingestion. Excess water at this stage of the life cycle is injurious to the worms. The filariform larvae burrow into the skin, enter a blood or lymph vessel, are carried to the lungs, pass upward to the mouth, and are swallowed, arriving in the small intestine, where they mature to adults.

Symptoms of infection start with ground itch, which occurs during the penetration of the skin by the filariform larvae. Creeping eruption may occur if human skin is penetrated by larvae of other species from animals, such as *A. braziliense* and others, which follow the same general life cycles in their own definitive hosts.

18.4.6 *Strongyloides stercoralis*

Strongyloides stercoralis is unique among helminths in having both free-living and parasitic generations. In the parasitic generation, parthenogenic females live in the mucosa of the small bowel, where they shed ova that hatch *in situ* into larvae. When liberated in the faeces, they develop into either infective filariform larvae or free-living males and females. The infective filariform larvae are ready to penetrate the body of the host. This can occur inside the intestine (internal autoinfection) or outside the body. The free-living generation larvae, on the other hand, reach maturity and complete their free-living life cycle repeatedly under favourable environmental conditions in essentially the same manner as that of any non-parasitic soil nematode. When environmental conditions become unfavourable, however, the rhabditiform larvae develop into the filariform larvae and become infective to humans.

Infection in humans and animals is contracted mainly by soil contact and penetration of the skin by larvae. Infection by the gastrointestinal route may also occur. Dogs and cats can be important reservoirs of human infection, as virulent strains may be introduced. Cross-infectivity between humans and dogs will depend on the infectivity within species and geographic strains of the parasite, which differ in their infectivity for different hosts.

18.4.7 *Angiostrongylus cantonensis* and *A. costaricensis*

Humans become infected with *A. cantonensis* and *A. costaricensis* by ingesting the third-stage larvae, either by consuming the molluscan intermediate hosts or

by consuming paratenic hosts that had fed on such infected molluscs. It is theoretically possible for humans to become infected by ingesting third-stage larvae liberated into water from dead or wounded molluscs, but such a source of infection is difficult to prove. The most common clinical feature of *A. cantonensis* infection in humans is meningitis 1–3 weeks after exposure, characterized by headache, moderate stiffness of the neck or back, paresthesia, little or no fever, and a pleocytosis consisting in large part of eosinophilic leukocytes.

Angiostrongylus costaricensis infection in humans (abdominal angiostrongylosis) is characterized by abdominal pain, mostly in the iliac fossa, prolonged fever, anorexia, and vomiting. Pathogenic lesions are found in the appendix and adjacent intestine and lymph nodes, consisting of granulomatous inflammation with intense eosinophilic infiltration. This nematode often reaches sexual maturation and releases ova into the intestinal tissues. The disease is endemic in Central and South America, but an autochthonous African case has been reported (Baird *et al*. 1987).

18.4.8 *Capillaria hepatica*

Capillaria hepatica (syn. *Calodium hepaticum*) lives in the host's liver, generally surrounded by a connective tissue capsule. Rodents are the primary initial hosts affecting humans, while cats, dogs, and rats are the principal transient hosts releasing ova in human habitats. The only known mode of spread to the definitive host, including humans, is ingesting embryonated ova. The ova must be released from the liver of the initial host through digestion in a transient (intercalary) host. The ova then pass out in the faeces, embryonate, and become infective. Embryonation occurs in about 4 weeks at 30 °C. Few verified cases of human infection with this nematode have been reported.

18.4.9 *Dracunculus medinensis*

Dracunculus medinensis is known as the guinea worm and is referred to as Moses' "fiery serpent" in the Bible. Female adult worms of *D. medinensis* range from 750 to 1200 mm in length and live in the connective tissue of humans and other vertebrates, where they migrate from one site to another. When the female is ready to discharge larvae (embryos), its anterior end emerges from a blister or ulcer, usually on the foot or lower limb, releasing large numbers of rhabditiform larvae when the affected part of the body is immersed in water. Larvae can move about in the water as long as 3 days until they are ingested by crustacean *Cyclops*. They moult twice in the intermediate host and are infective to a new host in about 2 weeks. If infected *Cyclops* (0.5–2.0 mm) are swallowed in

drinking-water, larvae are released, penetrate the intestinal and peritoneal walls, and inhabit the subcutaneous tissues. Infection with guinea worm is geographically limited. An ongoing eradication campaign has reduced the incidence of dracunculiasis, which is now restricted to rural, isolated areas in a narrow belt of African countries. The total number of dracunculiasis cases reported worldwide during 2002 was 54 638, of which about 76% were from Sudan (WHO 2003).

The only route of exposure is consumption of drinking-water containing *Cyclops* spp. carrying infectious larvae. The life cycle of *D. medinensis* can be broken by preventing the consumption of drinking-water that contains *Cyclops* spp., preventing the release of *D. medinensis* larvae (embryos) from female worms in infected patients into water, controlling *Cyclops* in water resources by means of fish, or inactivating *Cyclops* in drinking-water supplies by treatment with chlorine or copper sulfate.

18.5 TREMATODES

In general, trematodes are monoecious and require two intermediate hosts in their life cycle. The infectious stages of the trematodes to humans are metacercariae, which develop in the second intermediate hosts, with some exceptions. Ingesting raw second intermediate hosts, such as crabs (*Paragonimus*), fish (*Clonorchis*, *Opisthorchis*, *Echinostoma*, *Clinostomum*, heterophyid species), or vegetation (*Fasciola*, *Fasciolopsis*), depending on the choice by the larval trematode species, constitutes the source for human infections. Viewed in this light, infection of trematodes through drinking-water is unlikely.

18.5.1 *Schistosoma*

Schistosomiasis is a waterborne infection usually contracted by bathing in water that contains the snail intermediate host. There are a wide variety of snail hosts, each adapted to transmission of local strains of the schistosome species. Some snails are entirely aquatic, whereas others are amphibious. Some are abundant in small bodies of water, such as ponds and irrigation ditches; others are abundant in large lakes and in running streams. The amphibious snails are most abundant in and along banks of irrigation canals and drainage ditches, but can be drowned in flooded areas.

Many of the major economic developments in tropical areas are being frustrated by the increased prevalence of schistosomiasis as a result of water development (Ofoezie and Asaolu 1997; Chitsulo *et al*. 2000; Ross *et al*. 2001).

Schistosomes are unusual trematodes, in that the sexes are separate and there are no second intermediate hosts in their life cycles. There are a number of species of schistosomes that can infect humans, but most human infections are caused by one of the following three species: *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*. These primary species of human schistosomes have been well documented. The pathological lesions and clinical manifestations due to *S. haematobium* infection are distinct from those produced by the other two species. The adult worms of the former species migrate to the plexus or veins around the bladder, where they deposit their ova. The ova eventually escape into the urine, causing irregular haematuria, or they are engulfed in granulomatous lesions and papillomas. An accumulation of ova in the tissues of the bladder and ureters with the development of fibrosis may result in the formation of carcinoma of the bladder. The adult worms of the latter two species are found in the mesenteric and portal venous systems. The main pathogenic lesions are in the bowel and liver. They produce a focal colitis, which may present as a dysenteric syndrome. The extensive deposition of ova in the liver results in the development of multiple granulomas, periportal fibrosis, and, finally, hepatosplenic disease.

There are several other species in the genus *Schistosoma*, such as *S. intercalatum*, *S. bovis*, *S. mattheei*, and *S. rodhaini*, that can incidentally develop to maturity in humans and produce characteristic ova in the excreta.

18.5.2 Cercarial dermatitis

Avian schistosomes, including *Trichobilharzia ocellata*, developing in freshwater snails are known to be responsible in their cercarial stage for the production of papular eruptions of the skin of persons bathing in infected waters (Horak *et al*. 2002). Similarly, another type of avian schistosome dermatitis develops along saltwater beaches, with marine molluscs serving as the intermediate hosts and saltwater or migratory birds as definitive hosts. They are well documented as cercarial dermatitis or swimmer's itch (Cort 1950; Kirschenbaum 1979).

Other species known to cause cercarial dermatitis (Miyazaki 1991) include *Gigantobilharzia sturniae*, *Trichobilharzia brevis*, *T. physellae*, *Austrobilharzia variglandis*, and *Schistosoma spindale.*

18.5.3 *Fasciola hepatica*

Fasciola hepatica is found in most herbivores and averages 20–30 mm in size. *Fasciola hepatica* gives rise to cercariae during its asexual reproduction in *Lymnaea* snails. Cercariae, when released into the environment, swim to aquatic

vegetation and encyst as metacercariae. The vertebrate host, including humans, acquires infection by ingesting the metacercariae with water plants or drinking-water. Following maturation of the young flukes, the adult worms are found in the liver, gall-bladder, or associated ducts. They can cause severe damage, depending on the number of worms present and organs infected. The ova are passed with the bile into the faeces to continue the cycle.

There are two other related species in the family Fasciolidae: *Fasciola gigantica* and *Fasciolopsis buski*. The adults of the latter species are found not in liver but in the intestine of humans and pigs. Red caltrop (*Trapa natans*; water chestnut) is well known to carry metacercariae in China.

18.5.4 Miscellaneous

Cercarial dermatitis and ocular infection of the cercariae of *Diplostomum spathaceum* and mesocercarial invasion of *Alaria marcianae* have been reported. A fine review is provided by Smyth (1995).

18.6 CESTODES

The adult tapeworms of humans consist of a chain of a few to many ova-producing units (proglottids), which develop from the distal end of a scolex, which anchors the worm to the intestinal wall of its host. Cestodes infecting humans are found in two distinct orders: Cyclophyllidea and Pseudophyllidea. The former requires only one intermediate host, while the latter requires two intermediate hosts (copepods, aquatic vertebrates). In natural situations, the larval cestodes develop in mammals with which the respective final hosts have a predator–prey relationship.

Ova (embryophores) of some tapeworms are protected by thick shells and, in the most environmentally resistant species, are embryonated and infective when passed from the host. The genera *Taenia* and *Echinococcus* are among those most likely to be distributed in environments where they can infect humans and other animals. In areas of the world where the processes are inadequate for parasite destruction, dispersal of these tapeworm ova to the environment could constitute a serious public health hazard. Ova from both genera have been found in sewage.

18.6.1 Cyclophyllid cestodes

The order Cyclophyllidea contains two major genera that occur in humans: *Taenia* and *Echinococcus*. A high degree of host specificity is characteristic of

the adults of these cestodes. The range in intermediate hosts, however, is influenced by both phylogenetic and ecological factors.

18.6.1.1 Taenia solium and Taenia saginata

Taenia solium, the pork tapeworm, infects humans in both its adult and larval stages. Adults inhabit the human small intestine. Patients may be asymptomatic, but gastrointestinal disorders, including diarrhoea, flatulence, tympanites, and abdominal pain, are often reported. Humans become infected by larval *T. solium*, called cysticercosis, by ingesting food or water contaminated with embryonated ova. Massive invasion of skeletal muscles causes myositis, with pain, swelling, and weakness. Severe involvement of the myocardium causes heart failure. Clinical features of cerebral infection can include visual failure, seizures, episodes of abnormal behaviour, transient obstructive hydrocephalus, disturbed equilibrium, and other abnormalities. The degeneration of cysticerci in the brain results in a pronounced tissue reaction. Because of taboos concerning the use of pigs as food, *T. solium* is rarely found in Muslim and Jewish populations.

Ova of *Taenia saginata*, the beef tapeworm, may be distributed where cattle or sheep graze pastures that have been irrigated with untreated wastewater. Although transmission of *T. saginata* to cattle exposed to sewage wastes has been reported, little information is available relating to the magnitude of the threat to public health in either developing or developed nations. Pawlowski and Schultz (1972) reviewed the disease aspects of infections with tapeworms, including potential transmission of the beef tapeworm through sewage and sludge. However, additional data are urgently needed on the frequency of transmission to humans from utilization of variously treated or untreated sewage and/or sewage sludge. Larval beef tapeworms have not been found in humans.

18.6.1.2 Echinococcosis

Humans become infected by the larvae of four species of *Echinococcus*: *E. granulosus* (cystic hydatid disease), *E. multilocularis* (alveolar hydatid disease), *E. vogeli* (polycystic hydatid disease), and *E. oligarthrus* (polycystic hydatid disease). In the natural hosts, the larvae of the respective species are distinctive morphologically, but not in their basic organization. The range of pathological changes and clinical manifestations that develop in humans is largely attributable to sites of localization.

The characteristic feature of alveolar hydatid disease caused by *E. multilocularis* infection is the proliferation of the larvae in the liver, the primary site of localization in humans and in the natural intermediate hosts, by exogenous budding, invading irregularly and destroying the surrounding hepatic

tissue. The disease is chronic and usually asymptomatic until the lesion becomes large. Metastasis to the lungs and brain in humans may occur and is ultimately fatal.

The intermediate hosts, including humans, become infected through ingestion of ova shed in the faeces of foxes or dogs. The area inhabited by foxes may be grossly contaminated by their faeces. Dogs as synanthropic hosts appear to be a more important source of infection than wild foxes. Water contaminated by *E. multilocularis* is a major concern in Hokkaido, Japan (Yamamoto *et al*. 2001), where drinking-water plants have found their way into remote villages and are operated with scrupulous care.

Since the larval *E. multilocularis* produces large numbers of protoscolices in the natural intermediate hosts, such as voles, massive infections in the final host tend to occur.

Cystic hydatid disease induces a variety of clinical characteristics, attributable to the site of localization of the larvae of *E. granulosus* and complications. A single cyst in the lungs or liver may be asymptomatic unless it has become unusually large or ruptured. Leakage of cyst fluid may cause allergic reaction, including anaphylaxis. Suppuration of ruptured cysts is a frequent complication, especially in the lungs. Rupture of cysts in abdominal organs may lead to secondary dissemination in the peritoneal cavity. Metastatic foci may develop in the lungs or brain when cellular elements of the larvae enter the circulation. Cysts in skeletal locations may cause severe erosion.

Large numbers of cestodes may develop from a single cyst eaten by the final host; 60 000–70 000 adult cestodes have been recorded in individual dogs. Cystic hydatid disease is almost exclusively a consequence of contact with dogs harbouring the adult *Echinococcus*. The raising of livestock in association with dogs results in the establishment of a closed system; under such conditions, humans frequently become infected. Surface water and streams running in or near such fields raising livestock in association with dogs can be easily contaminated by the ova, and water may play an important role in the dissemination of the hydatid diseases, although the actual route of infection is still unclear. Economic losses due to cystic hydatid disease are of great concern throughout much of the world's farming country (Attanasio *et al*. 1985; Battelli 1997).

Echinococcus vogeli and *E. oligarthrus* have been reported to cause human polycystic echinococcosis in Latin America. The clinical presentation of the polycystic echinococcosis is very similar to infection with multiple cysts of *E. granulosus*. The latter is an extremely rare cause of human echinococcosis.

18.6.2 Pseudophyllid cestodes

Cestodes in the order Pseudophyllidea that infect humans include *Diphyllobothrium latum* and related species, such as *D. nihonkaiense* and *Diplogonoporus grandis*. Transmission to humans is by ingestion of hosts that harbour second-stage larvae (spargana), mostly fish.

Human infection with larval stages of *Spirometra erinaceieuropaei* can be acquired by ingestion of copepods in drinking-water. Other modes of transmission have been reported. Symptoms can develop rapidly after infection by cutaneous or mucocutaneous invasion, but may not develop for months or years if infection results from ingestion of larvae. Signs and symptoms depend on the site of migration and localization of the larvae.

There is still confusion concerning the classification of the diphyllobothriid larvae occurring in humans. Larval cestodes are not morphologically distinct, and there is little information regarding adult worms that develop from them.

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19

Human fascioliasis

S. Mas-Coma

19.1 INTRODUCTION

Fascioliasis is caused by two trematode species of the genus *Fasciola*: *F. hepatica*, present in Europe, Africa, Asia, the Americas, and Oceania, and *F. gigantica*, mainly distributed in Africa and Asia. Human fascioliasis was considered a secondary disease until the mid-1990s. This disease has great potential for expansion thanks to the large colonization capacities of its causal agents and vector species. It is emerging or re-emerging in many countries, including both prevalence and intensity increases and geographical expansion. Today, fascioliasis is the vector-borne disease presenting the widest latitudinal, longitudinal, and altitudinal distribution known (Mas-Coma *et al*. 2003).

In both species, the adult stage, which is relatively large in size (*F. hepatica*: 20–50/6–13 mm; *F. gigantica*: 24–76/5–13 mm), is a parasite of the large biliary passages and the gall-bladder. Disease is chiefly confined to the liver, so

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that the most important pathogenic sequelae are hepatic lesions and fibrosis and chronic inflammation of the bile ducts. The clinical periods include (i) the incubation phase (from "a few" days to 2–3 months), (ii) the invasive or acute phase (2–4 months), (iii) the latent phase (months or years), and (iv) the obstructive or chronic phase (after months to years of infection). Immature flukes may deviate during migration, enter other organs, and cause ectopic fascioliasis, most frequently in the gastrointestinal tract, but also in subcutaneous tissue, heart, blood vessels, the lung and pleural cavity, the brain, orbit, abdominal wall, appendix, pancreas, spleen, inguinal nodes, cervical node, skeletal muscle, and epididymis. The usual pathological effects of ectopic lesions are due to the migratory tracks causing tissue damage with inflammation and fibrosis (Chen and Mott 1990; Mas-Coma and Bargues 1997; Mas-Coma *et al*. 1999b, 2000).

True human endemic areas have been described in which fascioliasis chronicity and superimposed repetitive infections pose additional pathological complications (Valero *et al*. 2003). The clinical synergistic capacity of fasciolids in co-infection with other pathogenic agents is well known, immunological responses to pathogen antigens being markedly suppressed and concomitant infection being exacerbated following fascioliasis infection (Brady *et al*. 1999). Interestingly, the parasitological spectrum of protozoan and helminthic species found in the inhabitants of the human fascioliasis endemic areas, the multiparasitisms, and the associations between liver fluke infection and infection by other pathogenic parasites all appear to be similar in the different human endemic zones (Esteban *et al*. 1997a, 1997b, 1999, 2002, 2003). These synergistic associations of fascioliasis with other pathogens are believed to be at the base of the high morbidity and mortality rates of Aymara children inhabiting the Northern Bolivian Altiplano (Mas-Coma *et al*. 1995).

19.2 TRANSMISSION

The two-host life cycle of both fasciolids is similar and takes about 14–23 weeks. It comprises four phases (Mas-Coma and Bargues 1997; Mas-Coma *et al*. 2003):

A) The definitive host harbours fluke adults, producing eggs that reach the external milieu by way of bile and intestine; the definitive host is infected by ingestion of metacercariae; in humans, the flukes attain sexual maturity in 3–4 months, and their life span is between 9 and 13.5 years.
B) The transit between definitive mammal host and intermediate snail host includes the long resistance phase of the egg and the short active phase

of miracidium; eggs shed with the mammalian faeces will continue their development in fresh water of appropriate physicochemical characteristics (mainly temperature of 15–25 °C).

C) The development at snail level includes miracidium penetration, sporocyst, redial generations, production of cercariae, and shedding of the latter into water; the prepatent period (38–86 days) is dependent on temperature, higher temperatures reducing the period.

D) Transit between snail and mammal host includes the short swimming phase of cercaria and the long resistance phase of metacercaria; the shedding process takes place between 9 and 26 °C, independently of light or darkness; cercariae swim for a short time until contacting a solid support, mostly leaves of water plants above or below the water line, to attach and encyst; metacercarial cysts become infective within 24 h.

Liver fluke development is very dependent on environmental characteristics during phases B, C, and D and is markedly influenced by human activities during phase A.

19.3 GEOGRAPHICAL DISTRIBUTION

Recent studies have shown fascioliasis to be an important public health problem (Chen and Mott 1990; WHO 1995; Mas-Coma *et al*. 1999a, 1999b). Today, we know that fascioliasis can no longer be considered merely as a secondary zoonotic disease but must be considered to be an important human parasitic disease (Mas-Coma *et al*. 1999a, 1999b). Human cases have been increasing in 51 countries on five continents (Esteban *et al*. 1998). Recent papers estimate human infection up to 2.4 million (Rim *et al*. 1994), up to 17 million (Hopkins 1992), or even higher, depending on the hitherto unknown situations in many countries, mainly of Asia and Africa.

A global analysis of the geographical distribution of human cases shows that the expected correlation between animal and human fascioliasis appears only at a basic level. High prevalences in humans are not necessarily related to areas where fascioliasis is a great veterinary problem. The major health problems are known in Andean countries (Bolivia, Peru, Chile, Ecuador), the Caribbean (Cuba), northern Africa (Egypt), Near East (Iran and neighbouring countries), and western Europe (Portugal, France, and Spain) (Esteban *et al*. 1998).

19.4 EPIDEMIOLOGY

Three types of human endemic situations in areas presenting human fascioliasis have been established (Mas-Coma *et al*. 1999a):

(1) hypoendemic, with a prevalence of less than 1%; mean intensity less than 50 eggs per gram of faeces (epg);
(2) mesoendemic, with a prevalence of 1–10%; mean intensity of 50–300 epg; and
(3) hyperendemic, with a prevalence of more than 10%; mean intensity usually more than 300 epg.

The epidemiological classification also includes situations of (i) imported cases (human cases diagnosed in a zone lacking fascioliasis) and (ii) authochthonous, isolated, non-constant cases (sporadic human cases in an animal endemic area), as well as epidemic situations, with (iii) epidemics in animal endemic areas (outbreaks usually concern a very few subjects and appear in zones where previous human reports were always sporadic) and (iv) epidemics in human hypo-, meso-, and hyperendemic areas (a higher number of subjects may be involved, usually related to previous climatic conditions having favoured the transmission).

The Northern Bolivian Altiplano shows the highest prevalences and intensities known: prevalences in some communities of up to 72% and 100% in coprological and serological surveys, respectively (Hillyer *et al*. 1992; Mas-Coma *et al*. 1995, 1999c; Esteban *et al*. 1997a, 1997b, 1999; O'Neill *et al*. 1998), and intensities of up to >5000 epg in children (Esteban *et al*. 1997a, 1997b, 1999). Although fascioliasis is more prevalent and intense in children (with a peak in the 9–11 years age group), adult subjects are also infected, with prevalences higher than 40% and mean intensities of up to 752 epg (Esteban *et al*. 1997a, 1997b, 1999). Adult subjects either maintain the parasites acquired when young or can be newly infected because of the high infection risk (Esteban *et al*. 1999). Although of a lower level, prevalence and intensity situations found in other Andean and African countries (Peru, Egypt) were similar (Esteban *et al*. 2002, 2003).

The gender effect in fascioliasis is noteworthy. Prevalences and/or intensities in human hyperendemic areas appear to be significantly higher in females. In Andean countries, females shed pronouncedly and significantly more eggs than males (Esteban *et al*. 1999, 2002), and in Egypt, the prevalence appeared to be significantly higher in females than in males (Esteban *et al*. 2003). In Andean countries, however, prevalences do not differ between sexes (Esteban *et al*. 1999, 2002).

Several human hypo- to hyperendemic areas in the Americas, Europe, Africa, and Asia present a very wide spectrum of epidemiological characteristics related to the very wide diversity of environments (Mas-Coma *et al*. 2003). Within a human endemic area, the parasite distribution appears irregular, the transmission foci being patchily distributed and linked to the presence of appropriate water collections, and human prevalences appear to be related to the distance to water bodies presenting lymnaeids (Mas-Coma *et al*. 1999c).

19.5 FASCIOLID ADAPTATION CAPACITIES, DISEASE EXPANSION, AND DRUG RESISTANCE

The great expansion potential of fascioliasis is related to the large capacities of fasciolids to colonize and adapt to new environments, even with extreme characteristics, such as very high altitudes. Studies on the Northern Bolivian Altiplano (3800–4100 m) showed aspects favouring transmission, such as the longer cercarial shedding period and higher cercarial production; both aspects are related to the greater survival capacity of the infected lymnaeid snails (Mas-Coma *et al*. 2001). Another phenomenon also potentially related to the colonization power of fasciolids is their capacity to produce more larval stages when infecting specimens of the same lymnaeid species, but from another place (Gasnier *et al*. 2000).

The colonization abilities related to domestic animal management and export/import seem to be at the base of the present expansion of triclabendazole resistance, a serious problem, as this is the only drug currently available for human use. Triclabendazole resistance in animals was described first in Australia and later in Ireland, Scotland, and the Netherlands (Gaasenbeek *et al*. 2001). There are no drug alternatives for human treatment; drugs such as bithionol are no longer commercially available (Millan *et al*. 2000), and nitazoxanide, marketed in Mexico (Rossignol *et al*. 1998), and myrrh, registered in Egypt (Massoud *et al*. 2001), still require more studies on efficacy and tolerability. Although no resistance has been detected up to the present in human endemic areas (Talaie *et al*., in press), it may develop due to the extended use of triclabendazole for livestock, the tradition of human self-treatment with triclabendazole in human endemic areas owing to the very general availability of this veterinary drug, and uncontrolled livestock export/import.

19.6 RESERVOIR HOSTS

19.6.1 Normal definitive hosts

Fasciola hepatica is a common parasite of ruminants, especially sheep, goats, and cattle. Alternative hosts are horses, donkeys, mules, and camelids. Wild herbivorous mammals, such as buffalo, deer, wild sheep, wild pig, various marsupials, rabbit, hare, and nutria, are also susceptible hosts, as are various wild species in Africa, including monkeys. *Fasciola gigantica* is common in sheep, goats, cattle, and buffalo and has also been reported in camels, pigs, horses, donkeys, larger antelope, deer, giraffes, and zebras and occasionally in nutria, monkeys, and many other African wild animals (Mas-Coma and Bargues 1997).

Prevalences and intensities in the main reservoirs do not necessarily correlate with human infection. Prevalences and intensities may be relatively high in the main reservoirs when human cases are only sporadic (the situation in animal endemic areas), or they may be very high in humans but relatively low in animals (the situation in human hyperendemic areas). A good example is the Bolivian Altiplano, where prevalences in main (sheep, cattle) and secondary (pigs, donkeys) reservoir hosts are not high enough to explain the human prevalences detected: 49.1–87.0% (mean 61.6%) in sheep; 0–66.6% (23.8%) in cattle; 27.1% in pigs; 15.4% in donkeys; and 0–72.0% (15.4%) in humans (Buchon *et al*. 1997; Mas-Coma *et al*. 1997, 1999c; Grock *et al*. 1998; Esteban *et al*. 1999). Moreover, there is no significant difference in infectivity between isolates from different host species (Valero and Mas-Coma 2000; Valero *et al*. 2001a).

19.6.2 Adaptation to new definitive hosts

Fasciola hepatica has succeeded in expanding from the original European geographical area, thanks to the exportation of European livestock, to actually colonize the five continents, where it has adapted to other autochthonous mammal species, such as camelids in Africa, Andean camelids (also known as "aukenids") in South America, and marsupials in Australia (Mas-Coma *et al*. 2003). The capacity of *F. hepatica* to rapidly adapt to new definitive host species is illustrated by the examples of black rat, nutria, and pig.

In Corsica, there are habitats in which humans become contaminated but the normal definitive hosts (livestock) are not present. In those places, *Rattus rattus* has proven to be the reservoir host, with very high prevalences of up to 45.1% and intensities of 1–12 (mean 3.04) adults per rat in given foci (Mas-Coma *et al*. 2003). The black rat isolate proved to be viable with respect to both development in the snail (Mas-Coma *et al*. 2003) and subsequent black rat

infection (Valero *et al*. 1998a). *Rattus rattus* is able to shed eggs independently from the liver fluke isolate, shedding being continuous, with eggs appearing in the faeces daily throughout the rat life, uninterrupted during a 2-year period. Egg shedding by black rats shows an average number of eggs per fluke per day (850–2150) lower than in sheep (8800–25 000) or cattle (10 000–12 000), but much higher than in rabbits (19–69). Moreover, the chronobiological patterns were shown to favour parasite transmission, both seasonally and daily (Valero *et al*. 2002). Hence, *R. rattus* can contribute to the fluke life cycle and play a reservoir role in the disease.

A similar phenomenon has been detected in the nutria, *Myocastor coypus*, a rodent recently introduced in France. Prevalences detected were very high, 40.1%, in fascioliasis areas, with 90% of the infected nutrias shedding eggs. The average parasitic burden was 5.7 flukes per nutria, with 65% of the liver flukes being mature. The nutria isolate allowed the complete development of the fluke up to a subsequent infection in sheep. The nutria may therefore be considered a potential wild reservoir (Menard *et al*. 2001). Its expansion throughout France and neighbouring countries may thus contribute to liver fluke expansion.

The pig has been reported to offer considerable natural resistance to infection and has even been considered an unsuitable host for fasciolids. While natural parasitization by *F. hepatica* in pig occurs only occasionally in Europe, pig fascioliasis is common in other geographical areas, such as Africa and South America (Mas-Coma *et al*. 2003). Moreover, in the Bolivian Altiplano, *F. hepatica* adult development in the pig is similar to that in sheep and cattle (Valero *et al*. 2001a, 2001b), and there are no differences in metacercarial infectivity (Valero and Mas-Coma 2000). All this indicates that pigs must be taken into account when applying control measures.

19.7 FRESHWATER SNAIL VECTORS

19.7.1 Original and alternative lymnaeid vectors

Fasciola hepatica has a preferred snail transmitting species in Europe: *Galba truncatula*. Other European lymnaeids also found transmitting it under natural conditions are *Omphiscola glabra*, *Lymnaea (Stagnicola) palustris palustris*, *L. (S.) p. turricula*, and *Catascopia occulta.* Main snail hosts for *F. hepatica* in other continents are *G. truncatula* and *Pseudosuccinea columella* in Africa; *Fossaria humilis*, *F. bulimoides*, and *F. cubensis* in North America; *F. cubensis* and *P. columella* in Central America; *F. viatrix*, *L. diaphana*, *F. cubensis*, and *G. truncatula* in South America; *G. truncatula* and *Austropeplea ollula* (= *A. viridis*) in Asia; *L. tomentosa* in Australia; *L. tomentosa*, *P. columella*, and *G. truncatula* in New Zealand; and *A. ollula* in Hawaii, Papua New Guinea,

Philippines, and Japan. Alternative host species are *P. columella* in North and South America; *P. columella* and *A. ollula* in Australia; and *L. gedrosiana* in Iran (Bargues *et al*. 1997, 2001, 2003; Mas-Coma and Bargues 1997).

For *F. gigantica*, principal snail hosts are *Radix natalensis* in Africa; *R. auricularia* sspp. in the Near East, Middle East, Far East, and southern states of the former USSR; *F. cubensis* in the North American Gulf coast; *R. rufescens* in Asia and the Indian subcontinent; *R. rubiginosa* in the Far East and Malaysia; *R. swinhoei* in South-east Asia and the Philippines; and *A. ollula* in Hawaii and Japan. Alternative host species are *G. truncatula* in Africa; *R. caillaudi* in Egypt; *R. peregra* in the Near East, Middle East, and southern states of the former USSR; *R. gedrosiana* in Iran; *R. euphratica* in Iraq; *R. luteola* in Nepal; *R. bactriana*, *R. tenera*, and *R. subdisjuncta* in Turkmenia; *P. columella* in the North American Gulf Coast; and *A. ollula* in the Far East (Mas-Coma and Bargues 1997; Bargues *et al*. 2001).

The different specificities of *F. hepatica* and *F. gigantica* are epidemiologically very important, because of the different ecological requirements of their respective *Galba*/*Fossaria* and *Radix* vector species. The lymnaeids transmitting *F. hepatica* are species that show marked amphibious trends and that usually inhabit small or very small water bodies, such as those temporary water bodies depending on seasonal rain. Lymnaeids responsible for *F. gigantica* transmission are species preferring large and deeper water bodies rich in aquatic vegetation, such as typically permanent water bodies. Thus, transmission foci of both fasciolids are usually different and appear separate, even in the same endemic locality, and fascioliasis by *F. hepatica* is more related to seasonality than is fascioliasis by *F. gigantica*. However, exceptions are found in human hyperendemic areas such as those at very high altitudes, where *G. truncatula* is linked to permanent water bodies owing to high evapotranspiration rates (Mas-Coma *et al*. 1999c).

19.7.2 Lymnaeid colonization capacities and fascioliasis expansion

The amphibious “mud” snail *G. truncatula* spread from Europe, most probably with livestock exportation (i.e., in mud attached to feet of sheep and cattle). It has been reported in northern Africa from Morocco to Egypt, tropical highlands of Ethiopia, Kenya, and Zimbabwe to South Africa, the north-western coast of North America and New Zealand, and the Bolivian Altiplano (Bargues and Mas-Coma 1997; Bargues *et al*. 1997; Mas-Coma *et al*. 2001).

The expanding potential of *G. truncatula* is also related to its capacity for ecological niche widening, as observed on Corsica. This Mediterranean island maintains a low human hypoendemic, human contamination sometimes

occurring in places where fascioliasis transmission would *a priori* not be expected (beaches, fountains alongside roads in the mountainous inland, habitats with dense vegetation, river shores, etc.) (Gil-Benito *et al*. 1991a, 1991b). Such atypical habitats may be considered to be an ecological niche widening, as a consequence of the influences of the insularity phenomenon. This provides an understanding of the fascioliasis endemic throughout the island (Oviedo *et al*. 1992).

The rapidly colonizing, more aquatic, more heat-tolerant *P. columella* is originally from Central America, the Caribbean, and the southern part of North America. This species is today present in South America (up to Peru and Brazil), Europe, Africa, Australia, New Zealand, and even Tahiti. The capacity of *P. columella* to widen *F. hepatica* distribution was suggested by the continued low prevalence in cattle in southern Queensland (Baldock and Arthur 1985), an area generally unsuited for *L. tomentosa*, the main transmitting lymnaeid in Australia (Boray 1969).

19.7.3 New tools for lymnaeid classification and genotyping

The morphological and anatomical uniformity that numerous lymnaeid species show usually causes serious difficulties in specimen classification. Moreover, intraspecies variation of shell shape is particularly marked within lymnaeids, according to environmental conditions. Sequencing of the rDNA ITS-2 has proved to be the most useful tool for species classification and genotyping. ITS-2 analyses showed that the present knowledge on malacological features and systematic classification is far from appropriate. Genetic distances and sequence differences found allowed distinguishing the upper limit within a single species and how different sister species can be expected to be at ITS-2 level (Bargues *et al*. 2001, 2003). Moreover, rDNA markers (18S gene and ITS-2) are able to differentiate between fasciolid-transmitting and non-transmitting lymnaeids, as well as between those transmitting *F. hepatica* and those transmitting *F. gigantica* (Bargues and Mas-Coma 1997; Bargues *et al*. 1997, 2001).

Additionally, isoenzymes and microsatellites proved to be useful for population analyses. Both markers demonstrated that all lymnaeid populations inhabiting the Altiplanic endemic area are monomorphic, a clonicity related to self-reproductive processes deriving from a foundational original population (Jabbour-Zahab *et al*. 1997; Meunier *et al*. 2001). The original snails would have genetically transmitted their high susceptibility to their actual descendants by autofecundation, suggesting a large and homogenous susceptibility of the Altiplanic *G. truncatula* populations to the liver fluke (Mas-Coma *et al*. 2001).

19.8 HUMAN CONTAMINATION

19.8.1 Transmission foci

Transmission foci are patchily distributed and determined by lymnaeid populations inhabiting local waters. The main vector species, *G. truncatula*, is able to adapt to very wide and extreme physical and chemical conditions and to water bodies with a large range of aquatic vegetation. Thus, specific characterization of the transmission foci becomes very difficult, owing to the capacity of lymnaeids to inhabit different types of water bodies, such as small watercourses, natural and human-made canals, subsoil effluences from shallow phreatic layers, large and small rivers, flooding areas, shallow wells, pools, artificial fountains, overflowings, and clean as well as markedly eutrophic waters. Lymnaeids are usually found in stagnant water bodies or those with minimal water flow and very rarely in running waters (such as after intense rainfall) (Mas-Coma *et al*. 1999c).

19.8.2 Human infection sources

Human contamination takes place by ingestion of infective metacercariae. Metacercarial infectivity is dependent upon storage time, being lower when metacercariae are older; the maximum longevity was 31 and 48 weeks using doses of 20 and 150 metacercariae per rat, respectively, although in the latter case only a very low percentage was viable. Moreover, metacercarial viability and infectivity did not show differences between isolates from different reservoir species, demonstrating that flukes from secondary reservoirs such as pigs and donkeys involve the same potential risk as those from sheep and cattle (Valero and Mas-Coma 2000).

There are several contamination sources:

- *Ingestion of wild freshwater plants*: This is an important human contamination source in animal endemic areas. Among vegetables, freshwater plant species differ according to geographical zones and human dietary habits. Moreover, plant species involved are not necessarily the same in subjects infected “at table” (through vegetables making up part of the normal diet) as in subjects “infected in the field” (ingestion or chewing of vegetables taken directly from nature and not necessarily part of the usual human diet). Most human reports are related to watercress. However, the general term watercress includes different aquatic species, such as *Nasturtium officinale* (common watercress), *N. silvestris*, and *Roripa amphibia* (wild watercress). Wild watercress has been reported as the main source of human infection in

areas where fascioliasis in domestic animals is highly endemic. Other aquatic vegetables reported as vehicles of human infection are *Taraxacum dens leonis* (dandelion leaves), *Valerianella olitora* (lamb's lettuce), and *Mentha viridis* (spearmint) (Mas-Coma and Bargues 1997; Mas-Coma *et al*. 1999b). In the Bolivian Altiplano, several freshwater plants have been found to carry metacercariae: 56.3% Compositae; 50.9% *Eleocharis* sp.; 12.0% *Senicio* sp.; 10.3% *Vallisneria* sp.; 3.3% *Scirpus* sp.; and 2.6% Ranunculaceae. In this Andean zone, human infection appears to be related to traditional consumption of uncooked aquatic plants: *Juncus andicola* and *J. ebracteatus* (Juncaceae), *Mimulus glabratus* and *Nasturtium officinale* (Scrophulariaceae), *Nostoc* sp. (Cianofitas), and secondarily others (Mas-Coma *et al*. 1995, 1999c; Esteban *et al*. 1997a).

- *Ingestion of cultivated freshwater plants*: Metacercariae-carrying species may be so important in the human diet of a given area as to be produced at the family or commercial level, thus explaining infection of subjects living far from the endemic area. A study in France showed that home-grown, wild, and commercially grown watercress were the cause of 23, 8, and 2 cases, respectively. Watercress grown at home or commercially is related to outbreaks involving a few individuals (Gil-Benito *et al*. 1991a, 1991b). Metacercariae were found on 10.5% of green vegetables sold in the Samarkand market (Sadykov 1988).
- *Ingestion of wild terrestrial plants*: The long survival capacity and dryness resistance of metacercariae explain human contamination by consumption of wild terrestrial plants collected in dry habitats but submerged in water a few weeks or months before, as in places with temporary water bodies in endemic areas of Iran.
- *Ingestion of cultivated terrestrial plants*: The amphibious characteristics of vector species such as *G. truncatula* explain the transmission foci in plantations of non-aquatic vegetables needing frequent irrigation, such as *Eruca sativa*, *Lactuca sativa*, *Allium porrum*, *Petroselium sativum*, and *Portulaca oleracea*, on which attached metacercariae have been found in Egypt (El Sayed *et al*. 1997; Motawea *et al*. 2001). Thanks to transport of vegetables (both aquatic and terrestrial) from rural endemic zones to cities, plants carrying metacercariae can be sold in non-controlled city markets, giving rise to urban infection, as in Europe or Bolivia (Mas-Coma *et al*. 1999c). Metacercariae were found in 1% of lettuces of a local market in the Mantero valley, Peru (Bendezu 1969).
- *Drinking of beverages made from local plants*: In Iran, the inhabitants of the Caspian region eat fresh wild-grown watercress, other green leafy *Nasturtium* spp., and *Mentha* spp.; the raw plants are ground, mixed

with spices and olive oil, and served as an appetizer or condiment; a paste may also be prepared from these aromatic plants and stored for use over several months (WHO 1995). Similar local beverages are also produced in other human endemic areas, as in Cape Verde.

- *Drinking of contaminated water*: Consumption of natural water is often cited as a human infection source. In the Bolivian Altiplano, 13% of the metacercariae of all isolates are floating (Bargues *et al*. 1996). This becomes very important, owing to the very high number of cercariae-shedding lymnaeids that may be found: 31.6% prevalence in lymnaeids from Tambillo (Bargues *et al*. 1995); up to seven metacercariae in only half a litre of water from the small river crossing Tambillo (M.D. Bargues and S. Mas-Coma, unpublished data); and 20.5% prevalence among 462 lymnaeids collected in front of the school of Santa Rosa de Chaquil, Cajamarca province, Peru, where 47.7% of the children were infected (S. Mas-Coma *et al*., unpublished data).
- *Ingestion of dishes and soups made with contaminated water*: Water containing metacercariae may also contaminate food. Infection by ingestion of salads contaminated with metacercariae-carrying water has been reported (Cadel *et al*. 1996).
- *Washing of kitchen utensils or other objects with contaminated water*: Washing with contaminated water may be the source of inadvertent infection. In Egypt, women usually wash kitchen utensils and clothes at irrigation canals where lymnaeids are present and livestock are introduced for drinking or washing (Curtale *et al*., in press).
- *Ingestion of raw liver*: Recent results suggest that humans consuming raw liver dishes prepared from fresh livers infected with immature flukes may also become infected (Taira *et al*. 1997).

The importance of fascioliasis transmission through water is supported by many indirect results. There are significant positive associations between liver fluke infection and infection by other waterborne protozoans and helminths, such as *Giardia intestinalis* in Andean countries (Esteban *et al*. 1997a, 2002) or *Entamoeba coli*, *Chilomastix mesnili*, and *Schistosoma mansoni* in Egypt (Esteban *et al*. 2003). In many human hyperendemic areas of the Americas, people do not have a history of eating watercress (Hillyer and Apt 1997), and in zones such as the Asillo irrigation area of the Peruvian Altiplano, inhabitants do not consume freshwater plants (Esteban *et al*. 2002). In the Nile Delta region, persons living in houses where piped water is present had a higher risk of infection (Curtale *et al*., in press). In the Egyptian locality of Tiba, where an 18.0% prevalence was initially found (Esteban *et al*. 2003), human infection has

markedly decreased after the construction and utilization of so-called "washing units," in which the water is appropriately filtered.

Human infection is more frequently observed in years with heavy rainfall. Although human infections may occur throughout the year, seasonal distribution is typical in many areas. In Europe, human infection takes place in summer and autumn, and symptoms appear in winter. A prolonged and wet summer in Europe has often been followed by an outbreak. In northern Africa, acute human infections peak in August. Sometimes the seasonality is related to the ingestion of infected plants, with most human cases occurring during the watercress season (see review in Mas-Coma *et al*. 1999b).

The adaptation of lymnaeids to permanent water bodies makes transmission throughout the year possible, as observed in southern Europe (Valero *et al*. 1998b), Mediterranean islands (Oviedo *et al*. 1992), and Andean high-altitude areas (Mas-Coma *et al*. 1999c). Where seasonality occurs, temporary transmission is mainly related to lymnaeid vectors being able to quickly multiply and colonize temporary water bodies from rainfall and to estivate and hibernate during the non-appropriate periods.

19.9 COLONIZATION OF DIFFERENT ENVIRONMENTS

Human fascioliasis endemic areas include different human endemic/epidemic situations, human characteristics (demographics, races, diets, habits, traditions, and religions), domestic and wild mammal reservoir species, lymnaeid transmitting species, geographic zones (both northern and southern hemispheres), altitudes (from -27 m up to 4200 m), and climate conditions (hot and cold weather; seasonal and yearly constant temperatures; scarce to pronounced annual rainfall; low and high mean annual potential evapotranspiration; lack of a dry period to lack of a wet period through different dryness/humidity rates). These areas range from altiplanos to valleys, from islands to mainlands, from natural to artificial irrigations, from lakes to lagoons, from large rivers to small streams, and from permanent to temporary water bodies (Mas-Coma *et al*. 2003).

Climatic factors are decisive in fascioliasis transmission. There are climatic fascioliasis forecast indices that consider variations in factors such as air temperature, rainfall, and/or potential evapotranspiration. Several have been successfully applied to animal fascioliasis; the water budget-based system index and the wetness (Mt) index appear to be the most useful. After appropriate climate-diagram studies, modifications of both indices to fit the high-altitude and low-latitude characteristics proved to be useful in Andean human endemic areas. The modified water budget-based system index allowed the zones to be classified into low-, moderate-, and high-risk areas (Fuentes *et al*. 1999).

Already successfully applied to animal fascioliasis, remote sensing and geographic information systems have also proved to be useful for studying the distribution of human fascioliasis. The prediction capacity of the remote sensing map based on normalized difference vegetation index data, extracted from images from the US National Oceanic and Atmospheric Administration TIROS satellite, appeared to be higher than that from climatic forecast indices. Overlapping between real and predicted human prevalence ranges (transmission risk through normalized difference vegetation index) is notable (Fuentes *et al*. 2001).

Climate change and human modifications of the landscape also play a role in the geographic expansion of fascioliasis. Climatic anomalies associated with the El Niño–Southern Oscillation phenomenon and resulting in drought and floods are expected to increase in frequency and intensity (Githeko *et al*. 2000). Links to outbreaks of human fascioliasis in western South America, mainly Peru and Ecuador, may be expected. Fascioliasis colonization of irrigation systems, giving rise to human disease, has been recently described in the Puno Altiplano, Peru, and the Nile Delta, Egypt (Esteban *et al*. 2002, 2003).

19.10 CONCLUSION

All evidence suggests that the emergence–re-emergence of human fascioliasis is related to many factors, including the high potential of both liver flukes and lymnaeid vectors to adapt to new reservoir hosts, colonize new environments, and spread. The present globalization phenomenon may also be involved, by facilitating international export/import of livestock and enabling both liver fluke and lymnaeid vector transportation as well as expansion of drug resistance. In human endemic areas, results of field and laboratory multidisciplinary studies indicate that water — whether directly by drinking or indirectly by contaminating food (mainly vegetables) or objects capable of giving rise to inadvertent metacercaria ingestion (washing of kitchen utensils, clothes) — may be responsible for a more or less high percentage of human contamination, depending on the different environmental and human characteristics of the endemic areas.

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20

Leptospirosis and other potential zoonoses in water

C. Bolin, C. Brown, and J. Rose

20.1 INTRODUCTION

The environmental burden of excreta from domestic animals, wildlife, and human beings will increase in coming decades, and excreta are likely the largest source of pathogens for the environment. The potential for transfer of these pathogens to surface water and groundwater is evident. Current water treatment systems have been designed to address some of the well known waterborne diseases (e.g., cholera). However, pathogens that are important causes of waterborne illness today and those that represent potential emerging threats present significant challenges for current strategies to prevent waterborne illness. In this chapter, we provide four examples of emerging issues; some of these agents are also addressed from other standpoints in other chapters.

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20.2 LEPTOSPIROSIS

Leptospirosis is an important bacterial zoonotic disease throughout the world. The prevalence of leptospirosis in animals and humans varies in different parts of the world; in general, however, the disease is endemic in the tropics and is more seasonal in temperate climates. This is an example of a true zoonosis, in that the infection is maintained in wildlife and domestic animals and human beings are infected only when there is direct or indirect contact with the animal reservoir; human-to-human transmission is rare. *Leptospira* are shed in the urine of their wildlife or domestic animal reservoir hosts and contaminate the environment, including surface waters. The organism can survive for several weeks outside the host, provided that the environment is wet, close to neutral in pH, and protected from direct sunlight. The route of infection for mammals is by contact of the organism with mucous membranes, including those of the eye, nose, and mouth (Faine *et al.* 1999). For these reasons, leptospirosis is associated with contact with water in many parts of the world.

Outbreaks of leptospirosis in animals and humans are often associated with unusual rainfall events or flooding. Notably, there have been significant and highly publicized outbreaks of leptospirosis associated with El Niño rainfall or hurricanes in the western hemisphere (Trevejo *et al*. 1998; Sanders *et al*. 1999). Recreational use of fresh water is increasingly recognized to present a significant risk of exposure to leptospirosis and has been associated with several disease outbreaks (CDC 1997; Haake *et al*. 2002; Morgan *et al*. 2002; Sejvar *et al*. 2003).

Preventing contamination of the environment with *Leptospira* is not practical in many areas of the world because of the ubiquitous nature of the reservoir hosts. Prophylactic treatment with antimicrobials may be able to lessen the impact of leptospirosis associated with flooding events and recreational exposure to water in high-risk environments (Haake *et al.* 2002). However, in the tropics, where leptospirosis is endemic, such simple precautions as keeping animals out of the living quarters and food preparation areas, vaccination of domestic animals as appropriate, and use of protective apparel (e.g., shoes) may be of considerable benefit.

20.3 *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*

Mycobacterium avium subsp. *paratuberculosis* (hereafter *M. paratuberculosis*) is the causative agent of Johne's disease in ruminants, a chronic, debilitating intestinal disorder associated with diarrhoea, weight loss, decreased milk production, and death. The incubation period for Johne's disease is often 2–7

years, and infected animals begin shedding large numbers of organisms in their faeces before the onset of clinical signs. The common route of infection is oral, and organisms shed in the faeces of infected animals are the major source of exposure for other animals. It is estimated that about 40% of dairy herds in the USA harbour *M. paratuberculosis*.

There is evidence that *M. paratuberculosis* is a human pathogen, and it has been implicated as a potential cause of Crohn's disease. The association between *M. paratuberculosis* and Crohn's disease is controversial: scientists and clinicians argue for and against causation with considerable passion. However, there is an increasing body of evidence that *M. paratuberculosis* is present in the intestinal tissue of a significant portion of patients with Crohn's disease (Mishna *et al.* 1996; Bull *et al.* 2003). A recent report by the US NRC (2003) states, "There is increasing concern over the human-health implications of Johne's disease. The possibility that *M. paratuberculosis* infection could be a cause of some cases of Crohn's disease in humans, combined with concern that *M. paratuberculosis* is becoming widespread in the environment and the food chain, could transform Johne's disease into a serious public health problem."

The potential for environmental contamination with faeces from *M. paratuberculosis*-infected cattle is of concern from both animal and public health perspectives. Infected animals can shed about 10^8 organisms per gram of faeces, with a single cow shedding up to 10^{12} organisms per day. Therefore, the potential for environmental contamination with this organism is high, and this contamination is likely to contribute to new infections in cattle on farms and exposure of wildlife to this agent. Careful management and composting of manure are likely to significantly decrease environmental contamination with this organism. However, it is possible that significant numbers still gain access to surface water and groundwater near concentrations of infected animals.

Mycobacteria are, in general, hardy organisms that survive in the environment for long periods of time. Recent concerns regarding water as a source of exposure to so-called "environmental mycobacteria," including *M. avium*, *M. gordonae*, etc., have led to several studies examining the concentrations of these organisms in water and the effects of various water treatment procedures on the survivability of these organisms (du Moulin and Stottmeier 1983; du Moulin *et al.* 1988; Miyamoto *et al.* 2000; Taylor *et al.* 2000). The results are rather disturbing. In general, these organisms can be found in water systems at relatively high concentrations (e.g., 50 colony-forming units/ml) and are chlorine resistant, with the slower-growing organisms (e.g., *M. paratuberculosis*) more resistant to disinfectants than the more rapidly growing organisms. Studies specifically on *M. paratuberculosis* are few, but the organism has been isolated from municipal water supplies (Mishna *et al.* 1996), is heat resistant (Sung and Collins 1998), and is resistant to chlorine at

concentrations of 2 μg/ml for 30 min (Whan *et al.* 2001). This suggests that *M. paratuberculosis* will present a real challenge to even the best water treatment systems. Assessment of the risk of *M. paratuberculosis* in water supplies requires determination of 1) methods to isolate and detect this very fastidious organism in raw and treated water, 2) the numbers of organisms present in surface water and groundwater near sites of infected animals, 3) the concentrations of organisms likely to be in source water for human/animal consumption, and 4) the infectious dose of this organism.

20.4 MICROSPORIDIA, A RISK FOR SENSITIVE POPULATIONS

The microsporidia are obligate eukaryotic, single-celled, intracellular spore-forming parasites belonging to the phylum Microsporidia. There are over 1000 species, and 100 are capable of infecting both vertebrate and invertebrate hosts. Their role as an emerging pathogen in immunosuppressed hosts is being increasingly recognized, particularly as causes of opportunistic infections in persons with acquired immunodeficiency syndrome (AIDS) and in organ transplant recipients (Didier *et al.* 2000). Typical signs of infection include chronic diarrhoea, dehydration, and significant weight loss. Other health outcomes include keratitis, conjunctivitis, hepatitis, peritonitis, myositis, central nervous system infection, and renal disease. Treatments are available for certain species of microsporidia; however, some species remain resistant to therapy (Bryan 1995).

While a number of genera and species are currently known to infect humans, the most prominently studied have been *Encephalitozoon cuniculi*, *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *Encephalitozoon hellem*. There are two species of microsporidia associated with gastrointestinal disease in humans: *E. bieneusi* and *E. intestinalis*. The prevalence of microsporidiosis in studies of patients with chronic diarrhoea was reported to range from 7 to 50% worldwide (Bryan 1995). It is unclear whether this broad range represents geographic variation, differences in diagnostic capabilities, or differences in risk factors for exposure to microsporidia.

The microsporidia are considered emerging pathogens because new species have been identified as causes of disease in humans during the last 20 years. Because species of microsporidia that were recognized causes of disease in animals are now causing infections in humans, microsporidia are also considered zoonotic pathogens (Didier and Bessinger 1999). Recent genotyping studies are summarized in Table 20.1, and two things may be gleaned from these. First, distinct genotypes can be determined; thus, these

methods should be used in the study of human, animal, and environmental isolates. Second, animals, including humans, may harbour diverse genotypes; however, in surveys, the level of infection was low, and thus small numbers of positives were evaluated. Despite this limitation, it appears that human infections are likely associated with microsporidia originating from domestic animals, particularly cats and pigs.

Table 20.1. Studies of microsporidia (*Enterocytozoon bieneusi*) in faeces

Population studied (*n*)	Results	Reference
Pigs (109), cows (24), and HIV[a] patients (13)	35% + in pigs; four distinct genotypes; grouped 96.3–98.8% together with human genotypes, but none in common.	Breitenmoser *et al.* 1999
AIDS patients (13)	PCR[b] product screened against Genbank detected three genotypes (K, B [11 were B], and D). K has been identified in a cat, D in a macaque, and B only in humans.	Sadler *et al*. 2002
Humans (2), cats (3), pigs (5), and cattle (7)	No segregation could be demonstrated within humans, cats, and pigs. This study showed, with more sophisticated analysis of the genome, that genotypes A, B, and D from humans grouped with pigs and cats.	Dengjel *et al.* 2001

[a] Human immunodeficiency virus.

[b] Polymerase chain reaction.

Only one waterborne outbreak of microsporidial infection has been reported, in the summer of 1995 in France, resulting in approximately 200 cases of microsporidiosis, mostly in AIDS patients (Cotte *et al.* 1999). The species identified was *E. bieneusi* (Sparfel *et al*. 1997). While faecal contamination of drinking-water was never detected, contamination of the water supply from a nearby lake was suspected.

Microsporidia spores have been shown to be stable in the environment and remain infective for days to weeks outside their hosts (Shadduck and Polley 1978; Waller 1979; Shadduck 1989). Because of their small size (1–5 μm), they may be difficult to remove using conventional filtration techniques, and there is a concern that these organisms may have an increased resistance to chlorine disinfection. Initial studies using cell culture suggest that the spores are more susceptible to disinfection (Wolk *et al.* 2000). Ultraviolet doses used in water disinfection (6–9 mJ/cm^2) achieved high levels of inactivation (Huffman *et al.* 2002).

In the USA, there are minimal data on the occurrence of human strains of microsporidia in surface waters (Dowd *et al.* 1998b, 1999). Dowd *et al.* (1998a, 1999) described a PCR method for detection and identification of the microsporidia (amplifying the small subunit ribosomal DNA). They found the organism in sewage, surface waters, and groundwaters, but concentrations and prevalence were not described. The strain that was most often detected was *E. bieneusi*, which was associated with excretion from infected individuals into wastewater. Studies of surface water in France showed very low prevalence, with only 1 sample in 25 positive for *E. bieneusi* (Fournier *et al.* 2000).

20.5 VIRUSES AND SWINE

There are several viruses that may be associated with human disease, whereby zoonotic transmission, in particular via pigs and potentially water, appears to, or could, play a role (Table 20.2). Some of these viruses are currently reported as rare causes of human infection — for example, encephalomyocarditis virus, which causes a disease of swine with lesions in the heart, pancreas, and central nervous system, or Nipah virus, which causes respiratory and neurological signs in pigs and humans. The role of environmental and/or water contamination in the spread of these diseases may be somewhat speculative. However, the dramatic outcomes of these infections (e.g., acute myocarditis and death) (Petruccelli *et al.* 1991; Billinis *et al*. 1999; Parashar *et al.* 2000; Brewer *et al.* 2001), should these viruses continue to make the jump from animals to humans, are of concern, given that water contamination could occur and thus initiate widespread transmission.

Pandemic influenza is a disease that is known to be transmitted via animal reservoirs and aquatic bird populations (Webby and Webster 2001). Avian influenza viruses have been isolated from faeces and unconcentrated lake water, thus transmitting the virus via faecally contaminated water. While the avian virus replicates poorly in humans, pigs support both the human and bird strains. Thus, the role of pigs, birds, and water in the transmission of disease may be an important dynamic to consider.

Hepatitis E virus (HEV) is an enteric RNA virus isolated from humans and causes jaundice and clinical signs similar to those of hepatitis A virus (HAV). HEV is transmitted by the faecal–oral route and has caused devastating waterborne disease outbreaks, particularly in tropical and subtropical countries with inadequate sanitation (Aggarwal and Naik 1997; Balayan 1997). In Kanpur, India, in 1991, there were 79 000 cases of HEV due to sewage contamination of the drinking-water. The earliest confirmed outbreaks occurred in the 1950s in India (Bradley 1992).

Children are often asymptomatic, and the mortality rate is between 0.1 and 4% (Grabow *et al.* 1994). In pregnant women in their third trimester, the mortality rate can exceed 20% (Hurst *et al.* 1996). There has been speculation that HEV is endemic in various parts of the world, and subclinical cases may be contributing to the spread of the disease.

Table 20.2. Viruses associated with swine and potential water transmission

Virus	Interspecies infections?	Role of pigs	Potential for the role of water	Reference
Encephalo-myocarditis	Yes, between mammals (including a few humans)	Pigs are the most common and severely affected	Spread by the faecal–oral route and possibly through urine	Kirkland *et al.* 1989; Joo 1999
Hepatitis E (HEV)	Yes, between domestic animals, wildlife, and humans	Close genetic relationship of human with swine HEV	Faecal–oral transmission; known waterborne outbreaks	Smith 2001
Nipah	Yes, between wildlife and pigs and from there to humans	Outbreak in Malaysia in humans and pigs	Excreted in urine, some evidence of transmission via environmental contact	Parashar *et al.* 2000
Influenza A	Yes, between birds and mammals	Pigs may serve as an inter-mediate host for avian and human viruses	Virus excreted in high numbers by aquatic birds and has been detected in water	Webby and Webster 2001; Webster 2002

HEV is found in wild and domestic animals. Studies using genetic sequencing have found that human and porcine HEV are genetically similar and belong to the same genotype. Swine HEV is widespread in US swine herds (Smith 2001; Huang *et al.* 2002). Thus, zoonotic transmission seems quite likely, and at least one strain can infect across species barriers (Smith 2001). Recent studies in the USA have shown that swine veterinarians are at an increased potential risk of zoonotic HEV infections (Meng *et al.* 2002). The faecal contamination of water from pigs continues to challenge the human population with potential risks associated with the transmission of these agents. The survival and resistance of HEV to water treatment have not been well studied but are presumed to be very similar to those of HAV. As enteric viruses, they are stable in the water environment, particularly at lower temperatures. In addition, during water disinfection, the viruses are more resistant to chlorination than bacteria, although good inactivation can be achieved with free chlorine.

Chlorine disinfection does not inactivate viruses in wastewater as effectively as it does in drinking-water because of interference by dissolved organics and suspended particulates; the resulting disinfectant is combined chlorine, which inactivates viruses less effectively than free chlorine (Mahin and Pancorbo 1999). The enteric viruses are much more stable than the respiratory viruses such as influenza (Pirtle and Beran 1991).

20.6 SUMMARY AND CONCLUSIONS

In this chapter, leptospirosis, a well documented and emerging/re-emerging waterborne zoonosis, was discussed. Clearly, leptospirosis will continue to be an important source of waterborne infections associated with recreational water use, occupational exposure to fresh water, and unusual rainfall events in many parts of the world. In addition, information about selected pathogens that may be zoonotic, have the potential to challenge conventional water treatment systems, and are of emerging interest was presented. These latter agents will require considerably more investigation to determine their pathogenicity for humans, the role of animal reservoirs in the exposure of humans, and the role of water in transmission or dissemination in the environment.

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Section VI

Analysis of zoonotic microorganisms

A. Dufour and D. Till

Management of water quality has been applied mainly to receiving waters contaminated by point sources of human pollution. Waters affected by point sources of pollution are usually subject to regulation, because the pollution frequently impacts bathing beaches or shellfish harvesting waters, as well as drinking-water supplies. Water resources contaminated by dispersed, unidentified sources of pollution, the type usually associated with animals, have not been given special attention until fairly recently. This is the result of a greater awareness of emerging waterborne pathogenic zoonotic microorganisms and improved technical methods to measure water quality. This, in turn, has led to some technical problems that were not anticipated when microbial indicators of faecal contamination were first proposed as a means of monitoring water quality. These problems include the methodology and microbes traditionally

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used to monitor water quality and the recognition of emerging zoonotic pathogens as a waterborne risk, resulting, in some instances, from changing host population behaviour patterns.

At the beginning of the 20th century, Escherich noted that harmless, easily cultivated bacteria occurred in faeces. The organism was suggested for use as an indicator of the presence of faeces from warm-blooded animals in water. The practice of using *Bacillus coli* (later named *Escherichia coli*) as a measure of water quality was adopted, and, over the years, it was used in many forms, dependent on the practical methodology available. Coliforms, faecal coliforms, and *E. coli* were measured, respectively, over time as new specific methods became available. The measurement of *E. coli* in water served as evidence of the presence of faeces and even as a guide to how much faecal contamination was present. This approach appeared to work, because it was applied to waters near relatively small urban centres with poor infrastructure, little water treatment, and populations with little mobility. However, this approach to measuring water quality has been shown to have many inadequacies in identifying public health risk, no doubt related to extending its practical use to unintended applications that have never been validated.

Urban centres have expanded beyond their central confines, to the extent that suburban spread is encroaching on feral animal habitat. Another pressure not envisioned when microbial indicators, such as *E. coli*, were proposed is the commercialization of aquatic food resources harvested from waters that were frequently contaminated with human and animal faecal wastes. Similarly, the practice of confining large numbers of animals to feedlots has created a situation where very large volumes of faecal waste frequently reach waterways, leading to pollution of aquatic food sources and water resources used for recreation. In addition, much of the increased leisure time available to humans is spent on recreational water activities far from urban centres and nearer to natural animal habitats.

This population movement has led to regulations attempting to govern the safety of all surface waters, including those with non-anthropogenic sources of faecal contamination. However, the means by which we monitor surface waters to provide evidence that they are free of faeces — and, therefore, zoonotic enteric pathogens — has changed little in the last 100 years. The microbial indicator of faecal contamination has not changed, and the methodology for measuring these faecal indicator organisms has changed very little. The methods still rely on bacterial growth in culture media to enumerate their presence in water samples. This usually requires a 24-h period of growth before results are obtained, presenting a situation where the potential risk associated with a water body is detected long after a risk activity has occurred.

The risk of illness associated with exposure to foods harvested from animal-contaminated waters or the risk of illness due to direct exposure to these waters is real, but largely unquantified. Although we are fully aware of waterborne zoonotic illnesses through individual cases and outbreaks of illness, we have no way of predicting illness associated with animal-contaminated waters. Our current system for monitoring water quality is designed to protect human health from human-derived pathogens. Unfortunately, the source of faecal indicator bacteria used to monitor water quality is not specific to humans. Currently used indicator methodology cannot distinguish an *E. coli* from animals from an *E. coli* whose source is human. This shortcoming has led many countries to treat animal-contaminated water as if it were water contaminated by humans and therefore of equal risk.

Some solutions to these problems are presented in the chapters of this section. The means to define risk associated with waterborne zoonoses, techniques for identifying sources of animal pollution, and techniques for measuring the safety of animal-contaminated water are discussed with respect to providing tools and information for managing water resources.

21

Managing risk of waterborne zoonotic disease through water quality surveillance

D. Till, K. Field, and A. Dufour

21.1 WHAT IS THE RISK?

The extent of the worldwide disease burden resulting from potential or actual waterborne zoonoses is a growing concern. Until recently, the focus of concern for water-related illness and associated research have been directed towards contamination by human effluent. Microbiological water quality guidelines for recreational waters often included advice that exposure to animal faecal

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microorganisms is a lesser risk than exposure to pathogens of human origin. Guidelines for drinking-water concentrated on catchment protection and on the effectiveness of chlorine and other treatments for eliminating risk from human viruses. Accordingly, there have been very few studies carried out on the effect of animal faeces as a source of waterborne zoonotic infections.

Studies show that there is a swimming-associated pollution-related illness risk (WHO 2003), although results are equivocal for studies that have included findings for beaches impacted by animal faecal material. In a Hong Kong study (Cheung 1988; Holmes 1989), illness rates for two marine beaches impacted by animal (pig) wastes were lower than those for seven other beaches (although total swimming-associated illness rates were still statistically different from zero). In a Connecticut, USA, freshwater study (Calderon *et al.* 1991), no association of gastrointestinal risk with densities of faecal indicator bacteria was reported. Those results have been questioned, in that the data could be reinterpreted to imply that the faecally related health risks were very similar to those found in studies on waters impacted by human effluent (McBride 1993). In a New Zealand study of seven populous marine beaches, no substantial differences in illness risks were found between the human and animal waste-impacted beaches, but both were markedly different from the control beaches (McBride *et al.* 1998).

21.2 EMERGING PATHOGENS

In recent years, several so-called "new" or "emerging" pathogens of animal origin have presented as potential sources of waterborne infection. Few of these pathogens are of recent origin, and some may have been causing illness for a long time, but they were not previously identified due to a lack of suitable isolation and identification methods. These include *Campylobacter jejuni*, *Yersinia enterocolitica*, *Giardia lamblia*, and *Cryptosporidium parvum.*

In addition, there is the emergence of bacteria with new virulence factors, resulting in very potent new pathogens. One example is the pathogenic *Escherichia coli* strains, the enterohaemorrhagic *E. coli* (EHEC) suspected of taking up virulence genes from *Shigella* by horizontal gene transfer.

Members of this so-called new or emerging pathogens group all have an animal reservoir from which they are transmitted to humans, directly or via the environment. In contrast to the classical zoonoses, such as salmonellosis, these emerging zoonoses may not cause apparent clinical conditions in infected animals. Thus, apparently healthy animals can excrete to the environment disease-causing microorganisms of public health significance. Several of these pathogens are resistant to environmental stress (*Cryptosporidium*) and have a

low infective dose (EHEC). Even low-level contamination of water environments may lead to infections and illness in the exposed population.

Surface runoff and point source pollution from pastoral agriculture may introduce pathogenic microorganisms such as *E. coli* (EHEC), *Campylobacter*, *Cryptosporidium*, and *Giardia* into streams and rivers (Geldreich 1996), compromising their suitability for contact recreation and as a source of drinking-water. Small, unprotected rural drinking-water supplies may be a particular concern.

To perform a risk analysis for zoonotic pathogens in recreational water and drinking-water sources, it is necessary to understand the ecology of these organisms and their risk as infective agents. Results from epidemiological studies, together with ecological data, are the basis for effective resource protection and risk assessment for both recreational water use and drinking-water supplies. There are significant gaps in both epidemiological and ecological data available for effective risk assessment. What is known is that the increased incidence of waterborne infections such as campylobacteriosis and the challenge to traditional water treatment systems posed by cysts and oocysts of protozoa such as *Giardia* and *Cryptosporidia* present a situation whereby there is a heightened awareness of the disease risk from waterborne pathogens of zoonotic origin.

21.3 ANIMAL VERSUS HUMAN FAECAL CONTAMINATION

An inherent part of public health water quality surveillance is the evaluation of microbiological results from a water monitoring programme designed to identify unusual trends or patterns and episodes of human and animal faecal material entering waterways used as source waters for drinking-water, for recreational activities, and as a source of shellfish.

An essential element in the surveillance programme is the reporting of water monitoring results to regulatory agencies responsible for informing concerned parties of actual or potential health risk. The target audience for surveillance information will typically include public health officials at local and national levels, water suppliers, local administrators, communities, and water users.

Recreational waters, shellfish harvesting waters, and waters used as a source of drinking-water may be subject to faecal contamination from point sources (usually human domestic wastes) and from non-point sources (highly dispersed animal excreta). Public health authorities maintain the quality of waters for users either by intervention (e.g., requiring treatment) or by limiting access to water sources (e.g., protected catchments). Another approach is to regulate the quality

of the waters by limiting the level of faecal material that can encroach on these resources. Regulating water quality is the most widely used approach to protect public health. Monitoring the water using methods that quantify the density of indicator bacteria in water samples is common practice to ensure water safety. Faecal material and potential enteric pathogens that may be associated with faeces are limited by statute in recreational, shellfish harvesting, and drinking-water source waters to minimize illness in consumers of drinking-water or shellfish products and users of recreational water. There is no agreement on how these waters are monitored. In most shellfish harvesting water jurisdictions, water quality is measured using total coliforms or faecal coliforms. Recreational waters are monitored using *E. coli*, enterococci, faecal coliforms, faecal streptococci, or total coliforms. Sources of water that will be used for drinking-water are monitored in some jurisdictions using faecal coliforms, total coliforms, or *E. coli.* The actual indicators used in different environments are described in numerous diverse international and national guidelines. The World Health Organization is making serious efforts to harmonize approaches to monitoring recreational and other waters.

Water quality monitoring systems that use different indicator bacteria do have some things in common. The water quality limits for drinking-water sources and shellfish harvesting waters are not risk based — i.e., they are not based on a water quality–health relationship. Limits for recreational waters in many countries are based on a relationship between water quality and swimming-associated gastrointestinal disease. A second common feature is that the indicator bacteria cannot distinguish between faeces produced by humans and faeces discharged by animals. This is a significant shortcoming of currently used indicator bacteria. It is intuitive to believe that non-human faeces probably carry fewer pathogens that might be hazardous to humans. For example, viruses that are specific to humans do not normally occur in animals; therefore, the risk from animal faeces has been considered not to be equivalent to that associated with human faeces.

The dilemma is that the presence of such faecal indicators may or may not be an indication of actual risk from pathogens at that time and are of little use in determining if their faecal source is human or animal. Only detailed knowledge of the sources of faecal material in the catchment impacting on a waterway, be they human or animal in origin, and data as to the spatial and temporal loadings of expected pathogens from such sources can assist the assessment of a public health risk.

21.4 LIMITATIONS OF PATHOGEN DETECTION METHODS

The most straightforward approach to assessing public health risk is to monitor microbial pathogens directly. Although effective assays for many pathogens exist, currently these are often expensive and technically complicated. Pathogens may be rare, difficult to culture, and irregularly distributed, yet highly infectious, even at low doses. Because of this rare and uneven distribution, an enrichment step for culture-based detection or some type of stacked polymerase chain reaction (PCR), such as nested PCR for molecular detection, is often required. In most cases, this extra step makes accurate quantification difficult, potentially introducing culture or PCR bias. In addition, detection by PCR does not yield information about the viability/infectivity of the detected organism, as the target of detection is a gene sequence, not a viable pathogen.

The most important limitation to direct detection of pathogens is the number of assays for different pathogens that are required. Furthermore, faeces from both humans and animals may contain as-yet-unidentified pathogens or pathogens for which no assays exist. Newer technology promises the ability to assay for perhaps thousands of indicators and pathogens with a single gene chip or microarray. Microarrays consist of probe sequences (up to many thousands) immobilized in separate locations on a support such as a gel matrix bound to a glass slide. Either DNA or RNA recovered from the environment serves as the target for hybridization to the array of probes. Microbial ecologists have pioneered this approach for monitoring natural populations (e.g., see Guschin *et al*. 1997; Rudi *et al*. 2000; Liu *et al*. 2001; Small *et al*. 2001; El Fantroussi *et al*. 2003). They are working on overcoming the technology's current problems, such as non-specific hybridization (Guo *et al*. 1994; Mir and Southern 1999; Peplies *et al*. 2003). Straub and Chandler (2003) have discussed applications of microarray technology to the detection of multiple pathogens in water.

The availability of such advances will in the future provide a means to further evaluate the relationship of indicators to pathogens and enhance knowledge of pathogen loadings from human and animal sources. However, at present, such resources are limited in availability, very expensive, and, in many instances, still in development.

21.5 LIMITATIONS TO USE OF FAECAL BACTERIAL INDICATORS

Because of limits to direct monitoring of pathogens, it is standard practice to monitor easy-to-culture faecal indicators such as *Escherichia coli* and faecal

enterococci. Recently, molecular detection of indicator organisms has also come into use; for example, a rapid detection method for faecal pollution based on real-time quantitative PCR of enterococci has been proposed (Santo Domingo *et al*. 2003). Epidemiological studies have led to human health standards based on exposure to these indicators in drinking-water, recreational water, and shellfish waters (e.g., see Cabelli *et al*. 1982; Cabelli 1983; Dufour 1984; Cheung *et al.* 1990; McBride *et al.* 1998).

Limitations to the use of bacterial indicators have become apparent. For example, both *E. coli* and enterococci can survive and proliferate outside of animal intestines, in tropical soils and vegetation and temperate habitats such as freshwater algal wrack around the Great Lakes (Hardina and Fujioka 1991; Hagler *et al*. 1993; Anderson *et al*. 1997; Fujioka *et al*. 1999; Solo-Gabriele *et al*. 2000; Desmarais *et al*. 2002; Whitman *et al*. 2003). This calls into question their reliability as indicators in these habitats.

In addition, viral and protozoan pathogens are not well correlated with standard bacterial indicators (for some recent examples, see Wyer *et al*. 1995; Barrett *et al*. 2001; Jiang *et al*. 2001; Noble and Fuhrman 2001; Lemarchand and Lebaron 2003). The processes that control the survival and removal of microbes in water, such as competition, ultraviolet radiation, temperature, predation, and transport (e.g., see Rozen and Belkin 2001), differ among viruses and eukaryotes, and even among different types of bacteria. Thus, monitoring bacterial indicator species may not be sufficient to assess human health risk.

A number of investigators have suggested that the presence of indicator viruses is a better predictor of contamination by faecal viruses than is the presence of indicator bacteria. Commonly used viral indicators include coliphages (Funderburg and Sorber 1985; Havelaar *et al.* 1986; Borrego *et al.* 1987; Havelaar *et al.* 1990; Palmateer *et al.* 1991; Araujo *et al.* 1997; Paul *et al.* 1997) and bacteriophages of *Bacteroides fragilis* (Tartera and Jofre 1987; Tartera *et al.* 1989; Puig *et al.* 1999; Araujo *et al.* 2001). These have traditionally been assayed by plating on susceptible cell cultures, but there are now sensitive molecular assays for a large number of human viruses, including enteroviruses and adenoviruses (reviewed in Griffin 2003). There is still a lack of epidemiological data linking occurrence of viral indicators with pathogens; a lack of information on differential survival of viral groups, especially RNA versus DNA viruses; and a lack of knowledge about population/geographic distribution of viruses in faeces.

Since microbial indicators first came into use, microbial ecologists have amassed substantial information about bacteria in natural and artificial populations. The phrase “the great plate count anomaly” (Staley and Konopka 1985) was coined to describe the very large difference between the number of microbes counted by direct microscopic examination of a particular sample and

the number that can be grown on agar plates. Typically, culturable cells represent only a fraction of a per cent of total cells present. Furthermore, cultured strains do not adequately represent the diversity of types in the habitat. This is true even in well studied copiotrophic habitats, such as the lower intestines of humans and animals. Molecular surveys of bacterial diversity in faeces and the gut have revealed largely unknown strains (Wilson and Blitchington 1996; Pryde *et al.* 1999; Suau *et al.* 1999; Bernhard and Field 2000; Daly *et al.* 2001; Favier *et al.* 2002; Holben *et al.* 2002; Hold *et al.* 2002; Leser *et al.* 2002; Zoetendal *et al.* 2002). It has been known for some time that the community composition of microbial cells in a sample changes drastically when the sample is held in a container and incubated (Ferguson *et al.* 1984; Fuchs *et al.* 2000), even when the container is very large and incubation takes place under natural conditions. It cannot be assumed, therefore, that the indicator types present in a culture proportionally represent what was present in the original habitat or sample.

To avoid problems of culturability and culture bias, investigators typically profile microbial communities by a combination of PCR and sequencing (e.g., see Rappé and Giovannoni, in press). However, because PCRs from mixed templates such as microbial communities are often biased (Suzuki and Giovannoni 1996; Suzuki *et al.* 1998), this approach does not result in a proportional representation of the community. PCR-based surveys must be considered to be qualitative, not quantitative, unless specialized techniques such as real-time quantitative PCR are used and shown to be quantitative.

21.6 SUMMARY AND CONCLUSIONS

The increasing presence of zoonotic pathogens in surface waters provides new and unique challenges for water resource managers. Zoonoses, such as leptospirosis, *E. coli* O157 gastroenteritis, and cryptosporidiosis, have been well defined, and their animal sources have been linked to human disease caused by exposure to contaminated water. Other emerging zoonoses that may be transmitted by faeces through the waterborne route have not been as well defined. The presence of animal faeces in water is currently not identified, because traditional bacterial indicators of faecal contamination cannot distinguish human faeces from animal faeces. Because of their inability to distinguish human from animal faecal contamination, resource managers and regulators have elected to treat all faecal contamination as equally hazardous to human health. This approach frequently results in the closure of beaches and shellfish harvesting areas that are affected by stormwater runoff that carries faecal indicator bacteria of non-human origin. The risk of exposure to these waters contaminated by animals is unknown. Studies that have attempted to

define the risk associated with swimming in animal-contaminated water have not given a clear indication that there is an excess illness rate related to this type of exposure. These equivocal results do not lead to the conclusion that all faecally contaminated waters should be treated alike. New research to define the risk posed by animal faecal wastes to users of water resources and indicator systems that identify animal contamination of surface waters are needed. The availability of more research data that would meet the latter two information needs would significantly improve our ability to manage water resources.

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22

Faecal source identification

K.G. Field[1]

22.1 INTRODUCTION

When there is faecal contamination in water, it is necessary to determine the source, both to mitigate the contamination and to estimate human health risk, as the source will determine which pathogens are present. Both human and animal faecal contamination pose a threat to human health. For example, human faeces are commonly associated with the spread of *Salmonella typhi*, *Shigella* spp., hepatitis A virus, and Norwalk-group viruses; animal faeces spread many diseases, including *Salmonella*, *Escherichia coli* O157, *Giardia*, *Cryptosporidium*,

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and hepatitis E virus. Even technologically advanced parts of the world like North America have experienced recent severe waterborne disease outbreaks (e.g., *Cryptosporidium* in Milwaukee, Ohio, USA, in 1993; *E. coli* O157 in Walkerton, Ontario, Canada, in 2000). The human population may be exposed via drinking-water (Alary and Nadeau 1990; MacKenzie *et al*. 1994; Preston *et al*. 2000), swimming or bathing (Cabelli *et al*. 1982; Keene *et al*. 1994), irrigation, food preparation, and ingestion of contaminated shellfish (McDonnell *et al*. 1997; Fayer and Lewis 1999; Berg *et al*. 2000).

A variety of warm-blooded, and even some cold-blooded, animals contain faecal indicators in their faeces (e.g., see Harwood *et al*. 1999). Thus, the presence of indicator species in water is not sufficient to determine the source of faecal contamination.

Diagnosing the sources of faecal contamination in water is often called bacterial source tracking or microbial source tracking. Since naturally occurring microbes are ubiquitous in water, with bacteria occurring at an average density of 10^6 cells/ml in natural waters, and because the real concern is faecal pathogens in water, not microbes or bacteria, a preferable term is faecal source identification.

22.2 CHEMICAL METHODS OF FAECAL SOURCE IDENTIFICATION

Substances such as caffeine, faecal sterols and stanols, laundry brighteners, surfactants, fragrances, pesticides, and polycyclic aromatic hydrocarbons can be used to detect human and non-human faecal contamination and to determine urban sources of faecal contamination (Nichols *et al*. 1993; Leeming *et al*. 1996; Edwards *et al*. 1998; Sinton *et al*. 1998; Burkhardt *et al*. 1999; Standley *et al*. 2000; Isobe *et al*. 2002; Puglisi *et al*. 2003; Suprihatin *et al*. 2003). Standley and colleagues (2000) compared several of these so-called "molecular tracers" and concluded that a combined index of caffeine and fragrance levels was an effective identifier for human sewage; a ratio between particular steroids made an effective identifier for agricultural input; and a different steroid ratio identified wildlife input. Similarly, profiling of seven sterols in South Australian metropolitan catchments suggested areas of human, dog, and bird faecal impact (Suprihatin *et al*. 2003).

Although chemical indicators and molecular tracers can identify recent faecal inputs, their spread, transport, and persistence in water are unlikely to be correlated with that of pathogens. Currently, there are no epidemiological data that correlate these markers with human risk. In addition, some chemical indicators, such as laundry brighteners and caffeine, are culturally dependent and might not be effective outside developed areas.

22.3 MICROBIAL METHODS OF FAECAL SOURCE IDENTIFICATION

Microbial methods for faecal source identification can be divided into library-dependent and library-independent approaches. In this context, a "library" is a set of bacterial isolates from faecal samples, tested using the method of source discrimination; it is also called a "host origin database." Source identification occurs by a statistical comparison between patterns from the library and environmental isolates. Library-dependent methods include both phenotypic and genotypic tests.

Library-based approaches are labour-intensive, requiring extensive sampling both to prepare the library and to test environmental isolates. All library-based methods have complex requirements for adequate sample size, representativeness, and geographic stability (Wiggins *et al*. 2003; Harwood *et al*., in press; Myoda *et al*., in press). Limited data on geographic stability suggest that for most methods, libraries are not cosmopolitan, and thus a separate library for each locale or watershed may be required (e.g., see Souza *et al*. 1999; Hartel *et al*. 2002).

The average rate of correct classification (ARCC) is a statistical estimate of the ability of a library to correctly classify isolates pulled from the library. ARCCs reported in some studies have been quite high (e.g., see Dombek *et al*. 2000; Guan *et al*. 2002). However, the size of the library influences its ARCC. Small libraries have higher ARCCs than large libraries, but small libraries are not as representative and are therefore not as good at classifying novel isolates (from outside the library) as are large libraries (Wiggins *et al*. 2003). Thus, the ARCC is not a good estimator of the ability of the method to identify faecal sources and can be misleading. Because most methods of faecal source tracking have been assessed only by calculating ARCCs or by application in field studies, where the "real" answer is not known, the ability to compare the effectiveness of the methods is limited.

Microbial methods for faecal source tracking can also be divided into culture-dependent and culture-independent methods. Culture-dependent approaches require growing microbes from water samples. The microbes may be viruses or (more commonly) faecal indicator bacteria, such as *E. coli* or enterococci. The particular source identification method may require selective growth conditions, so that growth itself is diagnostic. Alternatively, a diagnostic test may be applied to the microbial isolates. All library-dependent methods are also culture-dependent.

22.3.1 Library-dependent, culture-dependent methods

22.3.1.1 Phenotypic methods: MAR and CUP

In the multiple antibiotic resistance (MAR) method, also called antibiotic resistance analysis, isolates of *E. coli* or enterococci are tested against panels of antibiotics in order to discriminate among human and various animal sources of faecal pollution; for some examples, see Parveen *et al*. (1997), Hagedorn *et al*. (1999), Wiggins *et al*. (1999), and Harwood *et al*. (2000). Antibiotic resistance is often borne on plasmids, is under strong selection, and is influenced by antibiotic exposure of the host population. Thus, it is not a geographically stable trait; libraries of strains from known sources must be constructed for each new geographic region being tested.

However, the MAR method is inexpensive and low-tech, making it readily available to a broad variety of investigators. In addition, its usefulness has been "proven," as it has been successfully applied to identify and mitigate faecal pollution originating from cattle (Hagedorn *et al*. 2003).

Carbon-source utilization (CUP) has recently been applied to faecal source identification (Hagedorn *et al.* 2003; Wallis and Taylor 2003; Harwood *et al*., in press). In CUP, faecal bacterial isolates are tested against a variety of carbon sources using a commercial kit. This method gave poor identification of sources and a high rate of false positives in a study in which it was applied to blind samples from known sources (Griffith *et al*., in press; Harwood *et al*., in press).

Several investigators have suggested serotyping indicator organism isolates to distinguish faecal sources (reviewed in Scott *et al*. 2002). Parveen and colleagues (2001) achieved a relatively good separation between human and non-human faecal isolates (in a library) with this method, but concluded that its expense made it more appropriate for application in combination with another method.

22.3.1.2 Genotypic methods

Ribotyping, repetitive extragenic palindromic polymerase chain reaction (REP-PCR), amplified fragment length polymorphism (AFLP), and pulsed-field gel electrophoresis (PFGE) are fingerprinting techniques producing bar code-like patterns for each bacterial isolate. In a recent comparative study that analysed samples of known composition, accuracy of faecal source identification by these methods was influenced by whether the investigators simply scored similar patterns as related or required that patterns matched exactly in order to identify them; the latter approach led to a higher percentage of correct source identifications (Myoda *et al.*, in press).

Ribotyping (e.g., see Samadpour and Chechowitz 1995; Parveen *et al*. 1999; Carson *et al*. 2001; Hartel *et al*. 2002; Myoda *et al*., in press) involves growing indicator bacterial isolates, extracting DNA, amplifying 16S rRNA genes, digesting amplification products, and detecting patterns of restriction fragments. This approach was popularized by extensive commercial application in the USA, and currently a number of studies using it are under way. The process is available in an automated format, although most investigators do it manually.

Several different versions of REP-PCR have been tested for faecal source identification, differing in choice of primers (e.g., see Dombek *et al.* 2000; Carson *et al.* 2003; McLellan *et al.* 2003). In this method, DNAs extracted from faecal indicator isolates are amplified with PCR primers complementary to conserved repetitive elements in bacterial genomes. The result is patterns of different-sized fragments, depending on the location of the conserved elements.

In AFLP, genomic DNA from faecal indicator isolates is digested with restriction enzymes, linkers are ligated to the restriction fragments, and fragments are amplified with PCR primers that target the linkers, generating fingerprints. Guan and colleagues (2002) recently compared this method with antibiotic resistance patterns and 16S rDNA sequencing with the same set of isolates and concluded that the AFLP approach had a relatively good ARCC, considering that their samples covered a broad geographic area; however, the method was expensive.

PFGE generates a bacterial fingerprint by digesting the entire genome with rare-cutting restriction enzymes and separating the resulting large fragments with a specialized gel apparatus made to separate large fragments of DNA. The equipment required is expensive and the gel separation step is slow, but the resulting fingerprints are the "gold standard" in another field, epidemiological studies of foodborne pathogens. This method is just beginning to be applied in source identification studies (e.g., see Myoda *et al.*, in press). McLellan and colleagues (2003) make the point that the fine level of genetic discrimination achieved by PFGE may not be necessary to discriminate source-specific clones.

The underlying assumption of these genotypic methods is that host-specific genetic structure exists across indicator bacteria populations (McLellan *et al.* 2003). In a comparative study using multiple REP-PCR assays and PFGE, McLellan and colleagues (2003) found multiple closely related subclusters of genotypes, which were mostly host-specific. However, the relationships calculated for members of the clusters varied depending on the primers or techniques used. Because of very high levels of diversity, both REP-PCR and PFGE would require a separate library for each geographic area.

22.3.2 Library-independent, culture-dependent methods

22.3.2.1 Viral methods

Phage of *Bacteroides fragilis* can distinguish human and animal faecal pollution, as certain strains of *B. fragilis* will grow bacteriophages only from human sewage and others will support phage growth from both human and animal faeces (Puig *et al.* 1999). Similarly, two serotypes of F+ RNA coliphages, Types II and III, are found in association with human faecal contamination, whereas Type IV is found in association with animal faecal contamination and Type I occurs in both human and animal faeces (Hartly *et al.* 1975; Furuse *et al.* 1981). Growth of these coliphages in an appropriate cell culture, followed by serotyping, identifies human and non-human faecal contamination in water. Recently, serotyping has largely been replaced by molecular typing (Hsu *et al.* 1995).

These viral methods are limited to discriminating between human and animal sources. Little is known about differential survival of the various types, which would affect the ability to discriminate over time. In addition, the markers appear to be irregularly distributed in populations and may work better in some geographic areas and when faecal sources comprise multiple individuals (such as from sewage) rather than single individuals (Noble *et al.*, in press).

22.3.3 Advantages and limitations of culture-based methods

Culturing faecal indicator organisms is relatively inexpensive and low-tech, making it broadly available. However, this advantage is lost if the source identification method that is applied to the cultured isolates is high-tech and expensive (e.g., PFGE and AFLP). Another advantage of culturing is that it provides an enrichment step, increasing the numbers of target microorganisms and providing single strains in isolation. Finally, culture-based methods often use standard public health indicators such as *E. coli* or enterococci, for which some information about survival and transport is available.

Disadvantages are that these methods are limited to testing easily cultured microbes. Many pathogens, and even the most common faecal bacteria, may be very difficult to grow. In addition, the composition of microbial communities changes drastically when cultured (e.g., see Ferguson *et al.* 1984). This “culture bias” has rarely been considered in faecal source identification. Phenotypic traits such as antibiotic resistance may be under strong selection, which would influence relative survival. Genotypic traits (e.g., ribotypes) are markers for clonal types, which may have phenotypic differences that affect their relative survival in culture.

If the culturing step did accurately reproduce the proportions of indicator organisms present in the original population, a significant advantage of culture-based approaches would be that they would allow ready quantification of different sources in the population. It should be possible to experimentally confirm or disprove this proportional survival, but this has not been attempted.

It is likely that some of the traits used for source discrimination in culture-based methods could be directly assayed from water samples without an intervening culture step. This would require much more specific tests (perhaps highly specific PCR primers) than are currently used for culture-based methods.

22.3.4 Culture-independent methods

In these methods, some marker is assayed directly from a water sample or from DNA extracted from a water sample, without an intervening culture step. Many of these methods assay specific genes by PCR; this approach is sometimes called "host-specific PCR."

22.3.4.1 Viral methods

A number of viruses can be monitored directly in water, without culturing (reviewed in Griffin *et al.* 2003). The presence of human or bovine viruses indicates the presence of human or cattle faecal pollution. Examples include human adenoviruses (Pina *et al.* 1998; Jiang *et al.* 2001), human enteroviruses (Tsai *et al.* 1993; Griffin *et al.* 1999; Noble and Fuhrman 2001), and bovine enteroviruses (Ley *et al.* 2002). Monitoring for viruses typically requires very large water samples; concentration of such large samples frequently concentrates PCR-inhibitory substances as well, which may interfere with detection (Jiang *et al.* 2001; Straub and Chandler 2003). To increase sensitivity, investigators may use nested PCR, which makes it difficult or impossible to detect quantitatively (Jiang *et al.* 2001). A real-time quantitative PCR assay has been used successfully to quantify enteric viruses (Monpoeho *et al.* 2000; Boehm *et al.* 2003). The viral methods are effective in detecting human sewage, although they may not detect faeces from individuals or small groups of humans (Noble *et al.*, in press).

22.3.4.2 Bacteroidetes faecal markers

Species in the genus *Bacteroides* have host-specific distributions that could make them useful as faecal source identifiers (Allsop and Stickler 1985; Fiksdal *et al.* 1985; Kreader 1995). However, as this group is anaerobic, it was not practical to use them for this purpose until the advent of routine molecular detection. *Bacteroides* and related genera (class Bacteroidetes) comprise a large

proportion of the faecal flora in warm-blooded animals, making them a relatively easy target for detection. In addition, they are genetically diverse, are limited to animal body cavities, are unlikely to survive long after release into receiving waters, and show species- or group-specific host distributions. Bernhard and Field (2000a, 2000b) used terminal restriction fragment length polymorphisms (T-RFLP) and length heterogeneity PCR (LH-PCR) to identify human and ruminant-specific Bacteroidetes sequences, which were used to design PCR primers to amplify human or ruminant markers out of faecally contaminated waters. Subsequently, clone library analysis and subtractive hybridization were used to design primers for other faecal sources (L.K. Dick, M.T. Simonich, S.P. Walters, and K.G. Field, unpublished data). These primers sensitively and specifically detect faecal pollution in small water samples and distinguish its source (Field *et al.* 2002; Bernhard *et al.* 2003). Primers are based on sequences present in faecal samples, not on sequences from isolated strains, taking advantage of dominant microbial constituents of faeces that have never been grown (e.g., see Wilson and Blitchington 1996; Suau *et al.* 1999; Bernhard and Field 2000b; Leser *et al.* 2002). Analysis of faecal samples from several locations in the USA and elsewhere suggests that the primers are widely geographically applicable (L.K. Dick, S.P. Walters, and K.G. Field, unpublished data). Dick and colleagues developed a quantitative PCR assay for the Bacteroidetes group and showed that Bacteroidetes dosage in sewage was well correlated with *E. coli*, opening up the possibility of using molecular detection of Bacteroidetes as a rapid quantitative indicator of faecal pollution (L.K. Dick and K.G. Field, unpublished data).

22.3.4.3 Toxin genes from E. coli

A heat-stable enterotoxin from enterotoxigenic *E. coli*, the STIb toxin, is associated with human faecal waste; its gene is targeted by PCR primers to detect human faecal pollution (Oshiro and Olson 1997). Similarly, the STII toxin gene is associated with pig faeces and is targeted by primers that detect pig faecal contamination (Khatib *et al.*, in press). The heat-labile enterotoxin, LTIIa, is associated with cattle faecal waste; its gene is the target of PCR primers to detect faecal pollution from cattle (Khatib *et al.* 2002). These markers are generally specific (with occasional exceptions; Field *et al.*, in press), are temporally and geographically stable, and occur at a high enough prevalence for culture-independent detection as long as a large enough number of cells is screened (Oshiro and Olson 1997; Khatib *et al.* 2002). They may also be detected following a culture step in which *E. coli* is enriched (Field *et al.*, in press). Because of the sporadic distribution of enterotoxigenic *E. coli*, this approach may not detect individual faecal samples (Field *et al.*, in press).

22.3.4.4 Bifidobacterium

Like the Bacteroidetes group, several groups have suggested using *Bifidobacterium* species to identify the source of faecal pollution (Resnick and Levin 1981; Carillo *et al.* 1985; Sinton *et al.* 1998; Rhodes and Kator 1999; Bernhard and Field 2000a; Nebra *et al.* 2003). Resnick and Levin (1981) found that members of the genus *Bifidobacterium* could not be cultured after 5 h in fresh water or 10 h in salt water. Carillo and colleagues (1985) also observed very low survival of *B. adolescentic* in a tropical environment and suggested that the genus could be used to detect only very recent faecal contamination. Bernhard and Field (2000a) developed human- and ruminant-specific T-RFLP and LH-PCR markers from uncultured *Bifidobacterium* in faeces. However, the *Bifidobacterium* markers were difficult to detect out of faecally polluted natural waters, suggesting that survival of this group after release is insufficient for this application (Bernhard and Field 2000a). Nebra and co-workers (2003) developed a probe that detects 16S rDNAs from *B. dentium*, a species thought to be limited to humans, and another probe intended to detect a number of animal-specific *Bifidobacterium* species. Following amplification of *Bifidobacterium* 16S rDNAs using general primers, they used their probes to differentiate human and animal samples. Although the *B. dentium* probe could detect most human sewage samples and a few individual human faecal samples, with a low number of false positives, the animal probe was unsuccessful. The amplification/probing approach does not lend itself to quantification. In addition, basing primers solely on published sequences from isolated strains may miss the most common *Bifidobacterium* strains in faeces, thus limiting the genetic variability that could be used for primer/probe design.

22.3.4.5 Community sampling

Animal species may have faecal bacterial communities that differ enough to allow them to be distinguished from one another by community fingerprinting methods (e.g., see Cho and Kim 2000; Satokari *et al.* 2001; Simpson *et al.* 2002; Tannock 2002; Zoetendal *et al.* 2002; Nagashima *et al.* 2003). In a comparison of small-subunit rDNA community fingerprints generated by T-RFLP, LaMontagne and colleagues (2003) differentiated cattle and dog faeces and sewage. Application of the method in a comparative study with samples of known composition resulted in low identification of sources with high false positives, however (Griffith *et al.*, in press). Experimental evidence suggests that PCR from mixed templates may be biased (e.g., see Suzuki and Giovannoni 1996; Suzuki *et al.* 1998; Becker *et al.* 2000). For this method to work for diagnosing sources of faecal pollution in water, PCR bias must be minimized and the PCR must be quantitative.

22.3.5 Advantages and limitations of culture-independent methods

Culture-independent methods have the advantage of sampling the entire population present in the sample, with no culture bias. In addition, they are simpler and quicker than culture-based methods; they may require only a few hours to detect faecal pollution and identify its source. They do not require prior preparation of a "library," as markers are in most cases universal. They are not limited to easy-to-culture microbes, but may instead use difficult-to-grow but common faecal microbes or mine the uncultured genetic diversity in faeces for source-specific markers.

A drawback of using any markers other than standard public health indicators is that their survival and correlation with standard faecal indicators and pathogens are poorly known. Since regulations are based on public health indicator bacteria, any other markers must be correlated with public health bacteria in order for managers to use them. Epidemiological studies that predict health outcomes are also needed.

A further limitation of the culture-independent methods is that markers for only a few animal species are currently available; wildlife species especially are not represented. Most of the culture-independent methods result in presence/absence data on marker occurrence; quantitative assays are needed. Finally, for any of these markers, it is important to test their geographic range and temporal variability.

A relatively small variety of gene sequences has been tested for use in host-specific PCR for faecal source identification. It is likely that many other appropriate targets will be identified. With primers or probes targeting multiple targets to identify each faecal source, perhaps combined in a microarray format with primers or probes for pathogens, faecal source identification could be rapid and reliable.

22.4 A COMPARATIVE STUDY OF FAECAL SOURCE IDENTIFICATION METHODS: THE SCCWRP STUDY

It is difficult to compare faecal source identification methods, as most have been applied only to analyse real-life samples from watersheds, where the "real" answer is not known. The Southern California Coastal Water Research Project (SCCWRP) and the US Environmental Protection Agency (EPA) sponsored a comparative source identification study (Griffith *et al.*, in press). Study participants were asked to identify the faecal source(s) in water samples containing human, cattle, dog, or gull faeces, sewage, or a mixture. Along with

replicate unknown water samples, participants were supplied with samples of the faeces used to create the unknowns. Study participants used many of the methods reviewed above, including coliphage and virus-based approaches (Noble *et al.*, in press), phenotypic culture-based approaches (MAR and CUP) (Harwood *et al.*, in press), genotypic culture-based approaches (ribotyping, REP-PCR, and PFGE) (Myoda *et al.*, in press), and non-culture-based approaches, including *E. coli* toxin genes, community sampling by T-RFLP, and several methods based on Bacteroidetes bacteria (Field *et al.*, in press). Methods were assessed according to their ability to identify whether samples contained or did not contain human faeces, identify each faecal source, and handle freshwater and saltwater samples and samples with humic acids.

Host-specific PCR (of *E. coli* toxin genes or Bacteroidetes markers) was very accurate at identifying samples with human faeces or sewage with no false positives. Several of the other methods, including the phenotypic methods and genotypic library-based methods, identified most or all samples with human input, but did have false positives. The virus-based methods worked well at identifying samples with sewage but less well at identifying samples with human faeces. None of the methods correctly identified all the sources in every sample. The host-specific PCR methods (of either *E. coli* toxin genes or Bacteroidetes markers) were relatively accurate at identifying the species for which they had markers, but did not have markers for all species. Most of the other methods had significant numbers of false positives, although some culture-based genotypic methods (ribotyping, PFGE) performed better than others.

This is the first comparative study using blind samples of known composition to compare faecal source identification methods. Although, due to limitations of the study, some methods did not perform optimally, several broad conclusions could be reached. First, the same approach did not perform equally well in the hands of different investigators, underlining the need for standardization. Second, the rate of false positives for culture-based, library-dependent methods was often higher than had been anticipated based on published ARCCs, underlining the weakness of this statistic. Third, each method had strengths and weaknesses, and no method performed perfectly. Some methods accurately identified human faecal contamination and would be useful when the principal research question is the identification of human input. Some methods were relatively rapid and accurate for some sources, but did not identify all the sources; these would be useful where the principal research objective is to identify the major sources of faecal contamination, to allow rapid mitigation. Other methods were more time-consuming and less accurate, but identified all sources; these would be appropriate where it was important to know all sources, perhaps for health risk assessment. No method was able to accurately quantify the sources.

An important finding of this study came out of the experimental design rather than source identification results. When making up unknown samples, the proportion of each type of faeces in the samples was quantified by estimating the concentrations of *E. coli* in the input human, dog, cattle, and gull faeces. However, since several source identification methods in the study were based on enterococci, not *E. coli*, enterococci concentrations were estimated as well. The relative proportions of the different types of faeces in samples estimated by the two methods were in many cases radically different (Table 22.1).

Table 22.1. Relative proportions of faeces of selected unknown samples from the SCCWRP faecal source identification study, as estimated by counts of *E. coli* and enterococci (unpublished data used with permission of John Griffith and Steve Weisburg, SCCWRP)

Sample	Faecal proportions according to counts of *E. coli*		Faecal proportions according to counts of enterococci	
C	Human 96%	Gull 4%	Human 18%	Gull 82%
E	Dog 86%	Cattle 14%	Dog 54%	Cattle 46%
F	Human 58%	Gull 42%	Human 1%	Gull 99%
H	Cattle 90%	Gull 10%	Cattle 32%	Gull 68%
N	Sewage 58%	Dog 42%	Sewage 4%	Dog 96%
T	Human 44%	Gull 56%	Human 4%	Gull 96%

The very large differences in apparent faecal contributions in identical samples, measured with *E. coli* or enterococci, illustrates a crucial shortcoming of using indicators to assess the risk of pathogen contamination. Unless the correlation of each pathogen to each indicator has been established, indicators cannot be used quantitatively to estimate risk.

22.5 AN IMPORTANT LIMITATION OF FAECAL SOURCE IDENTIFICATION METHODS

Little is known about proportional survival of different microbial groups or markers in water, yet this is basic to faecal source identification. For example, an older method of faecal source tracking, comparing the ratio of faecal coliforms to faecal streptococci, has been largely abandoned, because the two groups survive differently, causing the ratio to change over time (Pourcher *et al.* 1991; Sinton *et al.* 1993). The relative survival of faecal microbes or markers being used for source discrimination has not been investigated; this is a major shortcoming of all source tracking technologies, not just library-based methods. Before source identification can be used to help assess the risk of exposure to pathogens due to faecal contamination of water, researchers must measure the

survival of proposed source-specific markers, in relation to each other, in relation to standard faecal indicators such as *E. coli*, and in relation to pathogens.

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23

Rapid methods for the detection and enumeration of microorganisms in water

D.Y.C. Fung

23.1 INTRODUCTION

The application of rapid methods for water microbiology requires, first and foremost, effective and efficient sampling methods and techniques. Since the number of microorganisms in water is usually low, concentration of the microbes is an important aspect of water microbiology.

The most commonly used method is filtration. Usually, a 100-ml volume of water is filtered through a 0.45-µm-porosity, 47-mm-diameter nitrocellulose membrane and plated on a medium that is selective for specific organisms, such

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as total coliforms, faecal coliforms, and other pathogens. For turbid waters, the labour-intensive multiple-tube, most probable number (MPN) methods may have to be used. For protozoa and viruses, much larger volumes may be used. Elution and dissolution of filter matrix are required; other methods include desorption, elution, acid precipitation, and polyethylene glycol precipitation. Positively and negatively charged filters are also used to trap viruses. The most common method for the enumeration of microorganisms is the total viable cell count method, which uses selective and non-selective agars for growing live bacteria, yeasts, and moulds. This involves sample dilution, application of samples onto petri dishes, pouring melted agar, incubation of solidified agar samples for a specified time and temperature, and enumeration of colony forming units (CFU/ml), depending on the agar used and the colour, shape, size, and fluorescence characteristics of the microorganisms.

This chapter describes some efficient and rapid methods to ascertain total viable cell numbers in water as well as application of genetic and biosensor techniques to detect target pathogens in water for the protection of public health nationally and internationally.

23.2 MODIFICATIONS TO CONVENTIONAL SYSTEMS

In the past 20 years, several modifications of the conventional viable cell count method have been made. The spiral plating method is an automated system to obtain viable cell count. By use of a stylus, this instrument can spread a liquid sample on the surface of a pre-poured agar plate (selective or non-selective) in a spiral shape (the Archimedes spiral) with a concentration gradient starting from the centre and decreasing as the spiral progresses outward on the rotating plate. The volume of the liquid deposited at any segment of the agar plate is known. After the liquid containing microorganisms is spread, the agar plate is incubated overnight at an appropriate temperature for the colonies to develop. The colonies appearing along the spiral pathway can be counted either manually or electronically. The time for plating a sample is only several seconds compared with minutes as in the conventional method (Spiral Biotech 2003).

An ingenious hydrophobic membrane system was also developed with a square bacteriological filter printed with hydrophobic grids to form 1600 squares for each filter. A water sample can pass through the filter assisted by vacuum with the microbes trapped on the filter into the squares. The filter is then placed on pre-poured non-selective or selective agar and then incubated for a specific time and temperature. Since growing microbial colonies cannot migrate over the hydrophobic material, all colonies are localized into a square shape. The analyst can then count the squares as individual colonies. Since there is a chance that more than one bacterium is trapped in one square, the system

has an MPN conversion table to provide statistically accurate viable cell counts (Neogen, Inc. 2003).

A very popular self-contained film system has been developed and widely used internationally. In this system, appropriate rehydratable nutrients are embedded in a series of films in the unit. The unit is a little larger than a credit card. To obtain viable cell counts, the protective top layer is lifted and 1 ml of liquid sample is introduced to the centre of the unit; the cover is then replaced. A plastic template is placed on the cover to make a round mould. The rehydrated medium will support the growth of microorganisms after suitable incubation time and temperature. The colonies are directly counted in the unit (3M Company 2003).

Another convenient system consists of tubes of sterile nutrient with a pectin gel in the tube but no conventional agar. This liquid system is ready for use, and no heat is needed to "melt" the system, since there is no agar in the liquid. After an analyst mixes 1 ml of liquid sample with the liquid in the tube, the resultant contents are poured into a special petri dish coated with calcium. The pectin and calcium will react and form a gel, which will solidify in about 20 min. The plate is then incubated at an appropriate temperature for the proper time, and the colonies are counted the same way as for the conventional standard plate count method (3M Company 2003).

The four methods described above have been in use for almost 20 years. Chain and Fung (1991) made a comprehensive evaluation of all four methods against the conventional viable cell count method on seven different foods, 20 samples each, and found that the alternative systems and the conventional method were highly comparable at an agreement of $r = 0.95$. In the same study, they also found that the alternative systems cost less than the conventional system for making viable cell counts.

A relatively new method, called "Fung's Double Tube Method," has been developed to rapidly enumerate anaerobes such as *Clostridium perfringens* (Fung and Lee 1981). In this method, a larger tube is used to house a water sample (e.g., 1 ml), and then an appropriate amount of agar, such as SFP agar, is introduced into the larger tube. A smaller tube is then inserted into the large tube, thereby creating a thin agar layer between the two tubes. Colonies then formed can be easily enumerated in the system. Since *C. perfringens* grows very quickly at 42 °C, a *C. perfringens* count can be made in about 6 h from polluted environmental water samples. Hawaii is the only US state that utilizes *C. perfringens* as an indicator of faecal contamination of recreational water. Recently, during a research project in Hawaii, a filter system was introduced into the double tubes, and now almost any volume of water can be tested in the double tubes as long as the water (10 ml, 100 ml, or other volume) can be filtered through a membrane.

Several "real-time" instruments for viable cell counts have been introduced. The direct epifluorescent filter technique (Tortorello 1999) involves using vital dye (acridine orange) to stain live bacteria for obtaining viable cell counts in about 1 h. A new system filters cells on a membrane, then lets the cells grow for a few hours; the filter is then stained with vital dyes. Viable cell counts can be made by scanning the colonies by laser (Chemunex Corporation 2003). Another rapid system has been developed that utilizes bacterial ATP to report the growth of microcolonies trapped in a filter in a few hours (Millipore Corporation 2003).

Development of a miniaturized MPN method has been successful in recent years based on the original work of Fung and Kraft (1969). Irwin *et al*. (2000) developed a modified Gauss-Newton algorithm and a 96-well microtechnique for calculating the MPN using spreadsheets. This technique is less cumbersome than many traditional MPN procedures. Similarly, Walser (2000) developed a microtitre plate technology for the automation of microbiological testing of drinking-water. The system was validated for the analysis of aerobic mesophilic counts in drinking-water. The system can cope with low or high bacterial loads, ranging from 0 to 4.3 log CFU/ml. The system reduces the tedium and personnel influence of routine microbiological work and is applicable to many other determinations, such as faecal indicator and other microorganisms in water.

23.3 NEW MICROBIOLOGICAL TESTING TECHNIQUES

Many advances have been made in rapid methods and automation in microbiology in the past 20 years. Fung (2002) comprehensively reviewed the subject in the inaugural issue of the journal *Comprehensive Review in Food Science and Food Safety*, published by the Institute of Food Technologists. The history and key developments in the field were described. In addition, advances in sample preparation and treatments, total viable cell count methodologies, miniaturization and diagnostic kits, immunological testing, instrumentation and biomass measurements, genetic testing, and biosensors were described. Finally, US and world market and testing trends and predictions of future developments were included. Of particular interest are the developments in genetic testing and biosensors, which can be used in water microbiology. Traditional microbiological methods rely on growth characteristics of organisms at various temperatures, colony morphology in various cultural media, biochemical reactions in various carbohydrates, and immunological antibody–antigen reactions. These characteristics are influenced by environmental factors and growth conditions of cells.

23.3.1 Genetic methods: Polymerase chain reaction (PCR)

Genotypic characteristics of a cell are far more stable than phenotypic expressions. Genetic methods have been developing rapidly in the past two decades. First, DNA and RNA hybridization techniques were perfected and widely used to rapidly detect organisms such as *Salmonella*, *Listeria*, *Campylobacter*, etc. More recently, PCR has been widely used for the detection of a great variety of microorganisms in clinical, food, industrial, and water microbiology (Mrozinski *et al*. 1998). Basically, a DNA molecule (double helix) of a target pathogen (e.g., *Salmonella*) is first denatured at about 95 °C to form single strands, then the temperature is lowered to about 55 °C for two primers (small oligonucleotides specific for *Salmonella*) to anneal to specific regions of the single-stranded DNA. The temperature is then increased to about 70 °C for a special heat-stable polymerase, the TAQ enzyme from *Thermus aquaticus*, to add complementary bases (A, T, G, or C) to the single-stranded DNA and complete the extension to form a new double strand of DNA. This is called a thermal cycle. After this cycle, the tube is heated to 95 °C again for the next cycle. After one thermal cycle, one copy of DNA will become two copies. After about 21 cycles and 31 cycles, 1 million and 1 billion copies of the DNA will be formed, respectively. This entire process can be accomplished in less than an hour in an automatic thermal cycler.

After PCR reactions, one still needs to detect the presence of the PCR products to indicate the presence of the pathogen to be detected. In the original PCR procedure, PCR products were detected by electrophoresis, which is time consuming and laborious. Several new PCR protocols have recently been developed to efficiently report successful PCR reactions.

Mrozinski *et al*. (1998) described an automatic system for screening a family of PCR assays for pathogens, which combines DNA amplification and automated homogeneous detection to determine the presence or absence of specific targets. All primers, polymerase, and deoxyribonucleotide bases necessary for PCR as well as a positive control and an intercalating dye are incorporated into the single tablet. The system works directly from an overnight enrichment of the target organisms. No DNA extraction is required. Assays are available for *Salmonella*, *E. coli* O157:H7, *Listeria*, and *L. monocytogenes*. The system uses an array of 96 blue light emission diodes as the excitation sources and a photomultiplier tube to detect the emitted fluorescent signal indicating successful PCR reactions. This integrated system improves the ease of use of the assay. The inclusivity and exclusivity of this system reach almost 100%, meaning that false-positive and false-negative rates are almost zero. Additionally, this automated system can now be used with assays for the detection of *Cryptosporidium parvum* and *Campylobacter jejuni/coli* and for the

quantitative and qualitative detection of genetically modified organisms in soy and corn (Qualicon, Inc. 2003).

Vishnabhatla *et al*. (2000) described a self-contained PCR system that can report the successful PCR reaction by measuring fluorescence reaction in the experimental chambers. A special molecule is annealed to the single-stranded DNA to report the linear PCR amplification. The molecule has the appropriate sequence for the target DNA. It also has two attached particles. One is a fluorescent particle, and the other is a quencher particle. When the two particles are close to each other, no fluorescence occurs. However, when the TAQ polymerase is adding bases to the linear single strand of DNA, it will break this molecule away from the strand. As this occurs, the two particles will separate from each other, and fluorescence will occur. By measuring fluorescence in the tube, a successful PCR reaction can be determined (Applied Biosystems, Inc. 2003).

A new system using molecular beacon technology has been developed. In this technique, all of the reactions are in the same tube. A molecular beacon is a tailor-made hairpin-shaped hybridization probe. The probe is used to attach to target PCR products. On one end of the probe, a fluorophore is attached; on the other end is a quencher of the fluorophore. In the absence of the target PCR product, the beacon is in a hairpin shape and there is no fluorescence. However, during PCR reactions and the generation of target PCR products, the beacons will attach to the PCR products and cause the hairpin molecule to unfold. As the quencher moves away from the fluorophore, fluorescence will occur, and this can be measured. By using molecular beacons containing different fluorophores, one can detect different PCR products in the same reaction tube and thus perform "multiplex" tests of several target pathogens (Bio-Rad 2003).

One of the major problems of PCR systems is that of contamination of PCR products from one test to another. A system developed by the Pasteur Institute attempts to eliminate PCR product contamination by substituting the base uracil for thymine in the entire PCR protocol. Thus, in the reaction tube, there are adenine, uracil, guanine, and cytosine, but not thymine. During the PCR reaction, the resultant PCR products will be AUGC pairing and not the natural ATGC pairing. After one experiment is completed, a new sample is added to another tube for the next experiment. That tube contains an enzyme called uracil-D-glycosylase (UDG), which will hydrolyse any DNA molecules that contain a uracil base. Therefore, if there is contamination from a previous run, the AUGC-DNA will be destroyed before the beginning of the new run. Before a new PCR reaction, the tube with all reagents is heated to 56 °C for 15 min for UDG to hydrolyse any contaminants. During the DNA denaturation step, the UDG will be inactivated and will not act on the new AUGC-PCR products. This

system can detect *Salmonella*, *Listeria monocytogenes*, and other pathogens (Sanofi Pasteur 2003).

The nucleic acid sequence-based amplification (NASBA) technique has been perfected in recent years for water microbiology. It has advantages over the PCR technologies. Since the target is RNA, it can be used to detect RNA viruses and functional mRNA targets. It is isothermal and thus does not require a thermal cycler for the reaction. It is rapid and sensitive for detection of target molecules.

PCR, NASBA, and related genetic technologies can be powerful tools for water microbiology once the existing problems are solved and the systems are automated. Also, analysts have to be convinced to invest time and money to convert to these technologies for the routine analysis of water microbiology.

23.3.2 Biosensors

There are now a large number of biosensors available for detection of target microorganisms in a variety of food, water, clinical, and industrial samples. Ivnitski *et al*. (1999) provided a comprehensive overview of different physicochemical instrumental techniques for direct and indirect identification of bacteria, including infrared and fluorescence spectroscopy, flow cytometry, chromatography, and chemiluminescence techniques, as a basis for biosensor construction. Biosensor development and application are exciting fields in applied microbiology. The basic idea is simple, but the actual operation is quite complex and involves much instrumentation.

Basically, a biosensor is a molecule or a group of molecules of biological origin attached to a signal recognition material. When an analyte comes in contact with the biosensor, the interaction will initiate a recognition signal that can be reported in an instrument. Many types of biosensors have been developed, including a large variety of enzymes, polyclonal and monoclonal antibodies, nucleic acids, and cellular materials. In some applications, whole cells can also be used as a biosensor. Analytes detected include toxins (e.g., staphylococcal enterotoxins, tetrodotoxins, saxitoxin, and botulinum toxin); specific pathogens (e.g., *Salmonella*, *Staphylococcus*, and *Escherichia coli* O157:H7); carbohydrates (e.g., fructose, lactose, and galactose); insecticides and herbicides; ATP; antibiotics (e.g., penicillins); and others. The recognition signals used include electrochemical (e.g., potentiometry, voltage changes, conductance and impedance, and light addressable); optical (e.g., ultraviolet, bioluminescence, chemiluminescence, fluorescence, laser scattering, reflection and refraction of light, surface plasmon resonance, and polarized light); and miscellaneous transducers (e.g., piezoelectric crystals, thermistor, acoustic waves, and quartz crystal). Eggins (1997), Cunningham (1998), Goldschmidt

(1999), and others have produced excellent review articles and books on biosensors.

Recently, much attention has been directed to the development of "biochips" and "microchips," which may detect a great variety of molecules associated with waterborne and foodborne pathogens. Due to the advancements in miniaturization technology, as many as 50 000 individual spots (e.g., DNA microarrays), each spot containing millions of copies of a specific DNA probe, can be immobilized on a specialized microscope slide. Fluorescent labelled targets can be hybridized to these spots and detected. Biochips can also be designed to detect all kinds of waterborne bacteria by imprinting a variety of antibodies, or DNA molecules, against specific pathogens on the chip for the simultaneous detection of pathogens such as *Salmonella*, *Listeria*, *E. coli*, and *Staphylococcus aureus* on the same chip.

Biochips are an exceedingly important technology in life sciences, and the market value is estimated to reach $5 billion by the middle of this decade. This technology is especially important in the rapidly developing field of proteomics, which requires massive amounts of data to generate valuable information. The development of these biochips and microchips provides an impressive method for obtaining a large amount of information for biological sciences.

23.4 WATER APPLICATIONS: CONCLUSIONS

In regard to waterborne pathogen detection, there are several important issues to consider. These chips are designed to detect minute quantities of target molecules. The target molecules must be free from contaminants before being applied to the chips. In order to utilize these chips, water samples must go through extensive cell amplification (growth in culture) or sample concentration by filtration, separation, absorption, or centrifugation. Particles in a water sample will easily block the channels used in the microfluidic biochips. The necessity of preparing a water sample prior to analysis will not allow the biochips to provide "real-time" detection of pathogens in water at this time. Another concern is viability of the pathogens to be detected by biochips. Monitoring the presence of some target molecule will provide evidence only for the presence or absence of the target pathogen, not for the viability of the pathogen in question. Some form of culture enrichment to ensure growth is still needed in order to obtain meaningful results. The potential of biochips and microarrays for waterborne pathogen detection is great; at this time, however, much more research is needed to make this technology a reality in applied water microbiology.

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Section VII

Prevention and control of waterborne zoonoses

V.P.J. Gannon

Previous parts of this work have described specific bacterial, viral, and parasitic agents associated with waterborne zoonoses, the epidemiology of zoonotic diseases in various regions of the world, treatments for conditions caused by these pathogens, and, finally, analytical procedures for microbiological monitoring of water quality and safety and determining the host species sources of faecal contamination of water. The present section focuses on the prevention and control of waterborne zoonoses.

In the first chapter of this section, control of zoonotic agents in animal reservoirs is discussed. It is noted that there are certain generic procedures that can be applied to preventing the establishment of zoonotic pathogens in domestic animal reservoirs. These measures are 1) to establish groups of "clean" animals, 2) to keep the animals free of infection by preventing contact between

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them and other potentially infected animals of the same and also of other species, and 3) to provide clean housing, food, and water. Other tactics, such as selective breeding for disease resistance in animals, competitive exclusion, bacteriophage therapy, use of antimicrobials, and active and passive immunization, may also be helpful in controlling specific zoonotic pathogens in animal reservoirs. Control of zoonotic diseases in wild animal populations presents an even greater challenge than their control in domestic animal species. Vaccination schemes to limit infection with specific zoonotic agents in wildlife species and controlling the population levels of certain wild animal species are some of the steps that may be helpful in limiting zoonotic waterborne diseases.

Until recently, zoonotic pathogens have not been given a high priority by animal health agencies with responsibility for regulating international trade. This may be explained, in part, by the fact that many zoonotic diseases are not associated with disease or loss of production in the animal reservoir populations. However, there is a growing acknowledgement of the responsibility of animal health agencies in protecting consumers from zoonotic pathogens associated with both foodborne and waterborne disease. Importing countries are demanding that the food safety standards be met with respect to these pathogens. National and international agencies could better coordinate their activities and share resources to accomplish specific zoonotic pathogen reduction targets in animal reservoirs to limit both foodborne and waterborne disease.

The second chapter considers the important role of the proper disposal of animal wastes in the control of waterborne zoonoses. With increases in the world's human population and an increase in affluence of certain developing countries, animal meat and milk production will continue to increase in the foreseeable future. Animal agriculture will be vital in meeting the needs of national and international economies for increased production and production efficiency. The current trend towards the concentration of animal agriculture into regions where feed, labour, energy, and transportation costs are lowest will continue. However, this trend will be balanced by concerns about the effects of industrial animal production on the environment and on human and animal health. Proper disposal of animal wastes, including from abattoirs, has become a significant problem around the world from many perspectives, and not only with respect to the role that these wastes play in the transmission of waterborne zoonotic diseases. Other concerns centre around the role of these wastes in nutrient cycling, soil fertility, degradation of ecosystems, energy consumption, and greenhouse gas emissions. This "multimedia" nature of animal wastes has meant that farm storage, treatment, and disposal systems must satisfy competing and sometimes contradictory objectives; for example, transport of animal wastes between distant locations my be desirable from a nutrient cycling point of view,

but may also serve to distribute and enhance transmission of certain microbial pathogens present in these wastes.

Animal wastes are not uniformly contaminated with zoonotic pathogens; for example, wastes from young animals are likely to have much higher pathogen burdens. Unfortunately, only thermophilic animal waste treatment processes such as composting and anaerobic digestion have been shown to significantly reduce levels of microbial pathogens from these wastes. Furthermore, many zoonotic pathogens can remain viable in animal wastes and contaminated soils for long periods of time. Therefore, surface water protection from animal wastes applied to fields remains an extremely important step in preventing dispersion of waterborne zoonotic microorganisms. It is thought that heavy precipitation has played an important role in many outbreaks of waterborne zoonotic disease through the transportation of contaminated animal wastes and soil into surface and well water. Waters contaminated with animal wastes present a risk of waterborne zoonotic disease to individuals consuming untreated drinking-water and following accidental ingestion after recreational and occupational exposure.

The final chapter discusses drinking-water protection and treatment. In this chapter, the importance of using multiple barriers in water source protection, treatment procedures, and drinking-water distribution systems is discussed. The concept of hazard analysis and critical control points is being incorporated into "water safety plans" by many water treatment facilities to better define the hazards and critical steps from "source to tap," where failures could lead to unacceptable risks to human health. Technologies such as ultraviolet light treatment to reduce risks associated with chlorine-resistant forms of zoonotic pathogens such as *Cryptosporidium parvum* oocysts are also discussed.

Strategies to prevent waterborne zoonoses present a major challenge for public health authorities around the world. However, solutions to problems associated with these pathogens not only involve human health agencies but also must involve groups with responsibility for agriculture, public utilities, animal health, wildlife, recreation, climatology, the environment, municipal planning, and national and international trade. Strategies employed in these efforts must concentrate not only on the effects of animal agriculture on the environment and human health but on all aspects of the rapidly changing human–animal interface.

24

Control of zoonotic waterborne pathogens in animal reservoirs

V.P.J. Gannon

24.1 INTRODUCTION

There are a number of existing and potential control strategies to minimize health risks associated with zoonotic waterborne pathogens. These control steps include 1) prevention and control of zoonotic diseases in animal reservoir populations, 2) source protection efforts, including proper containment, treatment, and disposal of animal wastes and prevention of animal access to human water sources, 3) multi-barrier water treatment and distribution systems using traditional and new technologies to reduce or eliminate a wide variety of microbial and toxic risks and withstand extreme weather events, and 4) measures for protection and treatment of the most vulnerable members of communities from waterborne infections.

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This chapter concentrates on the first and arguably the most important step—that is, control of zoonotic pathogens in the host animal reservoir. Control strategies for waterborne pathogens that are most frequently associated with waterborne illness — the enteric bacteria *Salmonella enterica*, *Campylobacter jejuni*, and *Escherichia coli* O157:H7, the spirochete *Leptospira interrogans*, and the protozoa *Cryptosporidium parvum* and *Giardia duodenalis* — are discussed, as are the unique challenges associated with them. A brief discussion is also provided on the potential waterborne diseases associated with the agents responsible for a transmissible spongiform encephalopathy (TSE) in animals and humans.

24.2 RISK FACTORS ASSOCIATED WITH WATERBORNE ILLNESS

A number of circumstances set the stage for zoonotic waterborne diseases in communities. The following list provides a framework for consideration and a context for some of the prevention and control strategies to be discussed:

(1) The zoonotic waterborne disease agent is highly prevalent in the animal population. It may or may not be associated with recognizable and economically significant clinical disease in the animal reservoir. While young animals typically excrete large numbers of pathogens, an adult carrier state and/or persistence in the environment may help maintain infection in the animal population.

Cryptosporidium parvum and *Giardia duodenalis* infections are very common in domestic and wild animals (Thompson 2000; Ralston *et al.* 2003). Young animals such as calves are the most frequently identified sources of these zoonotic pathogens (Ortega-Mora and Wright 1994; Thompson 2000; Ralston *et al.* 2003). Ruminants such as cattle, sheep, and deer have been shown to shed *E. coli* O157:H7 (Chapman *et al.* 1997, 2001; Keene *et al.* 1997; Renter *et al.* 2001). *E. coli* O157:H7 and *Cryptosporidium parvum* are shed in larger numbers in the faeces and for greater periods of time by young calves than by adults (Harp *et al.* 1990; Zhao *et al.* 1995; Perryman *et al.* 1999; Besser *et al.* 2001; Gannon *et al.* 2002). However, adult cattle may excrete low numbers of these pathogens and act as sources of infections for young calves (Castro-Hermida *et al.* 2002; Gannon *et al.* 2002). *Salmonella enterica* and *Campylobacter jejuni* infections are also very common in a number of domestic and wild animal species. However, infections in poultry are

particularly high and also tend to be age-related, with infection much more common in chicks and poults (Gast and Beard 1989).

(2) There is a high animal density.

Several studies have shown higher rates of infection with waterborne zoonotic pathogens in regions with high domestic animal population densities. Infections with verotoxigenic *E. coli* (VTEC; *E. coli* O157:H7 and other related pathogenic serotypes of *E. coli*) have been reported to be higher in rural areas in Canada (Rowe *et al.* 1993) and Scotland (Sharp *et al.* 1994). Studies in the province of Ontario in Canada have shown that counties with higher cattle densities have higher rates of human VTEC infection (Michel *et al.* 1999). Similarly, Potter *et al.* (2002) reported higher rates of *Campylobacter jejuni* infection, particularly among children, in counties with high poultry densities in the state of Michigan in the USA.

(3) Biosecurity measures are minimal in animal reservoir populations. Animals are under minimal levels of confinement and free to wander through pastures and near watercourses. There is frequent contact with other animals of the same and different species, including wildlife. There is little effort placed on excluding or treating sick animals or on disease prevention through measures such as vaccination (see below).

(4) Conditions exist for the transport of pathogens found in animal wastes to water. There are poor waste management systems and infrastructure for storage of animal wastes. There is a high risk that animal wastes will be transported to water by runoff as a result of snowmelt or rainstorms.

Outbreaks of cryptosporidiosis and other waterborne diseases are known to follow extreme rainfall events (Curriero *et al.* 2001). In May 2000, an outbreak of waterborne illness associated with *E. coli* O157:H7 and *Campylobacter jejuni* in Walkerton, Ontario, Canada, caused illness in 2300 people and was responsible for 7 deaths (Hrudey *et al.* 2003). In 1992, another outbreak of waterborne infection associated with *E. coli* O157:H7, which occurred in Swaziland, caused an estimated 40 000 cases of gastrointestinal illness (Effler *et al.* 2001). In these last two outbreaks, it is thought that heavy rains transported bovine faeces contaminated with these bacterial pathogens into local drinking-water supplies.

(5) Animal wastes are not stored for long periods or treated prior to use in fertilizing crops. Water that is contaminated with animal wastes is used for irrigation or recreation. There is occupational exposure to contaminated water.

Recreational water has been identified as an important source of waterborne disease (e.g., Gilbert and Blake 1998). Lee *et al.* (2002) reported that while the number of outbreaks in the USA associated with drinking-water has declined over the past 20 years, the number of outbreaks associated with recreational water has increased and become more common than drinking-water-associated outbreaks (39 versus 59 in the period between 1999 and 2000). An increasing number of outbreaks of foodborne disease have been associated with consumption of raw vegetable crops, such as lettuce, radish sprouts, and fruit juices (Hilborn *et al.* 1999, 2000; Michino *et al.* 1999; Robertson and Gjerde 2001; Sagoo *et al.* 2003). Water contaminated with animal wastes used for irrigation or processing was the likely source of these agents in some of these outbreaks. Leptospirosis is commonly associated with occupational and recreational use of water (Lomar *et al.* 2000; Haake *et al.* 2002).

(6) Drinking-water is not treated, or treatment is minimal. Drinking-water systems are poorly designed, and there is a risk of surface water entry or leaching of water from underground waste storage systems.

A recent report on waterborne diseases in the USA from 1999 to 2000 points out that groundwater was identified as the source of infection in 28/39 (71.8%) outbreaks and that the majority of these outbreaks (18/28 or 64.3%) were associated with groundwater from wells that were private or non-community in nature and not regulated by the US Environmental Protection Agency (Lee *et al.* 2002). A recent study of waterborne disease outbreaks in England and Wales that occurred between 1970 and 2000 also reported private water sources as a significant source of infection (Said *et al.* 2003). In the latter study, the authors point out that while private water sources were associated with 36% of drinking-water outbreaks, they provided water for only 0.5% of the population.

Sobsey *et al.* (2003) reported that the risk of diarrhoeal illness in Bolivia could be reduced by 43% through chlorination of water stored in households.

(7) The zoonotic agent has a low infective dose for humans. Limited amounts of contamination can lead to widespread infection.

The median infective dose (ID_{50}) of oocysts of the protozoan parasite *Cryptosporidium parvum* varies considerably (from 12.1 to 2980), depending on the strain (Messner *et al.* 2001), and the infective dose of *Giardia duodenalis* is reported to be between 1 and 16 cysts (Rendtorff 1978). *E. coli* O157:H7 is thought to have a very low infective dose for humans (<50 organisms) (Strachan *et al.* 2001).

(8) Humans exposed to contaminated water are highly susceptible to infection because they are immunologically naive or immunologically compromised as a result of age or chronic disease.

Cryptosporidium parvum is known to cause a prolonged severe and life-threatening diarrhoea in immunocompromised individuals (Colford *et al*. 1996; Fayer *et al*. 2000; Slifko *et al*. 2000; Hunter and Nichols 2002). Young children and the elderly are most at risk for infection with *E. coli* O157:H7 (Karmali 1989). There also appears to be age- and exposure-related resistance to infection with these pathogens.

(9) The zoonotic agent is resistant to water treatment procedures that are commonly applied (e.g., resistance to chlorine demonstrated by oocysts of *Cryptosporidium parvum*). The microorganism can survive for extended periods of time and may even grow in the aquatic or terrestrial environment, even under harsh conditions. It is small enough to pass through some natural and human-made water filtration systems.

Cryptosporidium parvum and *Giardia duodenalis* are resistant to the levels of chlorination used in drinking-water treatment (Jarroll *et al.* 1981; Korich *et al.* 1990). *E. coli* O157:H7, like most other enteric bacteria, is not highly resistant to chlorine (Rice *et al.* 1999); however, certain strains may be more resistant than others (Zhao *et al.* 2001). Waterborne outbreaks normally occur where there has been no chlorination or a failure in chlorination (Friedman *et al.* 1999; Hrudey *et al.* 2003).

24.3 METHODS OF CONTROL OF ZOONOTIC WATERBORNE PATHOGENS IN ANIMAL RESERVOIRS

24.3.1 International and national zoonotic pathogen control programmes

International coordination of national animal health programmes for more than 164 countries is carried out through the Office International des Epizooties (OIE). The OIE currently maintains two lists of infectious diseases that member countries are trying to control and/or eradicate from their national domestic animal populations (http://www.oie.int/eng/maladies/en_classification.htm). The A list consists of "Transmissible diseases that have the potential for very serious and rapid spread, irrespective of national borders, that are of serious socio-economic or public health consequence and that are of major importance in the international trade of animals and animal products," and the B list includes "Transmissible diseases that are considered to be of socio-economic and/or public health importance within countries and that are significant in the international trade of animals and animal products."

Control of diseases on lists A and B is accomplished through a number of steps:

(1) National disease surveillance systems measure the disease burden associated with specific pathogens in animal populations within national boundaries.
(2) Control or eradication of specific diseases found through surveillance activities is accomplished using methods such as quarantine, test and slaughter, vaccination, and occasionally antimicrobial treatments.
(3) Animal importation control systems rely heavily on an assessment of the risk of importation of specific diseases from specific countries and on diagnostic testing of individuals or groups of animals before export or upon entry, preventive treatments, and quarantine.

While the OIE has attempted to include animal diseases that present public health risks in the compilation of these lists (e.g., anthrax, brucellosis), it is clear that the primary focus has been, until recently, on animal diseases of economic importance. Zoonotic pathogens associated with waterborne and foodborne diseases such as *Salmonella enterica*, *E. coli* O157:H7 and other enterohaemorrhagic *E. coli*, *Campylobacter jejuni*, *Giardia duodenalis*, and *Cryptosporidium parvum* are not included in these lists. Leptospirosis is a list B

disease. The lack of representation of many zoonotic waterborne and foodborne pathogens in these lists is explained by the fact that they are not frequently associated with severe animal disease. However, the OIE has recently recognized the increasing importance of food safety programmes to member countries in satisfying national and international trade requirements and the fundamental importance of controlling zoonotic pathogens in food animal populations as a cornerstone of these efforts. The OIE states that "The OIE's work programme for 2001–2005 recommends that 'OIE should be more active in the area of public health and consumer protection' " and notes that this should include " 'zoonoses and diseases transmissible to humans through food, whether or not animals are affected by such diseases.' " To implement this plan, the OIE has formed a Working Group on Food Safety, which will collaborate with the World Health Organization, the Food and Agriculture Organization of the United Nations, and subsidiary bodies, such as the Codex Alimentarius Commission. Such an initiative, if undertaken, is likely to be helpful in the control of some, but not all, zoonotic waterborne pathogens in food animal populations. While this initiative is very promising, it would also be helpful to have national and international organizations with responsibility for public health outcomes associated with zoonotic waterborne pathogens involved in the Working Group. However, it must also be kept in mind that not all countries are represented by the OIE, that zoonotic diseases in wildlife species and pets are considered only with respect to their effects on food-producing animals, and that, at times, these can be significant sources of zoonotic waterborne pathogens.

24.3.2 On-farm zoonotic pathogen control programmes

Recently, on-farm food safety programmes have been promoted by many national departments of agriculture. The successful implementation of these on-farm programmes may be helpful in the control of foodborne pathogens and would be predicted to have the secondary effect of either directly or indirectly limiting the levels of zoonotic waterborne pathogens in animal wastes. However, while nearly everyone agrees on the need for and value of these programmes, the control strategies or best management practices proposed are often generic in nature and emphasize steps such as good hygiene and animal waste handling and storage procedures. The means by which specific zoonotic pathogens can be reduced in domestic animal populations are not always clearly articulated, may not yet exist, or may require significant time for research and development before they can be implemented.

Despite the adherence to the ideals of hazard analysis and critical control point (HACCP) programmes (Mossel 1989) from farm to fork in ensuring food safety, relatively few countries have taken concrete steps to bring about their

implementation. One of the first examples of this type of on-farm initiative is the *Salmonella* control programme in poultry in Sweden, which began in 1961 (Eld *et al.* 1991; Wierup *et al*. 1992). The objective of the programme was to ensure that the whole food-chain, from feed to food, was free of *Salmonella*. A voluntary *Salmonella* control programme for flocks was established in 1970, and a compulsory programme to test all broilers for *Salmonella* before slaughter was established in 1984. The Swedish model places the onus on the producer by prohibiting the sale of poultry contaminated with *Salmonella*. It is necessary to start with chicks that are *Salmonella*-free. A nationwide use of a competitive exclusion culture (Hirn *et al.* 1992; see below) in poultry was initiated in 1981, as well as the use of feed that was verified as *Salmonella*-free (Malmqvist *et al.* 1995). Similar *Salmonella* reduction programmes for poultry were initiated in Finland, Norway, and Denmark and today include efforts to reduce *Salmonella* in swine (Wegener *et al.* 2003).

24.3.3 On-farm zoonotic pathogen control

24.3.3.1 Specific pathogen-free animals

The central aim of successful zoonotic pathogen control and eradication campaigns has been to produce specific pathogen-free animals. Experience has shown that it is much simpler to keep animals free of specific pathogens than to try to make them specific pathogen-free after infection has occurred. Despite best efforts, asymptomatic carriers may remain in herds or flocks after culling or treating of infected animals and perpetuate the infection; for example, pigs that are asymptomatic carriers of *Salmonella* are the bane of the European SALINPORK programme (Wegener *et al.* 2003). Unfortunately, some of the control procedures, such as vaccination and antimicrobial use, may control the level of pathogens excreted but do not eliminate the carrier state and will simply mask hidden infections. Therefore, the present Danish programme relies strictly on test and slaughter and not on vaccines. It is also for this reason that so much emphasis has been placed on "cleaning up" the primary breeder flocks and herds in *Salmonella* and *Campylobacter* control programmes in Nordic countries.

Zoonotic pathogen control programmes for the creation of specific pathogen-free animals must be cost-effective and use diagnostic procedures that can detect all infected animals to be culled. Serological procedures rather than microbial culture or detection methods have been found to be cost-effective in the Danish *Salmonella* control programme (Wegener *et al.* 2003).

Caesarean section-derived germ-free or gnotobiotic animals have been used in the swine industry to create disease-free animals. Research has also been conducted on breeding of pathogen-resistant animals (Adams and Templeton 1998; see also section 24.5.4 below).

24.3.3.2 Biosecurity

Physical biosecurity systems are best developed for poultry flocks and swine herds to control infectious diseases of economic importance to these industries (White *et al.* 1997). The housing systems are designed to keep animals free from pathogens by limiting entry of other animals of the same and of different species. Animals are kept pathogen-free by limiting introductions of new animals to the herd. If introductions are made, the animals are first tested for specific pathogens of concern. If they are negative, they must still remain isolated from the rest of the herd for a specific quarantine period. Rodent, wild bird, and fly control procedures are put in place, and human handlers must use a disinfectant boot bath and, in some cases, shower in and shower out between barns and farms. Animal feeds are often contaminated by enteric bacteria such as *Salmonella* (Crump *et al*. 2002); they may be heat processed to reduce this contamination and then must be stored in a manner that will prevent access by rodents and birds (Daniels *et al.* 2003). If animals are sent to slaughter, all animals are removed in one shipment, barns are thoroughly cleaned, and a rest period must pass before the next group of animals is introduced. In some cases, animals are vaccinated and/or antimicrobials are incorporated into the feed to provide an additional level of pathogen protection.

Biosecurity systems such as these are reported to reduce the risk of poultry shedding *Campylobacter* in their faeces (Gibbens *et al.* 2001). It is also likely that manure from animals raised with high levels of biosecurity present a lower risk of food and water contamination. On the contrary, it has been reported that Swedish organic free-range chickens have much higher levels of *Campylobacter* than chickens raised under conventional barn systems (Engvall 2001).

High levels of biosecurity may not be feasible in all countries and certainly not for all animal species. Much less effort has been placed on the rearing of ruminants such as cattle, sheep, and goats under conditions of high physical biosecurity. However, some management practices have been used to bring about a certain level of biosecurity and prevent the spread of pathogens. These include 1) limiting the introduction of new animals into herds, 2) moving mothers and calves away from calving areas onto “clean” pastures as soon as possible after birth, and 3) raising dairy calves in isolation from other calves and the rest of the herd. However, in many regions, ruminants are used to harvest plant growth from land unsuitable for crop production. As a consequence, they often have close contact with a number of wild and other domestic animal species and can readily become infected by a number of foodborne and waterborne pathogens. There is considerable evidence that cross-species transmission of waterborne pathogens such as *Giardia duodenalis*, *Cryptosporidum parvum*, *Campylobacter*, *Salmonella*, *E. coli* O157:H7, and

Leptospira interrogans occurs between cattle and wildlife species living in close proximity to one another (Keene *et al*. 1997; Lomar *et al.* 2000; Thompson 2000; Daniels *et al.* 2003; Liebana *et al.* 2003; Wahlstrom *et al.* 2003).

While ruminants have traditionally been raised on pastures, in many countries young ruminants are purchased following weaning and fattened on carbohydrate-rich diets in feedlots. These types of animal-rearing systems often depend on the use of a large number of animals to realize sufficient profit margins. Therefore, young animals from a large number of sources are transported long distances and mixed together in the feedlot environment. This frequently results in high levels of mortality among these animals due to respiratory and other diseases. Biosecurity needs for feedlots have relied heavily on the administration of antibiotics to animals upon arrival as well as the use of vaccines. Disposal of immense quantities of manure generated by feedlots, dairy, swine, and poultry operations has been a major challenge in many watersheds and is the subject of chapter 25.

24.3.3.3 Antimicrobials

Antimicrobials have long been used at low levels for the purposes of growth promotion in animal production as well as therapeutically to lower morbidity and mortality associated with specific disease conditions in animals. Use of growth promoters in feed has been viewed as an economical form of biosecurity for the agricultural industries in many countries for several decades. A number of studies have shown the benefits of incorporating antimicrobials into the diets of farm animals in reducing the levels of enteric bacterial pathogens associated with foodborne and waterborne diseases (Rantala and Nurmi 1973; Goodnough and Johnson 1991; Johnson 1992). Antimicrobials such as penicillin and dihydrostreptomycin have also proven useful in controlling leptospirosis infections and eliminating the carrier state in domestic animals (Alt *et al*. 2001). However, much concern has been expressed recently about the overuse of antibiotics in animal feeds for the purposes of growth promotion. Concern is greatest where there is the possibility of cross-resistance developing between antibiotics used in animal feeds and those used in human clinical medicine. The development in *Salmonella* of resistance to fluoroquinolones and extended spectrum β-lactamases as well as the emergence of multiantibiotic resistance are thought to be a direct consequence of antibiotic supplementation of animal feeds (Threlfall 2002). These antibiotic resistance determinants are encoded by mobile genetic elements that can quickly be transferred both within and between bacterial species. This has caused concerns about the long-term effectiveness of these measures and, more importantly, about the transfer of these genetic elements into a broader range of human pathogens. While there are attempts to

separate classes of antibiotics used in animals for growth promotion from those used in human therapeutics, between 1997 and 1999, the European Union implemented bans on five different antimicrobials: avoparcin, spiramycin, tylosin, bacitracin, and virginiamycin, which are used for growth promotion in animals (Frei *et al.* 2001; Emborg *et al.* 2003; Evans and Wegener 2003). While it was initially felt that these regulatory developments might increase levels of enteric pathogens in animal wastes, Evans and Wegener (2003) have reported decreases in *Salmonella* levels in poultry and pigs and equivalent levels of *Campylobacter* in poultry in Denmark 3 years after the ban on the use of these antimicrobials in feeds.

Cryptosporidium parvum is naturally resistant to most anticoccidial drugs (Coombs and Muller 2002). Joachim *et al.* (2003) reported that halofuginone decreases but does not eliminate faecal shedding of *Cryptosporidium* oocysts by calves and suggested that the drug, used together with good sanitation and disinfection procedures, may limit infection by this parasite in calves. Castro-Hermida *et al.* (2001) and Castro-Hermida and Ares-Mazas (2003) have reported that the cyclic oligosaccharides α- and β-cyclodextrins are effective in the treatment of *C. parvum* infections in young ruminants.

24.3.3.4 Immunoprophylaxis

While vaccination has long been used to prevent animal diseases, many of the zoonotic waterborne pathogens seem to have limited or no effect on the health of animals; swine and poultry frequently excrete *Salmonella* and *Campylobacter* and young calves excrete *E. coli* O157 in their faeces without apparent ill effect. The gold standard for vaccines has traditionally been prevention of disease and economic loss associated with animal disease. However, in the case of zoonotic pathogens, vaccines should not only prevent faecal shedding but also ideally eliminate the carrier state. Until recently, veterinary vaccines have been very crude and have consisted only of killed or modified live pathogenic organisms. This lack of sophistication is related to the low cost of production of these vaccines and difficulties with regulatory approval of recombinant vaccines. There is also concern about the loss in the quality of meat and poultry products as a result of vaccination via intramuscular routes, and more and more emphasis is being placed on adjuvants and routes of vaccination that stimulate mucosal immunity and prevent colonization and the carrier state.

It has been reported that infection of calves with zoonotic pathogens such as *E. coli* O157:H7 and *Cryptosporidium parvum* occurs within hours and days of birth (Fayer 1997; Gannon *et al.* 2002). Therefore, it may be more logical to mount a specific immune response in their dams through vaccination prior to birth so that protection against colonization and infection can be provided

passively through colostrum and milk. This approach was initially developed for protection of lambs and calves against K99-bearing enterotoxigenic *E. coli* (Sojka *et al.* 1978; Nagy 1980). Perryman *et al.* (1999) reported that the severity of *C. parvum* infection and oocyst shedding can be reduced by feeding calves immune colostrum obtained from dams vaccinated with a recombinant *C. parvum* surface antigen. Recently, feeding of monoclonal antibodies that were generated against surface antigens of *C. parvum* has also been shown to be beneficial in limiting persistent infections with this organism in SCID mice (Riggs *et al.* 2002).

Properly formulated vaccines have the potential to be valuable tools in controlling leptospirosis in domestic animal reservoirs. The degree of protection provided by *Leptospira* vaccines is serovar-specific and has led to the use of multivalent vaccines with antigens from several serovars. However, some of the multivalent vaccines on the market have not been shown to provide long-lasting protection against clinical disease or urinary shedding in cattle (Bolin and Alt 2001). Other vaccines with *Leptospira interrogans* serovar harjo alone or in combination with serovar pomona have been shown to decrease urinary shedding in cattle and are reported to decrease the incidence of leptospirosis in humans in contact with the cattle (Mackintosh *et al.* 1980; Bolin and Alt 2001).

24.3.3.5 Competitive exclusion

Rantala and Nurmi (1973) noted that day-old chicks can become colonized by a few colony-forming units of *Salmonella*; as the birds age, however, it is more and more difficult to infect them with the organism, and the number of *Salmonella* shed in the faeces decreases. They also showed that feeding the caecal contents of adult birds to young chicks made them resistant to colonization with *Salmonella.* In contrast to the use of antimicrobials in feeds, inhibitory effects on the shedding of *Salmonella* persisted long after the treatment was stopped. This method of combatting *Salmonella* in poultry, called "competitive exclusion," became widely used in Finland in their *Salmonella* control programme. Hirn *et al*. (1992) reported that less than 5% of the Finnish poultry flocks were *Salmonella*-positive and that 70–80% of the cases of salmonellosis in humans in Finland were acquired abroad. Stern *et al*. (2001) in the USA also used anaerobic cultures derived from mucosal scraping of the intestines of adult chickens in competitive exclusion experiments and demonstrated that their cultures reduce *Salmonella* in the caeca of chickens as well as faecal shedding of *Campylobacter*. The biological basis of competitive exclusion is poorly understood. However, it may be related to enhancing the immune function of the host, nutrient competition among bacteria, or the elaboration of toxic substances such as volatile fatty acids, antimicrobial

peptides, or bacteriocins (Joerger 2003). Recent studies on bacterial populations also suggest that certain bacterial hormones involved in quorum sensing allow "communication" among different bacterial species and may play a role in controlling population levels of specific bacterial species.

Cultures of microorganisms that limit pathogenic bacterial populations in the gut have been termed probiotics. Other microorganisms, such as lactobacilli and *Bifidobacterium*, and complex carbohydrates that promote the growth of populations of these members of the normal gut flora have been extensively studied as agents to control faecal shedding of enteric bacterial pathogens such as *Salmonella* in poultry (Stavric 1992; Gusils *et al.* 1999; Fernandez *et al.* 2002).

The strategy of using probiotic bacteria is also being explored for control of other bacterial pathogens in other animal species. Recently, Tkalcic *et al*. (2003) reported that a mixture of probiotic *E. coli* strains isolated from adult cattle reduced faecal shedding of *E. coli* O157:H7 and enterohaemorrhagic *E. coli* serotype O111:NM from young cattle within 8–30 days and 6–12 days following treatment, respectively.

24.3.3.6 Other pathogen control methods

Bacteriocins are bactericidal toxins produced by certain bacteria to eliminate other competing bacterial species and even subtypes within their own species. Bacteriocin-producing bacteria are being studied for the control of *E. coli* O157:H7 faecal shedding in cattle (Duncan *et al.* 1999; Schamberger and Diez-Gonzalez 2002) and faecal shedding of *Salmonella* in poultry (Wooley *et al.* 1999).

Bacteriophages are viruses that infect and lyse bacteria. They were first investigated for their role in controlling *E. coli*-associated diarrhoeal diseases in calves, lambs, and piglets (Smith and Huggins 1983; Smith *et al.* 1987). While the results of these studies using mixtures of bacteriophages were promising, the work was not pursued further until recently. Kudva *et al.* (1999) isolated bacteriophages that lyse *E. coli* O157 strains.

Studies have shown that enteric bacteria such as *Salmonella* spp. and *Campylobacter* spp. can be isolated from poultry drinking-water (Poppe *et al.* 1986; Stern *et al.* 2002) and *E. coli* O157:H7 from water and sediments in cattle water troughs (Shere *et al.* 1998; LeJeune *et al.* 2001; Van Donkersgoed *et al.* 2001). Workers have therefore postulated that drinking-water may be important in the spread of these enteric pathogens in herds and flocks and that measures such as chlorination of water may break the cycle of transmission, reducing infection rates and limiting faecal shedding of these pathogens. Unfortunately, chlorination of drinking-water has not been shown to significantly reduce faecal

shedding of either *Salmonella* spp. or *Campylobacter* spp. in poultry (Poppe *et al.* 1986; Stern *et al.* 2002) or faecal shedding of *E. coli* O157:H7 in cattle (LeJeune *et al.* 2004). There are several possible explanations for the lack of efficacy of chlorination of water in control of these enteric pathogens in animals. These include the following:

(1) Organic matter present in water from feed, animal secretions, faecal matter, algal growth, and detritus may limit the bactericidal efficiency of chlorine.
(2) While "free-living" or pelagic bacteria in water may be killed by chlorine, those present in biofilms in sediments (LeJeune *et al.* 2001) and on water surfaces are less susceptible to chlorine and persist and regrow when chlorine levels are low or once they are ingested.
(3) Finally, while water has been demonstrated to contribute to the spread of enteric bacterial pathogens (Shere *et al.* 2002), it is likely just one of many important routes of faecal–oral transmission in flocks and herds, and all routes must be controlled to result in significant decreases in transmission rates.

Recent studies have also shown that addition of sodium chlorate to drinking-water and feed reduces counts of certain enteric bacterial pathogens shed in the faeces of poultry, sheep, cattle, and pigs (Anderson *et al.* 2001; Callaway *et al.* 2002, 2003; Byrd *et al.* 2003). Chlorate is thought to exert its bactericidal effect directly on bacteria in the gastrointestinal tract. Many enteric bacteria possess the enzyme nitrate reductase to convert nitrate to nitrite. When sodium chlorate is provided in water or in feed, this enzyme reduces the chlorate to the bactericidal metabolite chlorite. Feeding sodium chlorite to animals has been suggested as a measure to reduce enteric bacterial pathogen populations in the faeces and gastrointestinal contents of animals prior to slaughter. However, it is unlikely to be adopted for use in controlling these pathogens during the growth stages of animals. Concerns also exist about chlorite toxicity to humans (Mantovani 1993), which may impede regulatory approval of this chemical agent for use in food animals.

24.4 CONTROL OF ZOONOTIC PATHOGENS IN WILDLIFE

In wild animal populations, parasite and host may live in an ecological balance with little or no obvious effect on the natural host reservoir population. Health effects are most likely to be observed with these zoonotic agents when human

hosts are accidentally exposed to them. Eradication programmes have been and continue to be successfully used to control infectious diseases in domestic animals and may be applicable to animal pests such as rodents and mosquitos. Contraceptive vaccines have been developed to bring about control of wildlife populations that may carry zoonotic diseases (Barber 2000; Smith and Cheeseman 2002; Mate *et al*. 2003). However, this approach is not acceptable for wildlife species that are thought to play an important ecological role or that are considered endangered. For example, it is thought that attempts to eradicate bovine tuberculosis have been frustrated by the inability to control *Mycobacterium bovis* infections in badger (*Meles meles*) populations in the United Kingdom (Delahay *et al*. 2003).

One of the few successful uses of a vaccine in wildlife has been in the control of fox rabies in Switzerland (Wandeler *et al*. 1988). In the vaccination programme, food baits impregnated with a modified live rabies virus were distributed in the countryside. Aguilar-Setien *et al*. (2002) reported on the potential use of a vaccinia-rabies glycoprotein recombinant virus aerosol for control of rabies in vampire bats (*Desmodus rotundus*). Attempts have been made to vaccinate racoons, skunks, coyotes, mongooses, and bats (Creekmore *et al*. 1994; Aguilar-Setien *et al*. 2002; Hanlon *et al*. 2002; Linhart *et al*. 2002) with either aerosol or bait vaccines against rabies virus. Vaccines have also been developed against brucellosis in feral swine and wild ruminants (Davis and Elzer 2002) and the plague bacillus, *Yersinia pestis*, in rodents. Aerosol or bait vaccines may be useful for the control of certain waterborne zoonotic pathogens.

Most of the leptospirosis in the world occurs in humid tropical climates and is thought to be caused by contamination of water with urine from infected wild or feral animal species. Control of leptospirosis in these species is challenging, given the variety of *Leptospira interrogans* serovars that are encountered in the wild animal reservoir species and the cost of vaccination and eradication programmes.

24.5 ARE TSES POTENTIAL WATERBORNE PATHOGENS?

24.5.1 Characteristics of TSEs

TSEs are a group of infectious diseases characterized by slow and progressive degenerative changes in the central nervous system. TSEs affecting humans include Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome, kuru, and fatal familial insomnia; those affecting animals include scrapie in sheep and goats, feline spongiform encephalopathy, transmissible mink encephalopathy, chronic wasting disease (CWD) in elk and deer, and

bovine spongiform encephalopathy (BSE) (Dormont 2002). It is hypothesized that these unique infectious agents are aberrant forms of a normal host protein. Prusiner (1982) first proposed the existence of this self-propagating protein, which he termed a "prion," as the causative agent of TSEs. Although the theory that prions are responsible for TSEs is now accepted by the majority of the scientific community, some scientists are still not convinced and suggest that an infectious agent such as viruses or virinos may be responsible (Manuelidis 2003). The normal prion protein is found in neural as well as many other body tissues and is thought to be a membrane-bound glycoprotein involved in copper transport into host cells and protection of these cells against oxidative stress (Clarke *et al.* 2001; Collinge 2001). The infectious form of the prion is thought to occur when the α-helical structure of the native protein is somehow converted to the β-sheet isoform. The altered prion protein differs from the native form in three important respects. Firstly, it is highly resistant to proteases and other physical and chemical agents, such as heat, ultraviolet (UV) light, and oxidants (Dormont 2002). Secondly, it is postulated that it catalyses the transformation of native prion protein to the infectious form using the infectious form of prion as a template, perhaps with the addition of an accessory factor termed "protein X." Finally, the highly resistant form of the protein accumulates over time in the host tissues and in some way leads to the characteristic neural pathology associated with TSEs. It is possible that the accumulation of the aberrant form of the prion protein leads to a failure of the normal prion protein role in the protection of cells against oxidative stress and to calcium cytotoxicity (Hur *et al*. 2002), but this is not certain.

BSE was first diagnosed in 1986 at the Central Veterinary Laboratory in Weybridge in the United Kingdom. It is thought that BSE may have originated spontaneously in cattle or as a rare type of scrapie present in sheep. Subsequently, cattle became infected as a result of feeding bovine- or ovine-derived BSE-contaminated meat and bone meal (MBM) to cattle. Once cattle became infected, they in turn contributed further to the contamination of MBM with the BSE agent. The highly resistant nature of the prion protein is thought to have allowed it to survive an altered rendering process used at the time in the production of MBM.

The protease and acid resistance of the prion may also allow it to pass through the stomach and into the intestinal tract. Once in the intestine, it is thought to be taken up by gut-associated lymphoid tissues and subsequently passed to lymphoid tissues such as the spleen and tonsils, where it enters the lymphoid-associated peripheral nervous system. It then makes its way to the central nervous system over the course of many months and years, depending on the TSE strain and the host species and genotype. The resistant nature of the protein may also be responsible for the ability of TSEs such as scrapie from

sheep to persist in soils for more than 3 years (Brown and Gajdusek 1991). There is strong epidemiological evidence supporting lateral transfer of scrapie and CWD in sheep and deer, respectively.

24.5.2 Risks of acquiring BSE from drinking-water sources

In 1997, evidence was presented that BSE was a zoonotic agent (Bruce *et al.* 1997). The BSE-associated disease in humans was termed "variant Creutzfeldt-Jakob disease" (vCJD) because of its similarity to the well recognized human TSE, CJD. It is assumed that humans with vCJD acquired the infection through the consumption of bovine nervous tissues (bovine offal, beef-on-the-bone) containing high levels of the infectious BSE agent. Shortly after this finding, it was suggested that nearly a million cattle in the United Kingdom would have to be culled and disposed of to control the BSE and vCJD epidemics in cattle and humans, respectively. As a result of this impending action, concern was expressed that the BSE agent present in infected material from the cattle to be slaughtered could enter surface water and groundwater (from slaughterhouses, rendering plants, and landfills) and pose a public health risk. The following is a list of some of the key components of this risk assessment (Gale 1998, 2001; Gale *et al.* 1998):

(1) *Concentration of BSE agent in bovine neural tissue.* It was assumed that the level of infectious material in the nervous tissues of cattle would be equivalent to that which occurred at the peak of the BSE outbreak as a worst-case scenario.
(2) *Infectious dose models for BSE.* It can be postulated that a certain threshold dose level of the BSE agent is required to cause vCJD in humans and that this dose is much less likely to be achieved as a result of a single consumption event of water rather than of bovine nervous tissue. This is because the agent is likely to be highly dispersed in water and concentrated in nervous tissues in beef. However, it is also possible that the threshold could be reached by a susceptible individual after years or months of low-level long-term exposure. However, even if this assumption is accepted, Gale *et al.* (1998) calculated that a threshold dose from the agent in water would be reached only after approximately 45 million years. Alternatively, it is possible that a specific threshold does not exist for the population and that a very low dose of the BSE agent could result in infection in a correspondingly small proportion of the population — i.e., those most susceptible would have a lower threshold dose. If this assumption is accepted and it is considered that the prion ID_{50} is divisible and cumulative, according to Gale *et al.*

(1998), one arrives at similar levels of risk for drinking water and eating beef-on-the-bone (10^{-8} versus 10^{-9} per person per year).

(3) *The interspecies barrier.* Mice can be infected with BSE by feeding them infected bovine nervous tissue; however, the ID_{50} by the oral route for mice is approximately 1000 times greater on an equivalent-weight basis than it is for cattle. A large part of the interspecies difference in ID_{50} may be related to the ability of the prion material to pass through the gastrointestinal tract and then enter gut-associated lymphoid and nervous tissues. While pigs can be infected with BSE-infected nervous tissue by the combined intracranial, intravenous, and intraperitoneal routes, they appear to be completely refractory to infection with this TSE by the oral route (Wells *et al*. 2003). Martinsen *et al.* (2002) recently reported that the BSE ID_{50} in mice can be decreased by lowering concentrations of hydrochloric acid in the stomach of mice using ranitidine. It is therefore conceivable that some humans may be more susceptible to infection than others and that this susceptibility could change with the physiology of the gastrointestinal tract.

(4) *Susceptibility to TSEs based on genotype.* It is known that there are differences in the genotype of prion proteins within sheep, mice, and human populations that make them resistant or susceptible to specific TSEs. Humans that are homozygous for methionine at codon 129 of the prion protein gene are more susceptible to CJD, and all cases of vCJD described thus far have this genotype (Will *et al.* 2000).

(5) *Dilution in water.* As mentioned above, humans with vCJD are thought to have acquired the infection through the consumption of bovine nervous tissues containing high concentrations of the BSE agent. The human ID_{50} has been calculated to be equivalent to 10^{13} BSE prion proteins (Gale *et al*. 1998). As mentioned above, it is thought that in contrast to beef products, the BSE agent is likely to be highly dispersed in water. Therefore, the daily intake of infectious prions by consumers of water is likely to be very low by nature of dilution when compared with BSE-contaminated nervous tissue in beef-on-the-bone.

(6) *Transport and solubility in water.* The prion protein is a typical membrane protein and has both hydrophilic and hydrophobic domains. This property limits its solubility in water and favours the formation of insoluble aggregates, promotes adhesion to hydrophobic particles, and would be predicted to limit its mobility in surface water or groundwater. Further, Brown and Gajdusek (1991) showed that a closely related TSE agent, scrapie, has very limited mobility in soils. Thus, risks of infectious prions entering groundwater or surface water from cattle carcasses buried for disposal are likely very low.

In support of these risk assessments, Cousens *et al.* (2003) reported that vCJD cases in the United Kingdom have not been geographically clustered, as would be expected if the source of infection was from an environmental source such as soil or water.

While much of the evidence suggests that the risks of infection with the BSE agent from drinking-water are negligible (Gale 2001), further research on our levels of exposure to TSEs in the environment and parameters affecting the infective dose in humans is required. A reasonable estimate of the ID_{50} for humans of BSE-infected material is required before a meaningful quantitative risk assessment can be conducted. However, essential elements necessary for this risk assessment model, such as the nature of the interspecies barrier for BSE infection in humans, are not known. In addition, a lack of understanding of the genotypic and physiological differences in susceptibility to this TSE within the human population has further complicated this task.

24.5.3 BSE eradication plan

The epidemic in the United Kingdom has resulted in 180 121 cases of BSE in cattle (http://www.defra.gov.uk/animalh/bse/index.html). The BSE epidemic appears to have reached its peak in 1992, and the number of cases has declined since then. At the time of this writing, 137 deaths have been associated with vCJD in the United Kingdom, and the peak of the epidemic appears to have been in 2000 (http://www.doh.gov.uk/cjd/cjd_stat.htm). In the United Kingdom, a number of steps have been taken to control the BSE outbreak in cattle and the vCJD epidemic in humans. The MBM ban for ruminant feed and culling of animals from infected herds were likely to have been the most important steps in controlling these epidemics. Many other countries have adopted similar measures to combat and/or prevent the entry of BSE. The MBM ban has been expanded to include all food-producing animals in certain countries. Measures to protect the human population from vCJD have included removal of specified risk materials (offal, heads, and spines) of bovine origin from the food-chain, restriction of slaughter of animals for human consumption to those under 30 months of age, and banning the importation of cattle or beef from countries with BSE. Both BSE and vCJD cases have declined due to these measures.

24.5.4 Scrapie eradication plan

Scrapie is a TSE endemic in sheep populations throughout most of the world (Australia and New Zealand are notable exceptions to this, with their sheep scrapie-free). It is often stated that scrapie has existed in sheep in the United

Kingdom for more than two centuries without an apparent public health impact (Baylis *et al.* 2002). However, as stated above, it has been hypothesized that BSE may be a rare strain of scrapie that has existed for some time at low levels in the sheep population in the United Kingdom prior to causing BSE in the cattle population. It is also certain that some sheep consumed MBM contaminated with the BSE agent before the MBM feed ban took place. It therefore seems likely that some sheep may have become infected with BSE through MBM and may pose a public health risk (Butler 1998).

Further, there is concern that the public health risk from BSE in sheep could be potentially greater than is posed by BSE in cattle. Firstly, the scrapie agent is distributed more widely in body tissues of sheep than is BSE in the tissues of cattle. Therefore, BSE in sheep could present a greater risk for acquiring a TSE for human consumers of mutton. Secondly, the epidemiology of scrapie in sheep is quite different from that of BSE in cattle. Studies on BSE in cattle suggest that there is little or no spread of the agent horizontally to herd mates and vertically to offspring; in scrapie in sheep and CWD in cervids, however, there is both vertical and horizontal spread of the agent within flocks and herds, and these agents appear to persist in pastures and act as a source of infection for other susceptible animals (Baylis *et al.* 2002; Detwiler and Baylis 2003; Salman 2003). This raises the possibility that BSE not only could be transmitted by consumption of mutton but also could become more widely dispersed in the environment. In response to this potential public health threat, a National Scrapie Plan has been implemented in the United Kingdom with the aim of eradicating scrapie (and BSE, if it exists) from the national sheep flock. The plan will use selective breeding for the scrapie/BSE-resistant prion genotype of sheep ARR/ARR (Houston *et al*. 2003).

24.6 SUMMARY AND CONCLUSIONS

The risk of zoonotic waterborne diseases can be resolved to some extent by minimizing the entry of animal wastes to source waters, by controlling animal movements, proper storage and disposal of farm animal wastes, using procedures that will minimize the survival of zoonotic pathogens, and limiting transport of these wastes in surface water runoff.

Control and, in some cases, eradication of zoonotic agents in animal reservoirs may be an efficient and cost-effective means of controlling drinking-water source and recreational water contamination with zoonotic pathogens. Most zoonotic pathogens are not uniformly distributed through animal populations but rather are present in specific animal species reservoirs. Further specific age classes or animals such as young calves may shed these organisms in much higher numbers than adults.

Control measures for domestic animals centre around the issue of increased biosecurity. This can be brought about by measures such as limiting access of other animal species through physical barriers, limiting cross-contamination among animals by using good sanitation practices and raising susceptible animals away from animals that may be carriers of zoonotic agents, keeping "closed herds," "all-in all-out" procedures, quarantine of new animals and surveillance-based test and slaughter procedures, providing food and water free of zoonotic agents, competitive exclusion, bacteriophage therapy, other feed additives, and active and passive immunization. These strategies can be applied with success at the farm level but are more effective on a national level with economic incentives for compliance.

Control of zoonotic diseases in domestic animal populations, while difficult, is more easily addressed than control of these diseases in wild animal populations. Wherever it is possible, there should be physical separation of wildlife from domestic animals; in addition, population control of wild species should be considered only in selected areas where there is a high rate of infection with a zoonotic agent in a reservoir population and there is a clear risk of contamination of surface water with pathogens and/or transfer of these pathogens to domestic livestock. Wildlife vaccination schemes to limit infection with specific zoonotic agents or for immunocontraception represent possible strategies for limiting certain zoonotic waterborne diseases.

The sudden emergence of zoonotic pathogens responsible for the outbreak of BSE in cattle and vCJD in humans in the United Kingdom has been a cause of concern for most public health authorities. A ban on feeding MBM to food-producing animals and a rigorous test and slaughter policy have brought both the BSE epidemic in cattle and the vCJD epidemic in humans under control in the United Kingdom. The small size and extreme resistance of these TSE agents to proteases, heat, UV light, and chemical treatments suggest that current water treatment technologies would have little effect in controlling these zoonotic agents, and TSE agents have been shown to persist in soils for years. However, the insoluble nature of prions is likely to limit their movement into water systems. Further, it is felt that a relatively small quantity of prions would be released following slaughter or rendering of cattle and that these prions would be diluted in the environment and would be highly unlikely to constitute an infective dose for a human over a lifetime.

24.7 RECOMMENDATIONS

Many zoonotic agents associated with waterborne disease are also associated with foodborne disease in humans. However, they frequently do not cause overt clinical disease in domestic animal populations. National and international agencies with health mandates that address control of waterborne and foodborne diseases should coordinate their activities and share resources to accomplish specific zoonotic

pathogen reduction targets. Programme objectives can be accomplished only by working with agricultural and conservation stakeholder groups and veterinary authorities that have experience in disease control in animal populations.

Agencies with health mandates that encompass control of waterborne diseases should also coordinate their activities and share resources to control contamination of water with animal wastes by working with agricultural and environmental stakeholder groups and agencies.

Control of waterborne zoonotic pathogens depends on the use of tools such as HACCP in water safety plans and risk assessments. Cost/benefit analyses must be performed before strategies are implemented, and the solutions proposed to limit waterborne disease must be communicated and found to be acceptable by stakeholders.

The most important component of any water treatment strategy is likely to be source protection. This can best be accomplished by taking steps to control zoonotic pathogens in animal populations (as outlined above), proper storage and disposal of animal wastes, and limiting physical access of animals to source waters.

Efforts should be focused on improving water treatment in rural areas and in small communities and improving the safety of water used in the irrigation of crops, in particular raw edible field crops.

Members of communities that are most at risk of waterborne diseases need to be identified and protected from waterborne disease. Those most at risk include the very young, the aged, the immunocompromised, individuals who are genetically most susceptible, and those with increased occupational or recreational exposure to waterborne pathogens.

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25

Control of zoonotic pathogens in animal wastes

V.P.J. Gannon, F. Humenik, M. Rice, J.L. Cicmanec, J.E. Smith, Jr., and R. Carr

25.1 ANIMAL AGRICULTURE SYSTEMS

A wide variety of animal agriculture systems contribute to animal waste production around the globe. The type of animal agriculture system depends on many things, including the culture, the economy of the region, ownership of the land base, fertility of the land, climate, and the animal species. According to the scheme of de Haan *et al.* (1997), three fundamental systems are recognized: 1) free-range or grazing, 2) mixed farming, and 3) industrial farming.

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25.1.1 Grazing systems

Grazing systems are estimated to occupy about 50% of the agricultural land in the world, but provide only 9% of the global beef and 30% of the mutton and goat meat production. In many grazing systems throughout the world, land is held in common, and only the herds of animals are owned by individuals or groups. However, there is a trend for increasing private ownership of grazing lands and restriction of land for grazing that cannot economically be used for other forms of agriculture.

In grazing systems in arid and semi-arid regions, animal densities are frequently low because vegetation cover is sparse. Therefore, animal wastes tend to be distributed over a large area. The resulting increased effect of sunlight, desiccation, predation, and antimicrobial substances produced by microbes in the soil would be expected to decrease pathogen numbers in animal wastes and reduce animal transmission of these pathogens and the zoonotic risks associated with these animal wastes. However, water is frequently a limiting resource in grazing systems, and animals, particularly in times of drought, congregate in areas with ready access to surface water. The resulting high density of animals leads to degradation of shore habitats and the input of animal wastes to surface waters. Sudden rainstorms then may rapidly transport large quantities of animal wastes into these surface waters. In Swaziland, an outbreak of waterborne *Escherichia coli* O157:H7 infection, affecting more than 40 000 people, was thought to have been caused by torrential rains that carried contaminated cattle manure into the communities' drinking-water and irrigation water source (Effler *et al.* 2001).

Restricting access of domestic animals to surface waters can reduce transport of animal wastes and soils containing zoonotic pathogens into surface waters. However, it is frequently not possible to exclude animals from the entire watershed, and the most that can be hoped for is limiting direct access by fencing off areas close to the water's edge and providing "buffer strips" of vegetative cover to diminish transport of animal wastes into the waters. Limiting access of animals to surface waters requires that other sources of water be provided through wells or diverted or pumped surface water. Animal activity can be concentrated in these areas if they are shaded. Expenses associated with these measures are likely to be impractical in many countries and resisted by the farming community if no incentives are provided. Limiting animal density in areas near surface waters prevents excessive grazing and removal of vegetative cover. Vegetative cover acts as a filter and limits transport of faecal materials and soil into surface waters.

Watersheds for large cities in developed countries are often fenced to prevent entry of domestic animals such as cattle and sheep. However, it can be argued that decreasing domestic animal populations in a watershed may lead to increases in the populations of wild and feral animals in the watershed that are just as likely to deposit wastes containing zoonotic pathogens. A waterborne outbreak of

toxoplasmosis in Victoria, British Columbia, Canada, is thought to have been caused by excretion of cysts in the faeces of cougars or feral cats within the limits of a fenced watershed (Aramini *et al.* 1999).

25.1.2 Mixed farming systems

Mixed farming systems are those in which plant crop production and animal agriculture occur on the same farm. Mixed farming systems are estimated to account for 54% of the global meat production and 90% of the milk production (de Haan *et al.* 1997). In mixed farming systems, plant crops provide some or all of the feed for animals on that farm. Farm animal wastes, in turn, are usually applied as fertilizers to the land used for growing feed crops. Therefore, there is cycling of certain nutrients between plant crop and animal production. Animal wastes are often stored prior to being spread on land.

25.1.3 Industrial farming systems

Concentrated and intensive systems are dedicated strictly to animal production. They are estimated to account for 37% of global meat production (de Haan *et al.* 1997). Farms usually have large populations of a single animal species housed in close quarters in open areas or enclosed buildings. These operations are referred to as concentrated animal feeding operations. In the USA alone, there are about 238 000 animal feeding operations that produce about 320 million tonnes of manure annually.

Manure and other wastes (such as respiratory secretions, urine, and sloughed feathers, fur, or skin) of various agricultural (livestock) animals may contain high concentrations ($>10^6$ per gram of faeces) of human pathogens (disease-causing microorganisms). Per capita faecal production by agricultural animals such as cattle and swine far exceeds that of humans, and the trend for production facilities to harbour thousands to tens of thousands of animals in relatively small spaces results in the generation of very large quantities of concentrated faecal wastes that must be effectively managed to minimize environmental and public health risks.

25.2 PATHOGENS

As shown in Table 25.1, animal pathogens posing potential risks to human health include a variety of viruses (e.g., swine hepatitis E virus), bacteria (e.g., *Salmonella* spp.), parasites (e.g., *Cryptosporidium parvum*), and helminths (e.g., *Ascaris suum*, *Taenia solium*), some of which are endemic in commercial livestock and difficult to eradicate from both the animals and their production facilities. Hence, pathogens in animal manure and other wastes pose potential

risks to human and animal health both on and off animal agriculture production facilities if the wastes are not adequately treated and contained. There are also growing public health concerns about the high concentrations of antibiotic-resistant bacteria in agricultural animals resulting from the therapeutic and growth-promoting use of antibiotics in animal production.

Table 25.1. Some human pathogens potentially present in animal wastes

Pathogen group	Examples
Viruses	Hepatitis E virus (swine), reoviruses, rotaviruses, adenoviruses,[a] caliciviruses,[a] influenza viruses (orthomyxoviruses)[a]
Bacteria	*Salmonella* spp., *Campylobacter* spp., *Escherichia coli*,[b] *Yersinia enterocolitica*, *Leptospira* spp., *Listeria* spp.
Protozoa	*Cryptosporidium parvum*, *Giardia lamblia*, *Toxoplasma gondii*
Helminths	*Ascaris suum*, *Taenia solium*, *Fasciola hepatica*

[a] While certain of these viruses may have originated from animal reservoirs, there is no evidence to suggest that they are commonly maintained in animal reservoirs and present in animal wastes. Humans and animals (including swine) usually have distinct strains of these viruses.

[b] Some strains of these bacteria are non-pathogenic, and others are pathogenic. The extent to which pathogenic strains occur in animal wastes varies with the animal species and other factors.

25.2.1 Pathways for pathogen movement on and off farms

Pathogens from animal manures and other wastes have the potential to contaminate water, land, and air if containment and treatment do not adequately manage the wastes. Pathogens are capable of persisting for days to weeks to months, depending on the pathogen, the medium, and the environmental conditions. Many treatment and management systems for animal manure are based on the principle of no discharge and the recycling of manure constituents on the farm. However, off-farm movement or transport of animal waste pathogens has occurred via water, air, and other media and is an infectious disease concern within the animal industry. Pathogen contamination of farm workers is also possible, and infection of farm workers can lead to further transmission of pathogens to family members and other contacts.

25.3 ANIMAL WASTE TREATMENT PROCESSES

According to US EPA (1999), there are six primary processes that can be employed to treat animal waste to reduce pathogen loads prior to land application or off-site transfer: composting, air drying, facultative lagoons/storage, anaerobic digestion, aerobic digestion, and lime stabilization.

25.3.1 Composting

Using either the within-vessel, static aerated pile or windrow composting methods, the temperature of the manure is raised to 40 °C or higher for 5 days. The temperature in the compost pile must exceed 55 °C for 4 h during the 5-day period. Where composting with turning and mixing of manure to elevate temperatures (at least 55 °C) is possible, 1 month of storage time is necessary.

25.3.2 Air drying

Drying of animal manure is a widely practised management approach in certain locations. However, little is known about the extent to which pathogens are inactivated in manure drying processes or during dry storage, because there have been few, if any, studies to document their effectiveness. Desiccation or drying to very low moisture levels (<1%) has been shown to result in >4-log inactivation of pathogens in municipal biosolids and in soils. Therefore, studies are recommended to determine the rate and extent of pathogen inactivation in drying and desiccation processes for animal manure.

25.3.3 Facultative lagoons/storage

Animal manure is treated or stored in a lagoon that is 0.3–0.9 m deep and has a large enough surface area to become naturally aerated so that cyclic aerobic and anaerobic conditions occur.

25.3.4 Anaerobic digestion

Anaerobic digesters can be covered, partially covered, or uncovered earthen or concrete units. They provide storage of animal waste while anaerobically decomposing and liquefying manure solids. End-products are in the forms of liquid supernatant, semisolid sludge, and waste gases. Anaerobic digesters work better in warm climates, where biological activity continues most of the year.

Anaerobic digesters that are totally enclosed with controlled temperatures can be used to produce biogas and reduce pathogens, but are difficult to justify due to high capital costs, high management requirements, and a lack of incentives for using the systems. Covered lagoons and anaerobic digesters can significantly reduce odours and releases of unwanted gases. Anaerobic digesters represent a more sophisticated waste disposal system than facultative lagoons, and the use of this technology has increased significantly in recent years as more large-scale animal production units are being built. Capture of methane for use as an energy source can be incorporated in the design of these systems.

25.3.5 Aerobic digesters

Animal manure is agitated with air or oxygen to maintain aerobic conditions. Mechanically aerated lagoons are sometimes used. Auto-thermal thermophilic aerobic digestion, biofilm reactors, sequencing batch reactors, and combinations of anoxic and aerobic treatments offer advantages of odour reduction and waste degradation and stabilization. Additional research is needed to optimize these systems for nutrient reduction, pathogen destruction, and energy use.

25.3.6 Lime stabilization

Lime or other alkaline treatment is used to bring animal wastes to a pH of 12 for at least 2 h of contact. Alkaline treatment has been highly effective in reducing pathogens in municipal biosolids, and promising results have been obtained when it has been applied to animal biosolids. Studies are needed to better characterize pathogen inactivation by alkaline treatments of animal biosolids with respect to solids composition, pH, and storage and handling conditions.

25.4 EFFICIENCY OF ANIMAL WASTE TREATMENT SYSTEMS IN PATHOGEN REDUCTION

The ID_{50} has not been precisely measured for the majority of waterborne pathogens. However, for many of these pathogens, a single organism may be sufficient to cause infection; for example, in the majority of recreational water-associated cases of *E. coli* O157:H7 infection, the organism has not been isolated from the water or has been recovered in very low numbers (Ackman *et al.* 1997; Bruce *et al.* 2003). Certain strains of *Cryptosporidium parvum* have also been shown to have a low ID_{50} (10^1–10^2 oocysts), particularly for the immunocompromised (Chappell *et al.* 1999; Messner *et al.* 2001). The consumption of just one embryonated helminth egg may be sufficient to cause infection.

Young animals are more likely than adult animals to shed pathogens, and they usually shed pathogens in higher levels in their faeces; for example, *E. coli* O157:H7 is shed by calves at levels ranging from 10^2 to 10^6 per gram of faeces, whereas the organism is rarely isolated from the faeces of adults (Zhao *et al.* 1995; Gannon *et al.* 2002; Shere *et al.* 2002). Sturdee *et al.* (2003) reported that more than 50% of farm-reared calves in one study area in England shed *Cryptosporidium parvum* oocysts at an average of 10^5/g. Therefore, wastes from young animals such as calves are more likely to contain high levels of these and other zoonotic pathogens. There may be some benefit in treating wastes from certain ages of animals and certain animal species differently and not mixing manure from a number of sources during storage and disposal (Dampney *et al.* 2002).

Many of the animal waste treatment procedures in common use operate at or below 35–45 °C (mesophilic) and in most cases are unlikely to reduce pathogen levels by more than 1–2 logs (Table 1 in Sobsey *et al.* 2003; Lorimor *et al.* 2003). In Table 25.2, examples of four swine waste treatment systems from a finishing swine operation over a 12-month period are presented. Of the four systems listed, the covered lagoon provided the greatest reduction of indicator bacteria (3.6-log reduction for faecal coliforms and *E. coli*) and bacteriophages (3.0-log reduction). Given these limitations, it would be necessary to use several treatment reactors or processes in series to reduce the high pathogen levels that may be encountered in the faeces of young animals to values approaching zero.

Some of the most promising technologies for reducing pathogens in animal wastes are anaerobic digestion and composting. They are reported to reduce the levels of certain pathogens such as *E. coli* O157:H7 by more than 10^4 (Jiang *et al.* 2003a; Sobsey *et al.* 2003). However, in order to achieve these and even greater levels of pathogen reduction, thermophilic conditions (45–70 °C) must be maintained during composting or biogas digestion (Sahlstrom 2003). Jiang *et al.* (2003a, 2003b) reported that 10^7 colony-forming units of *E. coli* O157:H7 per gram can be eliminated in compost if the temperature is above 50 °C for at least 1 week.

Ascaris suum and other helminth eggs are very resistant to environmental conditions and are more difficult to inactivate than other microbial pathogens. However, they are also inactivated over time by desiccation (Gaasenbeek and Borgsteede 1998), thermophilic waste treatment processes such as composting and anaerobic digestion (Plym-Forshell 1995; Vinneras *et al*. 2003), and lime treatment (Ghiglietti *et al.* 1995).

Table 25.2. Comparison of pathogen $\log_{10}$ reductions for various swine waste lagoons

	Covered lagoon/ storage pond	Conventional anaerobic lagoon/storage pond	Single cell anaerobic lagoon (ozone injection)	Single cell anaerobic lagoon (finishing operation)
Faecal coliforms	3.6	2.6	2.0	1.6
Escherichia coli	3.6	2.7	2.0	1.5
Salmonella spp.	2.7	2.4	0.8	1.8
Clostridium perfringens spores	2.5	1.7	0.7	0.8
Somatic coliphages	3.0	2.5	1.9	1.4
F-specific coliphages	3.0	3.1	1.4	1.1

25.4.1 Pathogen reductions by manure treatment and management processes

Estimated pathogen reductions in animal manures are summarized in Table 25.3. The reductions of some pathogens by some animal waste treatment processes have been determined in laboratory and pilot-scale field studies. In general, thermophilic processes, such as pasteurization, thermophilic digestion, and composting, are capable of producing extensive (>4 log) pathogen inactivation; therefore, the resulting treated residuals are likely to contain only low pathogen concentrations. Further studies are recommended to better characterize pathogen inactivation in thermophilic processes for manure treatment and to define the optimum conditions to achieve extensive pathogen reductions.

25.5 APPLICATION TO FIELDS

It is evident from the discussion above that animal wastes spread on fields that result from current storage and treatment procedures are likely to contain zoonotic pathogens. A variety of physical (sunlight, high and low temperature, desiccation, dilution, pH) and biological factors (antimicrobials produced by microbiota, predation, bacteriophages, nutrient competition) reduce microbial pathogen concentrations in soil over time. However, it is known that *Cryptosporidium parvum* oocysts, enteric bacteria such as *E. coli* O157:H7, and helminth ova are very hardy and can remain viable for many months in moist soils (Feachem *et al*. 1983; Olson *et al.* 1997; Jenkins *et al.* 1999; Roepstorff *et al.* 2001; Jiang *et al.* 2002; Ogden *et al.* 2002). Protection of surface waters and wells depends heavily on preventing the entry of animal wastes. Therefore, efforts should be made to limit transport of the animal wastes and contaminated soil by wind and water from the locations where these wastes have been applied.

A variety of approaches can be used to prevent or at least diminish the transport of manure and soils contaminated with zoonotic pathogens from fields to waterways. These strategies include measures to minimize tillage, delay of seed bed preparation, strip cropping, and maintenance of plant cover or plant residue to stabilize the soil (Dampney *et al.* 2002). Liquid manure can be injected directly into the soil. In addition, the use of annual or perennial vegetation at the borders of cultivated fields or streams, contour farming, and the use of diversion canals across slopes or diversion ditches with reed beds can limit transfer of soil and animal wastes associated with precipitation. Equipment with low trajectory should be used in spreading manure on land to prevent pathogen-contaminated aerosols from drifting into adjacent pastures or watercourses.

Table 25.3. Summary of animal waste treatment processes and estimated pathogen reductions (from Sobsey *et al.* 2003)

Treatment process	Estimated pathogen reduction (log_{10})	Comments
Physical processes		
Heat/thermal processes		
- Mesophilic	Typically 1–2	Depends on temperature, pathogen, contact time, pH, etc.
- Thermophilic	Typically >4	Depends on temperature, pathogen, contact time, pH, etc.
Freezing	Variable	Depends on temperature, pathogen, contact time, pH, etc.
Drying or desiccation	Typically >4 at <1% moisture; typically <1 at 5% moisture	Depends on pathogen, contact time, pH, etc.
Gamma irradiation	Typically >3	Varies with pathogen, dose, waste, etc.
Chemical processes		
High pH (>11)	Inactivation at high pH, e.g., alkaline/lime stabilization; >3–4	Varies with pathogen, contact time, pH, etc.
Low pH (<2–<5)	Inactivation at low pH, acidification; typically <2	Depends on pathogen, contact time, pH, etc.
Ammonia	Inactivation at higher pH where NH_3 predominates	Varies with pathogen, contact time, pH, other waste constituents
Biological processes		
Aerobic, mesophilic	Typically 1–2	Varies with pathogen, solids separation, contact time, reactor design, temperature
Aerobic, thermophilic (composting)	Typically >4	Depends on pathogen, solids separation, contact time, reactor design, mixing methods, temperature
Anaerobic, mesophilic	Typically 1–2	Depends on pathogen, contact time, reactor design, solids separation, temperature
Anaerobic, thermophilic	Typically >4	Depends on pathogen, contact time, reactor design, solids separation, temperature

25.6 POLICIES ON ANIMAL WASTES

Animal wastes have been referred to as "multidimensional" in nature (Zilberman *et al.* 2003), to emphasize that they are considered a renewable resource and valuable commodity by certain stakeholders and as a dangerous pollutant by-product of the agriculture industry by others. Treated animal wastes can improve the fertility of soil and help reduce chemical fertilizer and fossil fuel consumption. They can also be used in the production of energy from the methane that is generated during their anaerobic decomposition. However, problems with animal wastes include nutrient pollution (nitrogen and phosphorus), greenhouse gas emissions, unpleasant odours, and the fact that they act as a source of contaminants, such as zoonotic pathogens. To further complicate matters, a particular set of strategies may need to be used to minimize nitrogen and phosphorus contamination of the environment, others to minimize greenhouse gas emissions, and others to get rid of contaminants such as pesticides, drug residues, and microbial pathogens. The strategies intended to solve one set of problems may not be suitable for solving others; for example, transport of manure from one region to another to prevent accumulation of nitrogen and phosphorus may assist in spreading zoonotic microbial pathogens in animal populations. Conflicting agricultural strategies such as these are likely to have led to the meat and bone meal-associated bovine spongiform encephalopathy (BSE) outbreak in the United Kingdom.

Several possible solutions have been proposed to introduce technologies necessary to diminish agricultural water pollution. These schemes hope to satisfy environmental and public health concerns and at the same time maintain an economically viable agriculture industry. One proposal is to pass costs for violation of environmental laws onto the purchasers of the agricultural products (Zilberman *et al.* 2003). Another approach would be to provide "green" payments and other incentives for environmental services provided by farmers in animal waste control. However, the latter scheme would not be expected to be accepted in countries that could not compete in an agricultural "subsidy war."

Research must be supported to find cost-effective mechanisms to store, treat, and dispose of animal wastes that protect environmental quality and public health. The determination of the allowable level of a target organism from economically feasible animal grazing and manure treatment and management processes that will result in acceptable health risks is a major international need.

25.7 WASTES FROM DEAD ANIMALS AS SOURCES OF WATERBORNE INFECTIOUS AGENTS

Excreta from live animals are not the only source of waterborne infectious agents. Wastes from animals at slaughter (in abattoirs), on-farm deaths (from sporadic cases to nationwide die-offs), and the mass culling of infected animals are also potential sources of waterborne zoonoses. Abattoir wastes can contribute to the transmission of zoonotic diseases in several ways:

- Pathogens in the digestive contents, blood, or other contents can directly infect workers through inhalation of contaminated aerosols, accidental ingestion of contaminated particles, or contact with skin wounds (e.g., *Mycobacterium bovis*) (Department for Environment, Food and Rural Affairs 2003).
- Improper hygiene or operating procedures can lead to cross-contamination of meat products and subsequent ingestion by humans (or animals) (e.g., *E. coli* O157:H7; see Armstrong *et al.* 1996; Elder *et al*. 2000; Barkocy-Gallagher *et al*. 2001).
- Discharge or release of inadequately treated wastewater or solid wastes, such as gastrointestinal contents or animal carcasses, into the environment can lead to contamination of water sources (Sangodoyin and Agbawhe 1992).

Abattoirs and meat processing require large amounts of water, as shown in Table 25.4. The amount of waste produced in an abattoir can be very high (approximately 35% of the animal weight) (World Bank 1998). Wastes may include components that have high concentrations of pathogens — especially the contents of the intestine. Moreover, in wastes that are improperly stored or not immediately treated, pathogen numbers may actually increase. For example, Hepburn *et al*. (2002) found that *E. coli* O157:H7 could increase by 1–2 logs in ovine and bovine gut contents at temperatures ranging from 15 to 30 °C.

Table 25.4. Water use in the meat industry (from World Bank 1998)

Process/animal	Water use (m^3/tonne of product)
Pigs	1.5–10
Cattle	2.5–40
Poultry	6–30
Meat processing	2–60

Examples of practices that could lead to disease transmission include land disposal of inadequately treated abattoir wastes. Prior to the BSE outbreak in the United Kingdom, untreated cow and sheep blood was often used for fertilizer on grazing land (UK Government 2000). Each slaughtered cow produces 13.6 kg of blood, which needs to be disposed of safely. In the United Kingdom, the time between application of the wastes and allowing animals to graze was frequently only 4–6 weeks, instead of the recommended 4–6 months (UK Government 2000).

Improper disposal of the gut contents has the same potential as animal excreta for pathogen discharge into the environment (bacteria: see Borch *et al*. 1996; *Cryptosporidium*: see McEvoy *et al*. 2003). For every tonne of carcass weight, a slaughterhouse (beef) produces 5.5 kg of manure (not including rumen contents or stockyard manure) and 100 kg of paunch manure (partially digested feed) (Verheijen *et al*. 1996).

25.7.1 Control of disease transmission

Preventing disease transmission to abattoir workers or during mass culls of infected animals requires worker safety training, provision and use of appropriate personal protective clothing, and good hygienic practices.

Reducing contamination and cross-contamination during the processing of animals is best implemented within a hazard analysis and critical control point framework (Borch *et al*. 1996). Reducing faecal contamination of carcasses and subsequent cross-contamination of meat products includes developing and implementing processes for skinning, defeathering, and scalding; proper handling of organs with high microbial concentrations, such as intestines, tongue, pharynx, and tonsils; good cleaning and disinfection practices; and specific steps, such as scalding, organic acid washes, carcass pasteurization procedures, product temperature control, and irradiation, to reduce microbial hazards.

To reduce the potential for waterborne transmission, effective wastewater and water treatment technologies are also required (see chapter 26). Steps to minimize the use of water will also reduce the amount of contaminated wastewater produced. For example, dry animal pen cleaning and removal of manure from stockyards and during intestine processing without water will decrease the amount of contaminated wastewater (Verheijen *et al*. 1996; World Bank 1998). The elimination of wet transport (pumping) of wastes such as intestines will also reduce water consumption, risks associated with abattoir wastewater, and wastewater treatment costs (World Bank 1998).

25.7.2 Die-offs and mass culling of infected animals

Massive die-offs of animals from outbreaks of diseases such as anthrax in bovids and botulism in waterfowl are well known. In addition, mass culling of animals may be initiated by regulatory authorities as part of eradication or test and slaughter programmes to control the spread of specific infectious agents in animal populations (see chapter 24). These mass culling campaigns appear to be occurring with increasing frequency, and the numbers of animals involved are quite large. Several million animals were culled in the February 2001 outbreak of foot-and-mouth disease in the United Kingdom (Zinsstag and Weiss 2001), and nearly a million pigs were destroyed in the 1999 outbreak of Nipah virus in Malaysia (Nor 1999). Improper handling and disposal of culled animals could lead to contamination of water sources and potentially to transmission of disease to humans or other animals. Procedures for safely disposing of animal carcasses have been developed for a variety of infectious agents and may include mass burial and slaking carcasses in quick lime, incineration, rendering, or other techniques, depending on the type of outbreak. Special care should be taken during disposal to avoid contamination of water sources (e.g., burial at sufficient depth, in non-porous soil, and distant from groundwater and surface waters).

Disposal of animals potentially infected with BSE or other transmissible spongiform encephalopathies (TSEs), such as chronic wasting disease (CWD), poses a significant challenge. The infectious agents responsible for these TSEs are thought to be prion proteins. These altered host proteins are extremely resistant to processes normally used to inactivate microbial pathogens, such as low levels of conventional disinfectants, high temperatures, and enzymes that destroy proteins. Therefore, these agents are likely to persist in the environment and survive rendering, wastewater treatment, and water treatment. It has been reported that high concentrations of sodium hypochlorite and sodium hydroxide with moist heat (100 °C) for 1 min are required to inactivate BSE infectivity (Taylor 2002). For some pathogens (e.g., CWD), safe disposal techniques for carcasses have not yet been developed (USDA 2002).

Despite the highly resistant nature of prions, there are several arguments that suggest that there is minimal human health risk associated with the BSE agent in drinking-water (Gale 1998; see also chapter 24).

25.8 SUMMARY AND CONCLUSIONS

1. In grazing systems (including some mixed farming systems), transport of animal wastes and soil contaminated with zoonotic pathogens from pastures occurs most frequently during periods of heavy precipitation. Controlling animal density, limiting access of animals to surface water, and providing

"buffer strips" of vegetative cover are critical in preventing water contamination with these pathogens.

2. In industrial livestock systems and frequently in mixed farming systems, animal wastes are collected, stored, and sometimes treated prior to disposal on cropland. Manure from young animals may contain high levels of certain zoonotic pathogens. These pathogens can survive for months and perhaps even years in wastes applied to fields and in contaminated soil. Procedures such as composting and anaerobic digestion at high temperatures have been shown to be very effective in reducing the levels of zoonotic pathogens in animal wastes. However, these treatments are not commonly used because of economic considerations. Livestock producers must be provided with incentives to use effective waste treatment procedures. As in grazing systems, efforts must also be made to limit the transport of animal wastes and contaminated soils from fields into surface waters.

3. Regulatory authorities are tasked with increasing frequency with the disposal of large numbers of dead animal carcasses following massive culling operations used to control or eradicate specific animal diseases (e.g., BSE in the United Kingdom) or die-offs from animal disease outbreaks (e.g., avian botulism). These carcasses may be a source of zoonotic pathogens that could find their way into groundwater and surface water. Proper disposal of these dead animal carcasses and liquid and solid wastes from slaughterhouses often presents technical and regulatory challenges.

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26

Control of zoonotic diseases in drinking-water

J.L. Cicmanec, J.E. Smith, Jr., and R. Carr

26.1 INTRODUCTION

For over a century now, the process of providing hygienically safe drinking-water has focused on utilizing treatment processes as one of the significant steps to provide barriers to the passage of infectious disease-causing organisms to humans. This concept, referred to as the "multiple barrier" concept, is often considered to be the cornerstone of sanitary engineering; it is based on the premise that all of the steps and processes are interdependent and begin with source water protection and end with providing residual disinfectant for the water distribution system and safe transport to the consumer's tap.

Since there are many microbial agents that may be of concern to a drinking-water treatment system, and since it is recognized that developing a monitoring

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system for each of these would be practically impossible, prevention and multibarrier protection through treatment are the basics for successfully operating public drinking-water systems. Hazard analysis and critical control point and water safety plan approaches are also now being applied to determine the critical processes within the system where contamination can be measured and prevented. Some of the existing process control measures already in use in drinking-water treatment plants employ these principles. The basic feature to be emphasized is that many non-microbial assays, such as turbidity readings, particle counts, pressure drop, and disinfectant residuals, are currently being recorded at many facilities. These steps are followed since there is a need to restrict limited laboratory resources to critical monitoring points so that the results provide the greatest information and benefit.

This chapter briefly summarizes the conventional drinking-water processes and provides supporting data and examples where necessary.

26.2 COAGULATION/FLOCCULATION/ SEDIMENTATION

Coagulation is the process for promoting the interaction of small particles to form larger particles. The physical processes of coagulation, flocculation, and sedimentation occur during the solid–liquid separation when particles settle out under the force of gravity. Microbial agents (protozoa, bacteria, and viruses) act as particles in the coagulation and flocculation processes, and they tend to sorb to the settling particles and are thus removed. Excellent reviews of these processes are available (Letterman and Cullen 1985; Gregory *et al.* 1999). As well as testing for changes in turbidity and oocyst counts, researchers have also found that indigenous bacterial endospores can provide useful information regarding the removal of *Cryptosporidium* oocysts (Rice *et al.* 1996). In general, enhanced coagulation had the capacity to remove greater than 2 logs of *C. parvum* and endospores, and the optimized coagulant prepared especially for the removal of total organic carbon could remove more than 3 logs of *C. parvum* oocysts.

26.3 FILTRATION

With proper design and operation, filtration can serve as a consistent and effective barrier for microbial pathogens. In order to remove viruses, bacteria, and protozoan cysts, the filtration system must be able to remove particles that range from 10^{-5} to 10^{-1} mm in diameter. After a period of operation, when the head loss increases, the filter must be cleaned by backwashing. During the initial

period after backwashing, the filters perform poorly, and this is considered the ripening period. Passage of infectious agents during this period can be significant, so most plants stagger the backwash procedure for the various filtration units in their drinking-water treatment plant to at least dilute the lower-quality water being released. Other methods that are used to compensate for this initial poor performance are discarding the initially treated water, slow-start sequencing, or adding a filter aid to the wash water supply. Removal of microbial pathogens by filtration does not rely simply on physical straining. Instead, the process includes two steps: transport of particles from suspension to filter medium and then attachment of particles to the medium (Yao *et al.* 1971). Process variables such as the size and density of microbes, size and surface charges on the organisms and coagulant particles, depth of the filter medium, and filtration rate affect transport efficiency (Swertfeger *et al.* 1999). Unfavourable interactions between particles and filter medium must be avoided so that the particles can attach to the filter medium. Chemical coagulation prior to filtration is used to destabilize particles and is the most important factor for determining filtration efficiency. When rapid rate filtration is used independently as a simple strainer, it does not remove microbial pathogens very efficiently. On the other hand, slow sand filters can be very effective in removing microbial contaminants from water.

In a study by Ongerth (1990), it was determined that without chemical pretreatment, the removal of *Giardia* cysts averaged only 0.6 logs for conventional treatment and 0.44 logs for in-line filtration; when chemical coagulation was employed, the removal rates increased to 1.7 logs for conventional treatment and 1.17 logs for in-line filtration. Swertfeger *et al.* (1999) tested both single-medium and dual-media pilot-scale systems and found a 2.7-log removal for *Cryptosporidium parvum* oocysts, and there was no difference between winter and summer operations. By contrast, the removal of *Giardia* cysts, which are larger, was at least 4.4 logs, and more efficient removal was observed in summer than in winter.

26.3.1 Membrane processes

With regard to water treatment, certain types of membranes were originally considered for the removal of disinfection by-products (DBPs), as well as for the removal of very small particulates and pathogens. Reverse osmosis membranes are very tight membranes with molecular weight cut-offs below 200 daltons. They are typically used to remove salts from seawater, and, due to their tight membrane structure, they are operated at very high pressure (10–100 bar). Moving up the scale, next are nanofiltration membranes, which are generally considered to remove particles in the 200–1000 daltons range. They have been

designed to remove divalent cations. Nanofiltration membranes have been found to remove most DBPs, and they can be operated at lower pressures (5–9 bar). Ultrafiltration and microfiltration membranes are typically used only for the removal of particulates and pathogens. They remove particles in the 1000–500 000 daltons range. It is generally thought that ultrafiltration will remove viruses, but microfiltration will not.

26.4 DISINFECTANTS

Reagents such as chlorine, ozone, and potassium permanganate that work primarily as oxidants are commonly added early in the treatment process to maximize the contact time and to oxidize contaminants for subsequent removal during the treatment process. Oxidants also have special advantages for the removal or inactivation of undesirable taste and odour contaminants, although some odorous chemicals can be formed during treatment with chlorine (chlorophenols) or ozone (aldehydes) if the appropriate precursors are present.

Disinfection is a necessary component of most water treatment plants, especially those using surface waters, because granular filtration media alone do not remove most microbial pathogens from water. The principal factors that influence disinfection efficiency are the disinfectant concentration, contact time, temperature, and pH (depending upon the disinfectant). For example, chlorination is much more effective at neutral to acidic pH as opposed to basic pH, at which the predominant species is hypochlorite rather than hypochlorous acid. The practical application of the disinfectant concentration multiplied by contact time ($C \times T$) concept is useful for understanding and expressing disinfection kinetics and designing and operating systems. Other factors that may influence disinfection are encapsulation of organisms, aggregation, attachment to surfaces, and low nutrient growth.

Escherichia coli O157:H7, a highly virulent strain of a coliform that most commonly originates from cattle faeces, has emerged as a significant waterborne pathogen. Chlorination efficacy studies were conducted using seven environmentally isolated strains, with four wild-type *E. coli* strains shown for comparison (Table 26.1). At a relatively moderate treatment level of 1.1 mg of free chlorine per litre, pH 7.0, and a temperature of 5 °C, all strains were inactivated by 4.5 logs or greater within 120 s of exposure (Rice *et al.* 1999).

Campylobacter jejuni and *Arcobacter butzleri* are two among many other enteric pathogens that have also been associated with waterborne outbreaks. Blaser *et al*. (1986) examined the susceptibility of three strains of *C. jejuni* and found a 4-log reduction of bacterial counts after 1 min of exposure at a chlorine concentration of 0.1 mg/litre at pH 6 and 4 °C.

Table 26.1. Chlorine inactivation of *Escherichia coli* O157:H7 and wild-type *E. coli* (from Rice *et al*. 1999)

Isolate	Log_{10} CFU/ml[a]				Inactivation rate (s^{-1})	R^2
	Initial inoculation	Exposure time				
		30 s	60 s	120 s		
E. coli O157:H7						
N009-6-1	5.63	2.60	1.88	0.82	-2.96	0.82
N6001-8-10	5.78	2.52	1.44	0.72	-3.06	0.68
N6021-5-1	5.78	2.54	1.52	0.66	-3.06	0.54
N60049-26-1	5.68	2.35	1.40	0.54	-3.00	0.86
N6059-7-2	5.72	2.42	1.74	0.86	-3.02	0.72
N6104-5-9	5.62	2.40	1.69	0.72	-2.96	0.89
N6114-7-2	5.63	2.52	1.66	0.89	-2.96	0.82
Mean	5.69	2.48	1.62	0.74	-2.93	0.82
E. coli (wild type)						
A	5.53	2.66	1.80	1.52	-2.51	0.61
B	5.79	2.60	1.48	0.81	-2.68	0.60
C	5.68	2.48	0.92	0.84	-2.61	0.61
D	5.52	2.34	0.95	0.39	-2.50	0.61
Mean	5.63	2.52	1.28	0.89	-2.93	0.71

[a] CFU = colony-forming unit.

26.4.1 Chlorine

Chlorine is the most frequently used halogen disinfectant for the treatment of drinking-water in the USA. The use of chlorine has a long history in water treatment (Butterfield *et al.* 1943), and it has been used successfully in both drinking-water and wastewater applications. In fact, data for chlorine inactivation of various organisms are often used as the benchmark for establishing a specific microorganism's resistance to disinfection.

In terms of resistance to chlorine inactivation, animal viruses and bacteriophages are considered to be more resistant than vegetative bacterial cells. Hoff (1986) summarized his research on chlorine inactivation of poliovirus under oxidant demand-free conditions. At 5 °C, a 2-log reduction in viral counts occurred with a free chlorine residual of 0.6 mg/litre during 2 min of exposure.

Using *in vitro* excystation to determine cyst viability, Jarroll *et al.* (1981) determined that *Giardia* cysts were relatively resistant to chlorine inactivation. In their studies, they found that in order to obtain a 2-log reduction, at least a 10-min exposure with a free chlorine concentration of 1.5 mg/litre at pH 6 and 15 °C was

required. Data summarizing the effects of chlorination upon faecal coliforms are presented in Figure 26.1.

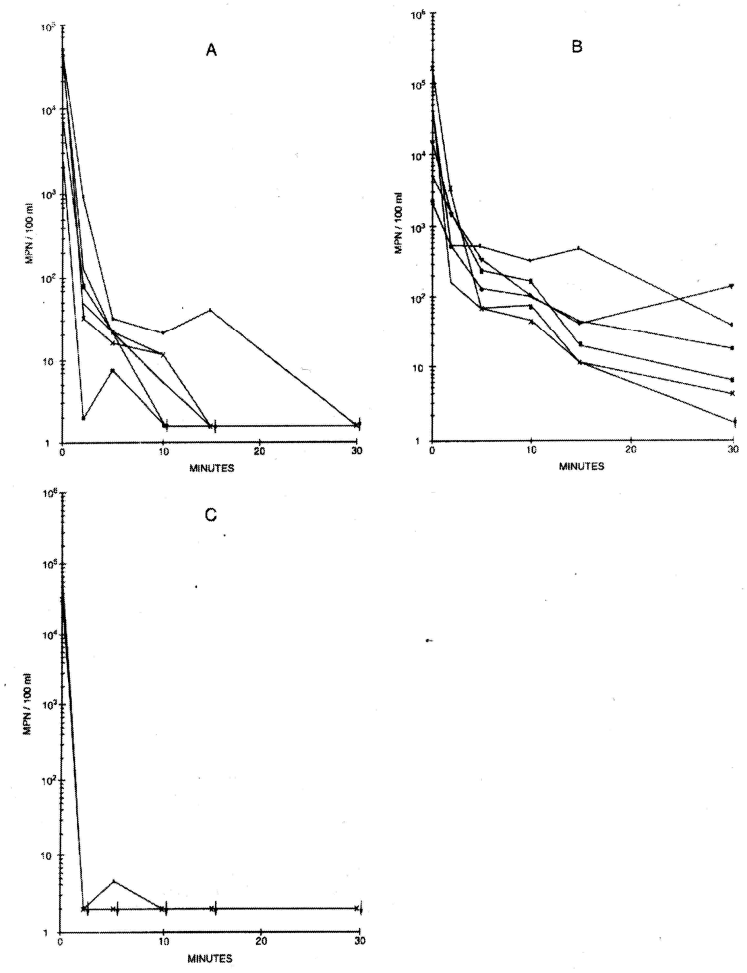

Figure 26.1. Effect of 0.5 mg chlorine on inactivation of coliforms in sewage effluent particles.

26.4.2 Chloramine

Chloramination of drinking-water has gained popularity because of concerns about the hypothetical risks of long-term consumption of chlorinated DBPs. Monochloramine is considered a weak biocide for most applications in comparison with free available chlorine, requiring exposure times 25–100 times longer than with chlorine to achieve comparable inactivation. Many municipal drinking-water treatment plants add ammonia to their finished water as it is being sent out for distribution. The ammonia will react with residual free chlorine to produce chloramines, which, although not as potent as free chlorine, will survive longer during distribution due to their lower chemical reactivity. This role as a "secondary" disinfectant after the water has already been adequately treated is useful for preventing "regrowth" of microorganisms during transit and may perhaps suppress some contamination that might occur during distribution.

26.4.3 Chlorine dioxide

Chlorine dioxide exists as an undissociated gas dissolved in water in the pH range 6–9, and its disinfection efficiency increases within this range with increasing pH. Because it exists as an undissociated gas, this oxidant is more vulnerable to volatilization than free chlorine or monochloramine. Chlorine dioxide is a potent disinfectant and is generally considered to have a biocidal efficacy comparable to or somewhat greater than chlorine under some conditions (Hoff 1986).

26.4.4 Ultraviolet light

A survey in 1992 (Kruithof *et al.* 1992) indicated that there were 500 drinking-water treatment facilities in Switzerland and 600 facilities in Austria that employed ultraviolet (UV) light in 1955 and 400 facilities in Norway using UV light as a disinfectant in 1975. The total capacity of these plants was 45 million litres per day. UV disinfection is becoming much more popular, since it has been shown to be very effective for inactivating cryptosporidia. The dosage of UV energy is expressed in units of $mW \cdot s/cm^2$. In order to achieve a 4-log reduction, 30 $mW \cdot s/cm^2$ is needed for *Bacillus subtilis* and *Salmonella typhi*, 10 $mW \cdot s/cm^2$ is need for *Cryptosporidium parvum* and *Giardia lamblia*, and 50 $mW \cdot s/cm^2$ is needed for MS-2 coliphage, the prototypic virus. Because of possible matrix effects, the treated water should have a turbidity reading less than 1 NTU, and it is essential that the output and energy transport are properly monitored to ensure sufficient biocidal activity. The principal advantages of UV treatment over chemical disinfectants are shorter contact time, its effectiveness

for *Cryptosporidium* and *Giardia*, and the lower production of DBPs. However, it does not provide residual protection within the water distribution system, so a secondary disinfectant would normally be required for water distribution.

26.5 SPECIAL CONCERNS FOR GROUNDWATER SOURCES

Groundwater is often reasonably protected from surface contamination and has undergone a measure of natural filtration during its passage and storage in the ground.

Bank filtration is a groundwater simulation process that has provided drinking-water of reduced bacterial loads in some situations when surface water is the proximate source of raw water. Bank-filtered water is the product of surface water seeping or being pumped from the bank or bed of a river or lake into subterranean locations. During ground passage of the water, the quality changes due to microbial, chemical, and physical processes and by mixing with the indigenous groundwater. The advantages of bank filtration are that it is a pretreatment step that can reduce water turbidity and enhances the removal of particulates in appropriate geological circumstances.

Schijven and Reitveld (1997) measured the removal of male-specific coliphages, enteroviruses, and reoviruses at three infiltration sites and then compared the measured values with those predicted by a virus transport mode. They found a 3.1-log reduction of bacteriophages within 2 m and a 4-log reduction within 4 m of fine dune sand. Phages were reduced 6.2 logs over 30 m of sandy soil, and enteroviruses and reoviruses were reduced to below detection limits over the same distance.

26.6 SPECIAL CONCERNS REGARDING AFO FACILITIES

Uncontrolled effluents from animal feeding operations (AFOs) can directly contaminate surface waters, and they can also migrate through overlaying soil and contaminate groundwaters. Should a spill or release occur from an AFO located upstream from a municipal drinking-water treatment plant, additional treatment measures may be necessary on a temporary or permanent basis to adequately handle the increased pathogen load. Measures such as filtration, ozonation, additional treatment with UV radiation, and holding untreated water in constructed lakes should be considered. The ability to use such measures is dependent upon prior planning and installation of these systems. However, in view of the serious health threat that can result from an accidental spill, it would

be reasonable that such measures be considered in the construction of new drinking-water treatment facilities or renovation of existing facilities that are downstream from AFOs.

26.7 DISTRIBUTION SYSTEMS

The distribution system is a key factor in the transmission of waterborne zoonoses. Failures in the distribution system — for example, through cross-connections or backflow — are responsible for a large proportion (as much as 37%) of waterborne disease outbreaks in some developed countries (Payment and Robertson 2004). For example, contamination of a water supply due to water main breakage and the repair of water meters in a small town in Missouri, USA, was a primary cause of an *E. coli* O157:H7 outbreak that affected 243 people in 1989 and 1990 (Swerdlow *et al*. 1992). A giardiasis outbreak at a campsite in Arizona, USA, was attributed to a cross-connection between a sewage pipe and the drinking-water distribution system (Starko *et al*. 1986).

Ingress of waterborne zoonotic pathogens into the distribution system can be controlled in two ways: through good design of the system and through appropriate management. For a more thorough discussion of managing microbial water quality in distribution systems, see Ainsworth (2004).

26.7.1 Design of distribution systems

Distribution systems need to be designed to handle peak water flow volumes (of which only a small percentage is used for drinking-water). At times of reduced water demand, low-pressure zones may be created that permit the entry of contaminants from the external environment. The network should be designed to minimize the potential for ingress of contamination, reduce water transit times, and maintain the appropriate level of residual disinfectant until the water reaches the tap. In large networks, it may be necessary to plan for pressure boosting at strategic locations and disinfectant booster (relay) dosing (Chambers *et al.* 2004).

Distribution pipes should be sited in appropriate locations (e.g., not immediately adjacent to sewer pipes or in septic tank fields), in the appropriate type of soil, and at a depth that reduces the potential for freezing and thawing. Pipes should be well constructed of suitable materials that reduce the potential for biofilm formation (approved materials may be specified by national approval schemes or by other certifying agencies, such as the National Sanitation Foundation in the USA). Other design considerations include eliminating low-flow dead ends or loops where water can stagnate and reducing the potential for cross-connections and backflow (Chambers *et al.* 2004).

26.7.2 Management of distribution systems

Distribution system management should take place within the context of a water safety plan (see chapter 5 and WHO 2004). Good practices to reduce the potential for transmission of disease through distribution systems involve standard maintenance procedures such as sanitary surveys to detect possible sources of contamination and cleaning, repair, and replacement of broken pipes, valves, etc. Special care needs to be taken during maintenance and repair of distribution systems to prevent the ingress of pathogens. For example, the *E. coli* O157:H7 outbreak described in section 26.7 most likely could have been prevented if hyperchlorination of the water supply had been used during the times when the repair work on the mains and water meters was being carried out (Swerdlow *et al*. 1992).

As discussed in section 26.7.1, it is necessary to maintain positive pressure in the distribution system to prevent contaminant ingress. This can be quite difficult, because distribution systems have to be designed for peak flow conditions (e.g., in the summer when water is also used to water gardens, fill swimming pools, wash cars, etc.). Maintaining adequate pressure may require additional pumping and the use of control valves during low-flow conditions (Chambers *et al.* 2004).

Optimizing water quality is also an important intervention in reducing the potential for transmission of waterborne zoonoses in the distribution system. Water quality parameters such as temperature, pH, oxygen, and nutrients affect the formation of biofilms, the effectiveness of disinfectants (including the length of time they will persist in the distribution system), and the rate at which pipes corrode. For example, high levels of organic carbon in the water facilitate the growth of microorganisms in the distribution system and react with chlorine to form toxic DBPs (Levi 2004). Some water quality parameters can be monitored on a real-time basis (e.g., chlorine residual in water throughout the distribution system) and thus provide data that can be used to make strategic management decisions to better protect public health in the event of system failure.

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Section VIII

Risk assessment and regulation

J.A. Cotruvo

Zoonotic microorganisms present perhaps the greatest past, present, and future risks to the safety of ambient water and drinking-water. Their worldwide distribution varies and is affected by climate and numerous natural and anthropogenic factors, and some microorganisms are emerging or re-emerging as disease risks. Some of the most significant risk factors include transboundary movements of people, food, and animals and increased intensive food and animal production, waste disposal, and cellular mutations, which ensure that the prevalence venues of these zoonotic microorganisms will continue to expand. Virtually every surface water source and many groundwaters are or will be vulnerable to one or more types of these ubiquitous microorganisms.

Analytical methodologies continue to improve our capabilities to detect and identify zoonotic microorganisms with greater specificity and rapidity and to trace them to their sources. Risk assessment is a useful tool that helps practitioners and regulators to determine the extent and magnitude of

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vulnerabilities and the consequences of exposure to specific zoonotic microorganisms in particular populations and to focus control actions at critical points in the chain. Nevertheless, public health protection ultimately requires strict reliance on the basic principles of prevention, traditional sanitary engineering applications, and multiple barrier protection. Hazard analysis and critical control points and water safety plan implementation are valuable anticipatory defensive systems for managing animal/food production facilities and public water supplies.

Regulation has the role of providing water quality and performance targets and ensuring that management practices and monitoring and treatment technologies will be installed and will function consistently. Controls and practices at the source, the feedlot and pastures, function by managing animal health to reduce the possibility that the animals are carriers or breeding grounds and shedding human and animal pathogens in their faeces. Waste collection and management practices reduce the potential for transport of contamination from human or animal sources to ambient waters and groundwaters that may be sources for drinking-water supplies. Filtration and disinfection technologies, when properly designed and applied, will control all known zoonotic organisms at the water treatment plant, but the goal is to utilize preventive management techniques at the source to reduce the challenge at the drinking-water treatment plant. Post-treatment recontamination can be prevented by continuous plant operation, residual disinfection, and distribution system design and maintenance.

Indicator organisms such as *Escherichia coli* and faecal coliforms and water quality measurements such as turbidity, although not perfect, are simple techniques that provide a high level of assurance of performance effectiveness of water treatment and barrier trains.

The ability to distinguish faecal organisms from human and animal sources will provide insights as to the origin of the microorganisms and source protection possibilities; in either case, however, prevention of contamination of the drinking-water will be the operative driving force.

27

A regulatory perspective on zoonotic pathogens in water

S.A. Schaub

27.1 INTRODUCTION

Human infectious diseases potentially associated with the faecal materials from a wide variety of animals are becoming a greater concern around the world. Humankind continues to rely on more and larger animal herds to feed its populations. In addition, humans are encroaching upon lands and waters once considered to be mainly animal habitats. These factors contribute to a greater opportunity for human exposures to pathogens from animal wastes. As a result, nations are confronted with greater impacts from historically prevalent diseases associated with animals and with new diseases entirely unknown even a few decades ago. While some waterborne pathogens that co-infect and cause disease in animals and humans have been around for decades or centuries (*Giardia lamblia*,

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Salmonella typhosa, *Ascaris*), others have recently found their way from animal populations to humans (*Cryptosporidium parvum*, *Escherichia coli* O157:H7, severe acute respiratory syndrome [SARS] coronavirus). Additionally, the close proximity of humans to animals and the customs or eating habits of certain populations have helped lead to co-evolution, jumping of host specificity, or genetic shifts in pathogen virulence factors. Humans are now becoming infected by pathogens that previously were confined to livestock or wild animals (Lassa and Ebola haemorrhagic fever viruses, hantavirus, *Mycobacterium paratuberculosis*).

27.2 NEED FOR REGULATORY APPROACHES TO CONTROL ANIMAL-BORNE PATHOGENS

Governments in economically developed societies are expected to play a role in the environmental control or the development and use of water or waste treatments to prevent human infectious diseases, regardless of their origins. Typically, governments establish criteria, standards, and control or treatment procedures for waterborne contaminants to provide national or regional reductions in pathogens in order to improve the level of health protection for the populace. Also, governments often impose monitoring requirements for specific pathogens or faecal indicators to ensure reduced human exposures to contaminated water. However, a lack of clear data on the likelihood and significance of human infectious diseases from animal faecal contamination of water resources has limited the ability of public health professionals and regulators to provide effective protection. There have not been many validated epidemiological links between human illness and water contaminated by animal sources. Regulators have limited justification to initiate more profound regulatory efforts to control zoonotic sources. As a result, although national, state, and local governments should have a role in the prevention or treatment of contaminated water, they may be paralysed to relative inaction and reactionary health protection policies.

The epidemiology of waterborne diseases has often been a major stumbling block to adequately assessing disease risks. An example is the Milwaukee, Wisconsin, USA, outbreak of cryptosporidiosis in the early 1990s. In that outbreak, the disease that infected a majority of the population of this city was first detected by an observant pharmacist who noted an excessive purchase of antidiarrhoeal medicines by the community. The public health community was not aware that an epidemic was occurring. This outbreak and others have demonstrated the lack of sensitivity and timeliness of our ability to detect outbreaks. Most investigations to provide an assessment of the movement of diseases through communities and to establish the sources of the pathogens have

occurred after the outbreak is well established or retroactively, after the outbreak has passed altogether.

27.3 REGULATORY CONTROL OF WATER RISKS

Zoonotic infectious diseases of faecal origin may contaminate the surface waters and groundwaters that humans use for drinking, recreation, fishing/shellfishing, irrigation, and aquaculture. Currently, animal sources of pathogens are often not specifically considered in possible new regulatory controls to protect waters or prevent human exposures because of the lack of regulatory tools to do so. Different regulatory approaches could be proposed for the future if, for instance, it is determined that there is a significantly reduced risk for human exposure or illness from animal-borne faecal pathogens in water compared with human-derived pathogens. Some of the data requirements and tools for considering zoonotic pathogen sources in future regulations include the following:

- analytical methods to sample, identify, and quantify specific pathogens or faecal indicators, including identification and use in control of animal pollution sources;
- evaluation of treatment effectiveness and health protection targets for discharge permits and total maximum daily loads (TMDLs) for specific contaminants;
- development or revision of protective criteria or standards that will ensure that water uses are protected; and
- improved monitoring strategies for animal-based point and non-point sources.

27.4 EXAMPLES OF REGULATORY APPROACHES

Some examples of current applications of regulations for microbial contaminants in an economically developed country are those of the USA. The approach for the monitoring and control of exposures to microbial hazards is handled differently for drinking-waters than for ambient waters used for other human purposes. The major reason is because drinking is considered a mandatory human water use, while other uses, such as recreation, are considered voluntary uses.

Under the *Safe Drinking Water Act*, the US Environmental Protection Agency (EPA) issues national drinking-water standards, which all public water utilities are required to meet. The standards are called Maximum Contaminant Levels (MCLs). Under current regulations, Maximum Contaminant Level Goals (MCLGs) of "0" are applied for enteric viruses, *Giardia*, and *Cryptosporidium.* However, actual routine monitoring for microorganisms is limited to total coliforms or *E.*

coli because of the high costs of conducting specific pathogen analyses in a routine manner and relatively poor precision and accuracy of analytical methods. The regulations rely on designed efficiency (performance) of the water treatment and disinfection technologies to attain the MCLG in the finished water. Total coliforms are routinely monitored in treated waters, and the detection of a single coliform per 100 ml triggers additional monitoring and the investigation of the treatment system. Repeated "detects" are reported to the states and to the US EPA.

On the other hand, under the *Clean Water Act*, the US EPA provides ambient water quality criteria, which rely on the application of faecal indicator microorganisms at prescribed target levels for health protection dependent on the water's use (e.g., recreational, shellfish growing and harvesting, and drinking). States adopt the criteria as standards for waters having designated uses that have to achieve a particular level of protection where human exposure is likely. These criteria are used to provide the swimmable and fishable requirements for US waters under section 304(a) of the Act. The US EPA is planning to establish future "drinkable" 304(a) criteria for *Cryptosporidium.* Below is a brief description of some of the essential components of the criteria for recreation and shellfishing:

- Recreational water quality criteria (primary recreation sites):

  (a) fresh water: 126 *E. coli* or 33 enterococci/100 ml using a running geometric mean over five weekly samples, which provide a protection level of 8 illnesses/1000 swimmer days;
  (b) marine water: 35 enterococci/100 ml using a running geometric mean over five weekly samples, providing a protection level of 19 illnesses/1000 swimmer days; and
  (c) single sample maximum values (upper-bound confidence intervals above the mean), which are less protective, but which can be applied at less frequently used recreational areas or in other primary contact venues (e.g., surfing, diving, water skiing).

The recreational criteria numbers were established for two indicators, based upon results of prospective epidemiology studies in which recreational water samples were measured for the indicators and persons bathing (with head immersion) were monitored over a 10-day period after the swimming event to determine if they experienced acute gastrointestinal disease symptoms. In the future, it is anticipated that new criteria and new or improved indicator microorganisms or methods will be based upon a companion study of new rapid indicator methods in conjunction with prospective swimmer epidemiology studies at freshwater and marine beaches.

- Shellfish growing water quality criteria for shellfish beds where the harvest will be sold for raw edible consumption:

  (a) identify and characterize growing areas; impacts of upstream wastewater treatment systems; required design of treatment system capabilities to protect waters and shellfish; size of shellfish water harvest zones; and treatment plant operation and maintenance procedures to ensure protection of the shellfish use;
  (b) approved unrestricted shellfish bacteriological quality classification:
      - total coliform: 70 most probable number (MPN)/100 ml (and <10% exceed 230 MPN/100 ml); or
      - faecal coliform: 14 MPN/100 ml (and <10% exceed 43 MPN/100 ml);
  (c) measure compliance by analysing at least 15 of the most recent adverse pollution conditions (minimum 5 per year) or the most recent 30 systematic random samples (minimum 6 per year) at stations located to evaluate actual or potential sources of pollution; and
  (d) establish National Pollutant Discharge Elimination System (NPDES) permit levels and monitoring for sewage treatment plants discharging to waters where shellfish may be grown for commercial sale or recreational fishing.

The shellfish criteria also provide additional target levels for waters that may be impacted by higher indicator levels, which may require periodic closures or even requirements for the "relay" or depuration of the shellfish stocks before harvest and sale from restricted waters.

Criteria for recreational and shellfish growing waters, using faecal indicators, have been effective tools to significantly reduce the levels of waterborne gastrointestinal disease. However, there may be shortcomings to the applicability of the criteria if animal sources of faecal waste are to be delineated from human wastes, assuming validation of the premise that human health risks from animal wastes are less than risks from human wastes. Unfortunately, actual human health risks are not well known or measured for the full range of zoonotic faecal-borne pathogens. Finding data that link animal faecal contamination with disease outbreaks from water exposure has been and will continue to be a significant challenge that will complicate future criteria development. Because of the lack of epidemiological evidence directly associating animals with waterborne disease risks, there has not been a concerted effort to determine when, where, or how to control sources or regulate these contaminants or to estimate their relation to human risks for revising criteria. Additionally, epidemiological studies conducted to correlate faecal indicators with disease risks or the pathogens themselves have

not been conducted on waters in which animal contamination is the dominant source for exposure.

Public health policy discussions have recently begun in the USA regarding the significance of animal-borne human pathogens, better definition of their health significance for the water environment, and the potential need to control these contaminants. There are a number of animal-based industries and some public health practitioners who do not think that the magnitude of animal faecal contaminant sources of human pathogens are very important or who believe that the levels of risk are significantly less than if the same pathogens were from human sources. There has also been speculation in the scientific community that animal sources of human pathogens may have less ability to infect and cause disease in humans than a human pathogen source. Possibly, human virulence gene expressions are shut off in pathogens derived from animal hosts. It has been suggested that if animal-borne human pathogens are less likely to cause human infection, then regulations or criteria might be relaxed if animals were identified as the source, while still being protective of human water uses. If regulators can determine the magnitude and distribution of disease risks from animal faecal sources, then it may be feasible to modify health protection targets in standards, criteria, and watershed management. Also, risks may be characterized differently based upon the relative probability of human infectious diseases being present in animal populations. It has been suggested that bacterial and parasitic diseases from animal sources are more likely to be a common human risk than viral diseases because they appear to have a wider range of hosts. This could be an important distinction for possible future regulatory approaches.

Another potentially important scenario exists concerning animal-borne human pathogens. That is, what are the implications of human faecal shedding of the pathogens that co-infect animals? Because of the increased proximity of humans to animals and the magnitude of the human waste load to water, if not adequately treated, this could provide a significant level of pathogens capable of infecting susceptible livestock, pets, and wildlife. Could human waste contamination of waters help maintain zoonotic infections and potential reservoirs for human pathogens, which can be spread to other animals and back again to humans in a cyclical process? This issue may also need to consider the implications of invasive species of pathogens and of animals. Also, cloned animals may need to be considered. Regulators may need to consider these potential ramifications to public health as we gain better data and knowledge about human contributions to zoonoses, the maintenance of infectious disease cycles, and the implications of anthropogenic activities to the health of animal populations.

With respect to the utility of the current US EPA criteria approach using classical indicators, there is also a question about the applicability of the criteria levels. The relationship of indicators and how they represent disease risk may

differ in faeces of various animal species. Some investigators have suggested that various animal species shed significantly different levels of the current indicators (per gram of faeces) than humans. However, it is not known if these differences are large enough to have a serious impact on the reliability of current health criteria or standards to accurately portray the probability of health risks if the targeted levels of indicators are maintained for all contamination sources. Future source tracking technologies, which are described below, may provide monitoring tools to determine the nature of faecal sources.

27.5 ISSUES TO ADDRESS BEFORE ESTABLISHING CONTROLS

Public health officials and regulators need to know the significance of the threats of disease from animal-borne human pathogens, whether from the livestock industry, from pets and feral animals, or from wildlife, in order to provide appropriate source mitigation, determine appropriate levels of treatment, or impose limits on waterborne exposure based on the risks. There are a number of factors that are important considerations in determining the likelihood and significance of the spread of infectious disease organisms from animals to humans and their control or regulation:

(1) the levels of pathogens released to a body of water and the relative degree of pollution of the water based on the size and strength of the waste source;
(2) the length of time the pathogen survives in the aquatic environment and the possibility of its growth in that environment (and also its survival relative to potential indicators);
(3) the effectiveness of available treatment and disinfection technology and practices to reduce or eliminate the pathogens, either at the source or before use of a contaminated water;
(4) the infectious dose for the pathogens to cause infection and/or disease for the particular exposure envisioned for particular water uses;
(5) the severity and duration of illness likely to be manifested in the persons exposed from a water use; and
(6) the effectiveness and longevity of immunity either from vaccinations or from previous exposures to the pathogens.

Each of these factors plays a significant role in the likelihood of infection and disease outcomes. Different factors may have a predominant effect on the outcome for each pathogen. Regulators and public health officials need to develop

a strategy to consider each of these factors in trying to reduce or eliminate the risk of disease to water users. As newly emerging or modified (natural or engineered mutations) pathogens from animal sources emerge as potential water contaminants, there may be increased demands to establish data and new tools, such as:

- analytical methods to determine their sources, environmental fate, and transport;
- applicability of class surrogates or indicators;
- water and wastewater treatability and discharge permits;
- dose–response relationships; and
- monitoring requirements for establishing criteria and standards, TMDLs, and pollutant (water quality) trading.

Regulatory decisions that would need to be considered include determinations of whether the pathogen itself needs to be the analytical target —whether it can be represented by a pathogen class surrogate (a member of a broad group of pathogens; e.g., total enteric viruses) or whether common faecal indicators can be applied. Some lingering problems with direct pathogen or pathogen class analysis are the typically high costs for sampling and analysis, especially for protozoa, viruses, and parasites. Additionally, there are typically greater levels of training, expertise, and laboratory equipment required. However, progress being made in development of molecular methods of analysis may help reduce the costs and complexity of performing direct pathogen monitoring. The advances in multiple array probe technologies may allow simultaneous detection of a wide variety of pathogens in a sample. Still other problems exist with some molecular approaches, in that it is difficult to determine if pathogens in a water sample are dead or alive. Finally, the long time required to sample and perform analyses is a major roadblock, especially where it is important to make a quick determination about whether or not it is safe to use a water, particularly for recreational and crop irrigation uses.

27.6 REQUIREMENTS FOR CRITERIA WHERE ANIMAL PATHOGENS/INDICATORS ARE NEEDED

It is likely that, for years to come, there will be a requirement for sampling and analysis of faecal indicator bacteria from humans and animals. There will also be a need for the development, validation, and application of other indicators for different exposures and diseases associated with water uses. Faecal indicator methods are relatively inexpensive, are easy to learn to use, have “low tech”

laboratory equipment requirements, and typically provide a measure of viability of the organisms in the sample. New research is developing rapid methods that provide results within a few hours. Other research in microbiological source tracking (MST) methods is providing promising new indicator technologies (antibiotic resistance, *Bacteroides* indicator, polymerase chain reaction [PCR] probes, coliphage typing, etc.) that can differentiate faecal sources and have the potential to detect and discriminate human versus livestock versus various wild animal contamination. Other work is evaluating the efficacy of faecal or wastewater chemicals to help discriminate faecal or wastewater sources. Some non-microbial indicators under investigation are faecal sterols, detergents, fabric whiteners, and secretory immunoglobulins. These chemical indicators could be useful as added tools to determine the sources of faecal and wastewater contaminants, and some have the potential to be applied as "dipstick" or on-line monitoring methods.

It is likely that monitoring for enteric disease risks will be more sophisticated in the future than it is today. As technologies develop, it will be possible to use "tiered" monitoring approaches, where cheap, rapid, and even on-line methods would be used for screening water samples. Then, progressively more targeted, sophisticated methods would be used on positive samples to further delineate sources and viability, and even to detect and quantify specific pathogens of concern. Alternatively, a combination of different indicators may be analysed simultaneously in sampling protocols to provide an index of faecal pollution strength and source, similar to other environmental quality monitoring applications (e.g., air quality indices).

There continues to be a real requirement for analytical methods for water, whether for pathogens or indicators, to be used with increased levels of confidence to support regulatory needs to reduce health risks. Historically, new methods to be used in a regulatory context are first validated by outside laboratories to demonstrate that they can be used effectively by someone other than the method developers. Validated methods are then collaboratively tested on the water medium in which they will be used to establish their analytical performance. Collaborative testing of methods typically involves a designated group of qualified laboratories that use a single testing protocol and strictly follow the analytical procedure. Typically, representative subsamples of the water medium (either spiked or unspiked for the microorganisms of concern) made from single preparations of various waters of interest would be distributed to the laboratories from single preparations. The collective multilaboratory sample results are analysed to establish the precision, accuracy, and inter- and intralaboratory bias for use of the method. Often there are predetermined performance goals for acceptance of a new method, which must be met by the collaborative study in order for it to be used in a regulatory setting.

27.7 HEALTH CRITERIA AND STANDARDS SETTING

In setting health criteria or standards for microbial health risks in water in the USA, the selected analytical methods must first achieve required acceptance levels, as described above. Then the method must be experimentally evaluated to establish its relationships to health-protective numerical targets in order to define acceptable occurrence levels for criteria. When there is a requirement for a regulation for a specific pathogen, it may be necessary to conduct human or animal dose–response studies, where the dose administered (quantified by the pathogen method) is evaluated in terms of specific health effects, such as infection or disease end-points. The relationship of measured dose to health effects can then be defined to establish the protective levels required for regulation. On the other hand, for health criteria, health risks are often derived from prospective epidemiology studies or outbreak evaluations, where an indicator method is measured in the water during the time and at the location that the human exposure is occurring. A correlation is sought for the indicator level associated with the acceptable level of a targeted disease syndrome (e.g., acute gastrointestinal disease for recreational criteria).

In the future, regulatory decisions may need to consider the capability we have to detect and discriminate animal faecal pollution from human sources and also the ability we have to determine the relative risks associated with human versus animal-borne pathogens. If risks are not equivalent, then the question becomes what metrics are available that regulators can use to distinguish between the sources or the risks: MST methods; virulence factor activity relationships; or dose–response data that may demonstrate differences in infectivity and disease outcomes for animal versus human pathogen sources. If adequate decision-making tools or methods to achieve the above are not available or feasible, then it may be appropriate to continue to regulate all faecal wastes equivalently, a conservative approach.

The current regulatory approaches that could be applied in the future to better control animal faecal contamination of water and reduce human exposure are as follows:

(1) reduce contaminants at their sources by on-site treatment of wastes (e.g., concentrated animal feeding operations [CAFO]; NPDES discharge permits) or otherwise deny their entry to ambient water resources;
(2) reduce human exposure during use of ambient waters by treatment of the water before use (drinking-water source treatment/disinfection); or
(3) intervene in the use of the contaminated water (e.g., beach and shellfish bed closures).

A number of approaches specific to the protection of drinking-water consumers are available. These could be adjusted to consider different animal faecal contaminant risks to health (if such were demonstrated) to provide significant reductions in treatment requirements while still protecting the health of consumers:

(1) source water protection programmes;
(2) source water criteria at intakes, such as the US EPA's consideration of 304a criteria for *Cryptosporidium*;
(3) modified treatment and disinfection processes and tools to meet water treatment requirements (such as proposed for removing *Cryptosporidium* at the plant under the US EPA's Long Term 2 Enhanced Surface Water Treatment Rule);
(4) maintenance of the integrity of distribution systems; and
(5) systematic identification and risk assessment for potentially problematic emerging pathogens, such as the US EPA's Contaminant Candidate List for potential future drinking-water regulation.

There are also a number of approaches to the protection of direct ambient source water uses such as for recreation, irrigation of fruits and produce to be consumed raw, shellfish growing, drinking-water sources, and aquaculture. A major emphasis of protection of these uses is the establishment of discharge permits for animal wastes such as in CAFO and animal feeding operation (smaller-scale animal operations) wastes, but also for rendering plants and abattoirs. TMDLs are applied to protect uses and can consider both point and non-point discharges of animal wastes. A new approach being used for some pollutants in the USA is the application of "pollution trading," whereby one pollutant source with costly treatment requirements could trade pollution "credits" with another source having less costly needs. In the future, these pollution trading approaches could utilize microbiological TMDLs as a tool to meet the trading needs. It will be interesting to see if faecal indicator trading has a place in this overall contaminant management scheme to ensure that infectious disease levels are kept in check.

Most economically developed countries have ambient water criteria for various water uses to protect their populations from infectious disease. There are efforts to look at new ways to apply criteria to uses. In the USA, the EPA is looking at the establishment of integrated microbiological criteria wherein a single criteria approach and a set of faecal indicators (or pathogens) are monitored for all ambient water uses. In this integrated application, the acceptable health criteria levels for the uses may be different, but the indicators or pathogen methods could be standardized. This approach could also apply tiered monitoring methods and

faecal pollution indices (based upon several different indicators considered together to establish a numerical index of contamination levels).

Any new approaches to monitoring discharge permits, non-point sources, TMDLs, or ambient criteria to discriminate animal-borne human pathogens may need to rely on improved or new methods to implement discriminatory approaches to animal contaminants. A number of analytical methods for monitoring these various water-based regulatory needs are being investigated. New molecular methods such as PCR, reverse transcriptase-PCR, Multiplex probes, and various immunological and biochemical techniques for specific pathogens or pathogen classes are being developed and evaluated for their efficacy to detect, identify, quantify, and even determine the presence of pathogen virulence factors. Also, improved molecular methods for microbial indicators of faecal contamination are being developed. Within the realm of indicator methods is the current US EPA focus on validation of rapid (<2 h) methods to detect and quantify faecal contamination levels. The availability of rapid methods would be a significant improvement as tools for real-time NPDES monitoring and for ambient water quality monitoring, where rapid data availability could influence decisions on treatment requirements and on safety of water uses.

A number of analytical methods have been identified that have capabilities to discriminate (to various degrees) animal versus human faecal contamination. MST methods are mostly in the development and evaluation stage. Some methods rely on antibiotic resistance patterns; others are direct measurements of specific pathogens or new indicators, such as coliphage and *Bacteroides* sp. bacteria; but most rely on molecular techniques, such as PCR probes of specific unconserved areas of the genome and immunological or biochemical techniques that determine differences in the genotypic or phenotypic expression of indicators/pathogens. There has been an attempt in the USA (private sector and US EPA-funded) to establish a prescribed set of protocols to evaluate the efficacy of the MST methods. A major hurdle may be the inability of the methods to work adequately in discriminating sources beyond a single watershed or geographical region. To be useful as regulators' tools, the source tracking methods will need to be capable of providing at least regional or preferably national capabilities to discern differences in animal versus human faecal contamination sources.

27.8 CONCLUSIONS

Strong epidemiological evidence that animal-borne human faecal pathogens are a significant risk for human exposure in faecally contaminated water is lacking. There is a need for more data to make informed scientific judgement about the significance and magnitude of zoonotic faecal pathogens as agents of human infection from contaminated water. Until there is adequate evidence for or against

the concept of quantifying different risks, it will not be possible to alter regulatory approaches that would consider animal faecal contaminant sources as different from human sources.

Currently, animal-associated human pathogens are not discriminated from human sources for various water uses, such as recreational, shellfishing, and drinking-water source waters. Additionally, microbial indicators of faecal contamination do not differentiate between human and animal sources. Targeted research efforts are needed to provide discriminatory analytical tools to help advance regulation if they are warranted.

There are a number of data gaps regarding the adequacy of approaches for monitoring and analysis in criteria and standards setting, as well as a need to validate and standardize current and new methods. For animal pathogen sources, it is necessary to establish whether or not pronounced differences exist for exposures to animal faecal sources; environmental fate and transport of zoonotic pathogens; their treatability in drinking-water and wastewater; differences in the infectivity and virulence of zoonotic pathogens for humans; dose–response; and epidemiological correlations. Also, there is a need to determine if animal-borne pathogens are adequately covered under current regulations and treatment approaches, as well as determining what the appropriate indicators are.

Current research efforts may allow regulations in the future that will incorporate the ability to discriminate animal faecal sources from each other and from human sources based upon MST. These technologies may have promise for use in better defining TMDLs and possibly for implementing pollution trading approaches.

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28

The Stockholm framework for guidelines for microbial contaminants in drinking-water

R. Carr and J. Bartram

28.1 INTRODUCTION

Following a major expert meeting in Stockholm, Sweden, the World Health Organization (WHO) published the document *Water Quality: Guidelines, Standards and Health; Assessment of Risk and Risk Management for Water-related Infectious Disease*. This document creates a harmonized framework for the development of guidelines and standards, in terms of water-related microbial hazards (Fewtrell and Bartram 2001). This framework involves the assessment of health risks prior to the setting of health targets, defining

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basic control approaches, and evaluating the impact of these combined approaches on public health status (Figure 28.1).

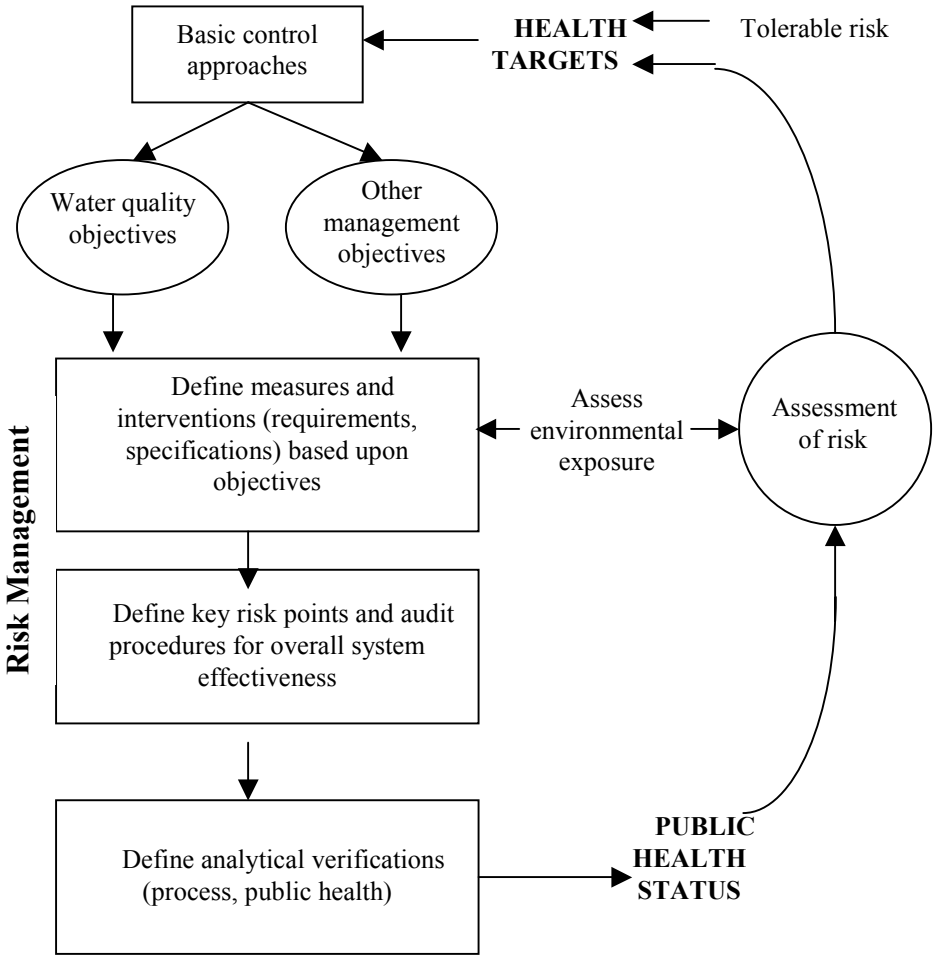

Figure 28.1. Stockholm framework for assessment of risk for water-related microbial hazards (from Bartram *et al*. 2001).

The framework allows countries to adjust guidelines to local social, cultural, economic, and environmental circumstances and compare the associated health risks with risks that may result from microbial exposures through wastewater use, drinking-water, and recreational/occupational water

contact (Bartram *et al.* 2001). This approach requires that diseases be managed as a whole package and not in isolation. Comparisons can be made between disease outcomes from one exposure pathway or between illnesses by using a common metric, such as disability-adjusted life years (DALYs).

Future WHO water-related guidelines will be developed in accordance with this framework. This chapter contains a summary of the framework components (Table 28.1) and a discussion of DALYs and tolerable risk. For a more detailed discussion of the framework, see Fewtrell and Bartram (2001).

Table 28.1. Elements and important considerations of the Stockholm framework (adapted from Bartram *et al*. 2001)

Framework component	Process	Considerations
Assessment of health risk	Hazard assessment Environmental exposure assessment Dose–response analysis Risk characterization	Best estimate of risk — not overly conservative Equivalence between risk of infection and risk of disease Health outcomes presented in DALYs, facilitates comparison of risks across different exposures and priority setting Risk assessment is an iterative process — risk should be periodically reassessed based on new data or changing conditions Risk assessment is a tool for estimating risk and should be supported by other data (e.g., outbreak investigations, epidemiological evidence, microbiological risk assessment, and studies of environmental behaviour of microbes) Process dependent on quality of data Risk assessment needs to account for short-term under-performance
Tolerable risk/health targets	Health-based target setting based on risk assessment Define water quality objectives	Needs to be realistic and achievable within the constraints of each setting Set based on a risk–benefit approach, should consider cost-effectiveness of different available interventions Should take sensitive subpopulations into account Index pathogens should be selected for relevance to contamination, control challenges, and health significance (it may be necessary to select more than one index pathogen)

Framework component	Process	Considerations
Risk management	Based on health-based targets: Define other management objectives Define measures and interventions Define key risk points and audit procedures Define analytical verifications	Risk management strategies need to address rare or catastrophic events A multiple-barrier approach should be used Monitoring — overall emphasis should be given to periodic inspection/auditing and to simple measurements that can be rapidly and frequently made to inform management Hazard analysis and critical control points (HACCP)-like principles should be used to anticipate and minimize health risks
Public health status	Public health surveillance	Need to evaluate effectiveness of risk management interventions on specific health outcomes (through both investigation of disease outbreaks and evaluation of background disease levels) Procedures for estimating the burden of disease will facilitate monitoring health outcomes due to specific exposures Burden of disease estimates can be used to place water-related exposures in the wider public health context to enable prioritization of risk management decisions Public health outcome monitoring provides the information needed to fine-tune risk management process through an iterative process

28.2 DISABILITY-ADJUSTED LIFE YEARS (DALYS)

DALYs are a measure of the health of a population or burden of disease due to a specific disease or risk factor. DALYs attempt to measure the time lost because of disability or death from a disease compared with a long life free of disability in the absence of the disease. DALYs are calculated by adding the years of life lost to premature death (YLL) to the years lived with a disability (YLD). YLL is calculated from age-specific mortality rates and the standard life expectancies of a given population. YLD is calculated from the number of cases multiplied by the average duration of the disease and a severity factor ranging from 1 (death) to 0 (perfect health) based on the disease (e.g., watery diarrhoea has a severity factor ranging from 0.09 to 0.12, depending on the age group) (Murray and Lopez 1996; Prüss and Havelaar

2001). DALYs are an important tool for comparing health outcomes because they account for not only acute health effects but also delayed and chronic effects, including morbidity and mortality (Bartram *et al.* 2001). When risk is described in DALYs, different health outcomes can be compared (e.g., cancer vs. giardiasis) and risk management decisions can be prioritized.

28.3 WHAT IS AN ACCEPTABLE (TOLERABLE) RISK?

According to Hunter and Fewtrell (2001), the following criteria can be used to judge whether a risk is acceptable:

- it falls below an arbitrary defined probability;
- it falls below some level that is already tolerated;
- it falls below an arbitrary defined attributable fraction of total disease burden in the community;
- the cost of reducing the risk would exceed the costs saved;
- the cost of reducing the risk would exceed the costs saved when the "costs of suffering" are also factored in;
- the opportunity costs would be better spent on other, more pressing public health problems;
- public health professionals say it is acceptable;
- the general public says it is acceptable (or, more likely, does not say it is not acceptable); or
- politicians say it is acceptable.

Tolerable risks are not necessarily static. As tools for managing water-related disease transmission improve, levels of risk that are tolerable may decrease. Tolerable risks can therefore be set with the idea of continuous improvement. For example, smallpox was eradicated because it was technologically feasible to do so, not because of the continually decreasing global burden of disease attributed to this disease.

The control of *Listeria monocytogenes* in ready-to-eat food products provides another example. *Listeria* causes listeriosis, a serious, although rare, foodborne disease (Codex 1999). In the 1980s, effective management procedures were developed that demonstrated that finished, ready-to-eat food products could be produced with very little or no *Listeria monocytogenes* present. In the USA, a policy of "zero" tolerance for *Listeria* in these products was adopted. As a result of the implementation of this policy, the incidence of and mortality due to foodborne listeriosis in the USA declined by 44% and 49%, respectively, over a period of 4 years (Billy 1997). In many developed countries, similar reductions in foodborne listeriosis cases (99% of listeriosis is estimated to be foodborne; Mead *et al.* 1999) have occurred due to adoption of good management practices and HACCP programmes by food processors,

improvement of the integrity of the cold chain in storage/transport, etc., and better risk communication to susceptible consumers (Codex 1999).

28.4 TOLERABLE MICROBIAL RISK

For water-related exposures, WHO has determined that a disease burden of 1×10^{-6} DALYs per person per year from a disease (caused by either a chemical or infectious agent) transmitted through drinking-water is a tolerable risk (WHO 2004). This level of health burden is equivalent to a mild illness (e.g., watery diarrhoea) with a low case fatality rate (e.g., 1 in 100 000) at approximately a 1 in 1000 annual risk of disease (10^{-3}) to an individual (a 1 in 10 risk over a lifetime) (WHO 1996, 2004; Havelaar and Melse 2003). The US Environmental Protection Agency (EPA) sets a tolerable risk in relation to infection, rather than disease, of less than 1 *Giardia intestinalis* infection in 10 000 people per year (a 10^{-4} risk) from drinking-water (Regli *et al*. 1991). However, based upon background rates of gastrointestinal disease in the general population, Haas (1996) argued that an acceptable risk of infection of 10^{-4} per person per year was too low and that even a risk of infection of 10^{-3} per person per year was too low.

The US EPA set the tolerable risk level using the risk of infection rather than the manifestation of disease. This is an important distinction, because there are a number of factors that determine whether infection with a specific pathogen will lead to a disease, including the virulence of the pathogen and the immune status of the individual (see Prüss and Havelaar 2001 for a further discussion of infection versus disease). For example, hepatitis A infections in children are predominantly asymptomatic (no apparent symptoms), but the same infection in adults often does lead to disease symptoms (WHO 2000). Asymptomatic infection can be confirmed by microbiological examination of stool specimens and in some cases by detection of a serological response (Teunis *et al*. 1996). However, infection is harder to detect in the general population because there are no obvious disease symptoms to track. For this reason, it is more difficult to measure compliance with and/or enforce a guideline value set with infection as an end-point compared with one based on disease, and such a guideline value is less precise in terms of public health protection.

Tolerable risk can be looked at in the context of total risk from all exposures, and risk management decisions can be used to address the greatest risks first. For example, it would have very little impact on the disease burden if the number of cases of salmonellosis attributed to drinking-water are halved when 99% of the cases were related to food.

For water-related exposures to microbial contaminants, diarrhoea or gastrointestinal disease is often used as a proxy for all waterborne infectious diseases. Mead *et al*. (1999) estimated that the average person (including all age groups) in the USA suffers from 0.79 episodes of acute gastroenteritis (characterized by diarrhoea, vomiting, or both) per year (a 7.9×10^{-1} annual risk of gastrointestinal illness). The rates of acute

gastroenteritis among adults worldwide are generally within the same order of magnitude (Table 28.2). However, children — especially those living in high-risk situations, where poor hygiene, sanitation, and water quality prevail — generally have a higher rate of gastrointestinal illness. Kosek *et al.* (2003) found that children under the age of 5 in developing countries experienced a median of 3.2 episodes of diarrhoea per child per year (a risk of 3.2 per year).

Table 28.2. Diarrhoea cases per year by age group and country income level (adapted from Murray and Lopez 1996)

	Country income level[a]		
Age (years)	Low	Middle	High
0–4	4.5–5.0	2.3–4.0	1.8
4–15	0.6–0.9	0.1–1.2	0.1
15–80+	0.2–0.3	0.2–0.3	0.1
Average	0.8–1.3	0.6–1.0	0.18–0.22

[a] As defined by the World Bank (2003).

28.5 CONCLUSION

To effectively manage waterborne disease, it is necessary to consider the total burden of disease from all water-related exposure routes (drinking-water, contact with recreational water, the use of wastewater in agriculture, and contamination of other food items, e.g., shellfish). The Stockholm framework provides a tool for developing guidelines and standards to manage the disease burden from all water-related exposure routes. Framing health outcomes in terms of DALYs allows one to compare the health impacts associated with different diseases in a population, such as an infrequent case of cancer versus more frequent cases of mild diarrhoea, and thus enables priority setting to maximize health protection. Health risks should be put into the context of tolerable risk, which can be redefined as new technologies or other developments facilitate the reduction of health impacts from waterborne exposure routes or specific pathogens.

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29

Quantitative microbial risk assessment issues

G.B. McBride

29.1 INTRODUCTION

Epidemiological studies and surveillance activities are a vital part of region-wide and country-wide efforts to understand the human health risks associated with waterborne zoonoses and to set appropriate public policy (see chapter 10). Another approach is to use quantitative risk assessment (QRA). The essence of QRA lies in its four main steps:

(1) hazard assessment;
(2) exposure assessment;
(3) dose–response analysis; and
(4) risk characterization.

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The concentration of zoonotic microorganisms (Step 1) and the extent of exposure (Step 2) vary between individuals, locations, and occasions. Simple calculations of average risks using average concentrations and exposures can therefore be seriously misleading. Accordingly, it is necessary to use random statistical sampling from the distributions of these variables to build up a risk profile.

In performing these calculations, QRA draws on information from epidemiological studies and surveillance activities (in selecting the pathogens of concern in Step 1; quantifying the degree of exposure in Step 2). It also draws on material available in the scientific literature (especially for Step 3). The risk characterization (Step 4) is useful in its own right, as a means of indicating important aspects of waterborne transmission of zoonoses. It may also be used to modify and refine epidemiological and surveillance efforts, thereby leading to more effective public policy and animal husbandry practices that lessen risks of waterborne transmission of zoonotic microorganisms.

This brief chapter gives an example of the application of QRA to waterborne zoonotic organisms (*Campylobacter*) and highlights some strengths and weaknesses of the approach. Details of the application methodology are not given, as they are well described in existing texts (Haas *et al*. 1999; Haas and Eisenberg 2001).

29.2 CAMPYLOBACTERIOSIS CASE-STUDY

The incidence of campylobacteriosis is of concern in New Zealand, with the surveillance system showing that it currently comprises over half the country's notifiable disease burden (see chapter 12). An epidemiological study by Eberhart-Phillips *et al*. (1997) has indicated that undercooked chicken is a major risk factor, but it has also identified other factors, including contact with animals and use of rainwater as a source of water at home. The study by Eberhart-Phillips *et al*. (1997) did not seek information on contact with recreational water, so the importance of that factor could not be assessed. Since that study, *Campylobacter* species have often been found in water samples at recreational sites. Given that it is a potentially waterborne disease and that farmed animals commonly have direct access to waterways, there has been interest in assessing the degree to which contact with recreational water may play a role in the transmission of *Campylobacter*.

This question is best addressed by a QRA approach, as has now been reported (McBride *et al*. 2002). The study by McBride *et al*. (2002) focused on infection, rather than illness, as its health outcome, for two reasons. First, avoiding infection also avoids illness, but the converse is not true — there can

be many passive infected carriers of this microorganism, whose shedding can cause infection *and* illness in others. Second, the available dose–response relationship for infection is more straightforward than the relationship for illness (as discussed below).

The main results are summarized in Table 29.1, which shows the percentile of time that risks reach a given case rate. This has been done by Monte Carlo simulations of exposure of 1000 people (each with a different degree of exposure) on each of 1000 occasions — a million calculations in all. Furthermore, two separate target populations were considered. In the first case, the *local population*, all 1000 attended the same beach on any given occasion, so on that occasion they were all exposed to the same (randomly selected) *Campylobacter* concentration; on another of the 1000 occasions, they were all exposed to another *Campylobacter* concentration; etc. In the second case, the *dispersed population*, all 1000 people attended a different beach on any given occasion, so on that day they were each exposed to a different *Campylobacter* concentration.

As a result, the risk profiles for the two populations are quite different, although the mean number of cases over the million exposures was about the same (about 40 500). For the local population, the risk is usually completely negligible, because the beach is uncontaminated for a majority of the time. However, when the beach is somewhat contaminated, substantial infection rates could occur if recreational activity was occurring. On the other hand, there is always some contamination at some beaches, so there is always some risk at some beaches, and so the dispersed population has a much flatter risk profile.

The results for the dispersed population have been used, along with the notified illness rate, estimates of under-reporting rate, and the proportion of passive carriers, to deduce that 4–5% of the actual number of *Campylobacter* infections could be the result of exposure to recreational fresh water (it is coincidental that the average case rates in Table 29.1 are also in this range).

At first sight, it may seem that this figure (4–5%) does not suggest an important transmission route, and the QRA results could be ignored. Two matters suggest otherwise. First, this result is for freshwater exposure only. It does not consider recreational exposure to estuarine and coastal waters or exposure via the consumption of raw shellfish (commonplace in the New Zealand setting, with its rich coastal resources). Second, water is an effective conveyer of microorganisms from one location to another. For example, infection in an animal herd towards the top of a watershed can be transmitted rapidly downstream to other herds, which may also become infected via contaminated water, further increasing the risk of waterborne transmission.

These results have been used in public policy, especially in the setting of new microbiological water quality guidelines for freshwater recreational areas, using

the moderate correlations observed between concentrations of *Campylobacter* and the much more easily assayed *E. coli* (Ministry for the Environment and Ministry of Health 2003).

Table 29.1. Two types of risk profiles (McBride *et al*. 2002)

	Campylobacteriosis infection risk (per 1000 recreational events)	
Percentile[a]	Local population (1000 people at the same beach each day)[b]	Dispersed population (1000 people at 1000 different beaches each day)[c]
Minimum	0	25
2.5th	0	29
5th	0	31
10th	0	33
15th	0	34
20th	0	36
25th	0	37
30th	0	38
35th	0	38
40th	0	39
45th	0	40
50th	0	41
55th	0	42
60th	1	43
65th	3	44
70th	9	44
75th	18	45
80th	26	46
85th	72	47
90th	131	49
95th	329	52
97.5th	435	54
Maximum	491	62

[a] Percentage of time that the infection rate is up to the value shown in each row.

[b] Each of the exposed "see" the same random pathogen concentration at any one time.

[c] Each of the exposed "see" a different random pathogen concentration at any one time.

There are, of course, some uncertainties in this work, and these need to be recognized.

29.3 UNCERTAINTY IN QRA

QRA is designed to accommodate uncertainty and to quantify the consequences of that uncertainty. Yet it is itself uncertain! Consider the fundamental elements:

(1) the choice of appropriate zoonotic pathogen(s);
(2) the quantity of the pathogens deposited by animals;
(3) their transport and inactivation over the landscape to water bodies;
(4) the efficacy of water supply or wastewater treatment processes (e.g., waste stabilization ponds, stream riparian retirement);
(5) the pathogen concentration in the water conveyed to humans — for drinking, for recreation, or indirectly following filtration by shellfish;
(6) the degree of exposure (exposure duration and ingestion/inhalation rate during recreational exposure), or the amount of shellfish consumed;
(7) a dose–response relationship showing probabilities of infection related to the received dose; and
(8) calculation methods.

To a greater or lesser degree, there is uncertainty in all these items, many of which have been addressed elsewhere in this book and need no further elaboration here. Here we particularly address the last two issues.

29.3.1 Dose–response

Some waterborne zoonotic pathogens have been the subject of clinical trials, including *Salmonella* (multiple non-typhoid strains), *E. coli* (non-enterohaemorrhagic strains, except O111), *Campylobacter jejuni*, *Cryptosporidium parvum*, and *Giardia lamblia* (Teunis *et al.* 1996; Haas *et al*. 1999; Haas and Eisenberg 2001). Data from these trials are fitted to dose–response curves (generally a single-parameter exponential model or a two-parameter beta-Poisson model). Issues arising are as follows:

- Subjects in clinical trials are usually restricted to healthy adults, so the young, infirm, and immunocompromised are not represented.
- Trials typically administer few — if any — low doses (Rollins *et al*. 1999; Tribble *et al*. 1999). Consequently, there is considerable uncertainty in the dose–response relationship at low probabilities of infection or illness (Teunis and Havelaar 2000). Yet such doses are often typical for exposure to contaminated water and therefore crucial in the risk assessment calculations. For an example, see the beta-Poisson *Campylobacter jejuni* relationship on Figure 29.1 (from the beta-

Poisson model reported by Medema *et al*. 1996); only one dose lies below the calculated median infective dose.

- Trials are generally restricted to one strain of an organism. The infectivity of that strain may differ from that of other strains. For example, trials have been performed on different strains of the same organism, with substantially different infectivities being found (i.e., for *Cryptosporidium parvum* — Okhuysen *et al*. 1999; Teunis *et al*. 2002a, 2002b). Similarly, there are hints of the same for *Campylobacter jejuni* (Bacon *et al*. 1999; Stewart-Tull *et al*. 1999).
- Infectivity may also be affected by the manner in which the pathogen is stored and passaged in the laboratory.
- Dose–response curves for infection given dose are monotonic-increasing, whereas curves for illness given infection may not be (Teunis *et al*. 1999). Regarding the illness curves, examples can be found for three possible alternatives: an increase in the probability of illness with increasing dose (salmonellosis), a decrease with higher doses (campylobacteriosis), and a probability of illness (given infection) independent of the ingested dose (cryptosporidiosis). These alternatives may reflect different modes of interactions between pathogens and hosts. For example, a decreasing illness probability with dose may be found if higher doses elicit progressively stronger defence reactions in hosts, thereby preventing further damaging activities in those hosts (Teunis *et al*. 1999). Further dose–response studies would be necessary to clarify the strength of these findings. (As noted above, this is one of the reasons for making infection the endpoint of an analysis, rather than illness. The other reason is that avoiding infection also avoids illness, but the converse is not true.)
- Clinical trials have not been conducted for many zoonotic waterborne pathogens, so they completely lack dose–response data.

Ways to address these issues need to be found in order to improve QRA — and, in some cases, to even make it possible to perform it.

29.3.2 Calculation methods

While good advice is now available in texts (Haas *et al*. 1999; Fewtrell and Bartram 2001), some calculation issues remain to be addressed — or, at least, reinforced. These issues include the following:

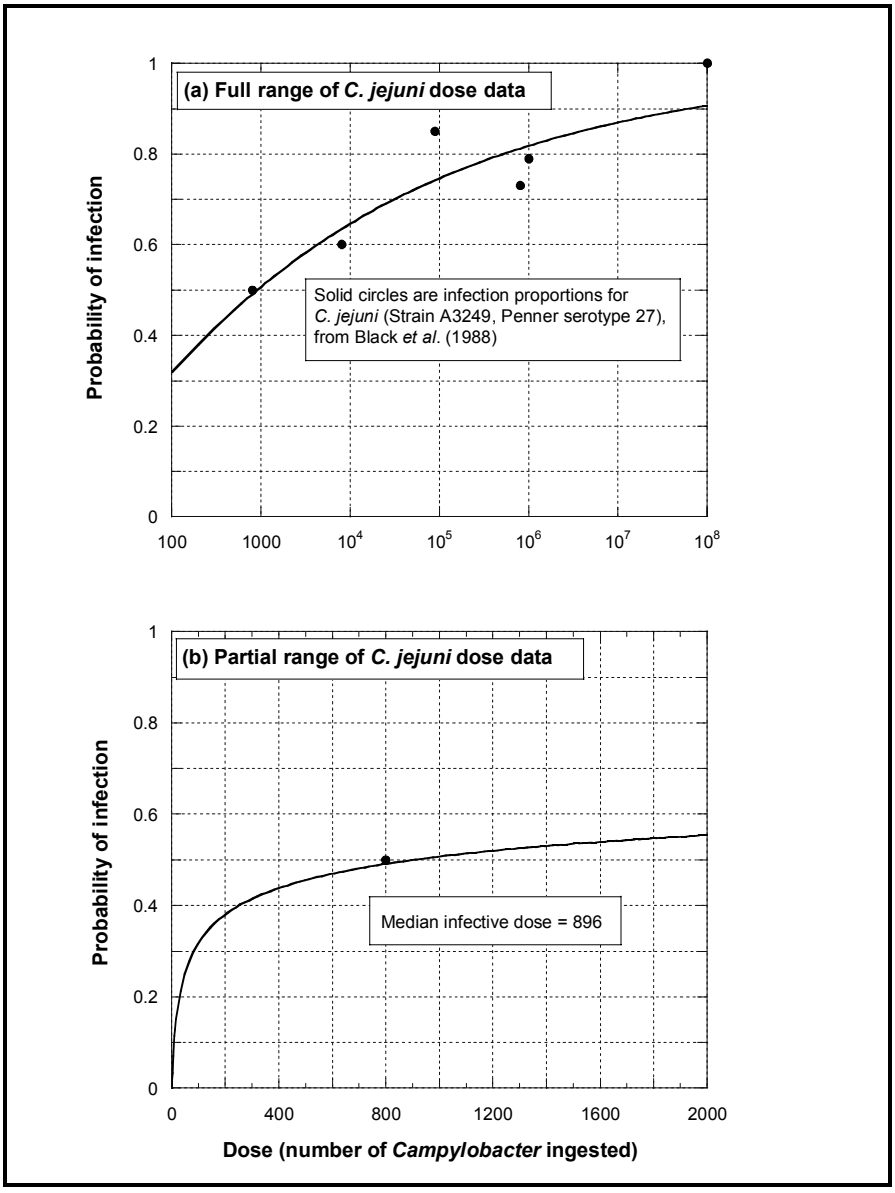

Figure 29.1. Dose–response curve for *Campylobacter jejuni* over full and partial range.

- Some data are known to be variable (e.g., pathogen concentrations in water). These are characterized by statistical distributions. To calculate risk, these distributions need to be combined with dose–response curves.
- Risk will be underestimated if that combining is effected merely by comparing the median infective dose with an average actual dose.
- Calculation of risk therefore does not result in *a* number, but in a *distribution* of numbers showing a risk profile (e.g., see Table 29.1).
- Combining distributions can, in some cases, be done using formal analytical methods. However, numerical methods are usually needed, such as Monte Carlo random sampling, for which commercial software is available. Care must be taken if any of the variables are correlated (Haas 1999).
- Statistical distributions cannot be directly fitted to most probable number data (e.g., for *Campylobacter*). These data are defined on a number system in which no two numbers have equal occurrence probabilities under random selection; some numbers are actually impossible. Instead, a "binning" procedure can be deployed, with a distribution being fitted to the bins (e.g., McBride *et al*. 2002).
- Concentration distributions can be multimodal, so selecting a unimodal distribution from the standard set available in most software will underestimate the occurrence probability of extreme values, leading to potentially serious underestimations of risk. In such cases, either mixture distributions or empirical distributions must be used.
- The target population must be defined. In addressing regional or national policy issues, the target population may be exposed at many sites. In that case, the calculation for any particular time of exposure should assign a different, random pathogen concentration to each exposed person. That being so, the risk profile will tend to be rather flat, because at least a few sites may be sufficiently contaminated at any one time to give rise to infection. On the other hand, if the interest is in a particular site, then all people at that site can be at risk.
- While the above reasoning calls for a number of people (e.g., 1000) being exposed at any one time, it can often be satisfactory to use just one exposed person at each time. This makes for rapid calculation. However, for very infective organisms (e.g., with a single-figure median infective dose), this strategy produces error. In an extreme case, consider a pathogen with a probability of infection given ingestion of a single particle of 0.34. If that one person is predicted to have ingested a particle, then that translates to about 340 predicted cases of infection per 1000 individuals; otherwise, there will be no cases. This is not tenable. What in fact will happen is that a few

of the 1000 people may become infected — many less than 340, but more than none at all.

- Presence/absence data are problematical for risk analyses. One must use "added zeros" distributions and expert elicitation of bounds on concentrations (McBride *et al*. 2002).
- Dynamic epidemiologically based risk assessment models may be used to take account of secondary (person-to-person) transmission, long-term and short-term immunity, and environmental population dynamics (Haas and Eisenberg 2001; Soller *et al*. 2003).
- Risk profiles are best understood by policy-makers as predicted cases of infection per unit of the exposed population (e.g., per 1000 people).

29.4 CONCLUSIONS

QRA is but one technique in overall risk analysis. It has the potential to be particularly useful, especially for scenario modelling — the "what if?" approach. For example, if the efficacy of installation of wastewater reticulation and treatment systems or of riparian retirement can be established, then quantitative risk models can be used to look at likely improvements to human health. Yet many gaps remain to be filled before QRA can be used with confidence to help in setting policy and managing use of water resources. The greatest deficiency appears to be the absence of dose–response data for many waterborne zoonotic pathogens. All this being so, the case for performing quality audits of waterborne risk assessment (Macgill *et al*. 2001) is well made.

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Section IX

Future emerging waterborne zoonoses

30

Waterborne Zoonoses: Emerging pathogens and emerging patterns of infection

V.P.J. Gannon, C. Bolin, and C.L. Moe

30.1 INTRODUCTION

Sharma *et al*. (2003) have defined an emerging pathogen as "any new, re-emerging, or drug resistant infection whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the future." This definition is derived from a report published by the Institute of Medicine of the National Academies in the USA in 1992 (Lederberg *et al*. 1992). Smolinski *et al*. (2003) of the Institute of Medicine have published

 Waterborne Zoonoses: Identification, Causes and Control. Edited by J.A. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D.O. Cliver, G.F. Craun, R. Fayer, and V.P.J. Gannon. Published by IWA Publishing, London, UK. ISBN: 1 84339 058 2.

another work entitled *Microbial Threats to Health: Emergence, Detection and Response* to address current concerns associated with emerging pathogens.

Emerging pathogens are characterized by their increasing prevalence and have the potential to become endemic, epidemic, and even pandemic in nature. Clinical illness associated with these emerging pathogens, compared with other closely related pathogens, may be more severe, transmitted more rapidly or widely within populations, or more difficult to prevent or treat. Each of these features raises the awareness and attention paid to the emerging pathogen by members of the scientific, public health, regulatory, and political communities. The emerging pathogen may be a unique organism that is very distantly related to other known human pathogens (e.g., Ebola virus) or may simply represent a change in phenotype of a common organism, such as a new serotype, a new virulence attribute, or a different antimicrobial resistance pattern (e.g., multiple antibiotic-resistant *Mycobacterium tuberculosis* and *Salmonella enterica* serovar Typhimurium). However, the emergence of specific pathogens may also result from the recognition of a new subtype of a familiar pathogen that has long been responsible for specific human diseases but only recently associated with them by the scientific community (e.g., *Helicobacter pylori* and stomach ulcers). Increasing attention to and widespread ability to recognize and diagnose an infectious disease are often accompanied by an apparent increase in the isolation of the pathogen and prevalence of associated infections. This increase in diagnoses may simply be an artefact of increased awareness and the spread of new diagnostic or laboratory testing capabilities. However, the recognition of emerging infections as a result of increased surveillance may also indicate that earlier data had underestimated the prevalence of disease associated with a particular pathogen. Therefore, the "emergence of a pathogen" includes the element of recognition as well as the potential recent evolution of a unique pathogen.

Several authors in this book have highlighted the importance of ongoing surveillance activities in providing a better understanding of the impact of waterborne zoonoses and in the identification of emerging new pathogens and trends in waterborne diseases (e.g., chapters 5, 11, 12, and 19). Commitments are required from both developed and developing countries to build, sustain, and coordinate global epidemiological surveillance systems to monitor the emergence of waterborne zoonotic pathogens. In addition, laboratory studies are needed to support the identification and risk assessment of new pathogens as well as new phenotypic variants of pathogens traditionally responsible for waterborne zoonoses. For example, there has been much concern on the part of water authorities when oocysts of *Cryptosporidium parvum* have been identified in water samples. However, recent research suggests that there are considerable

differences in the risks to human health posed by closely related but distinct subtypes of *Cryptosporidium parvum*, and improved methods for identification and characterization of these organisms would be beneficial in the management of risks associated with these parasites (chapter 16).

The sudden appearance and true increases in the prevalence of zoonotic pathogens are thought to be the result of changes in pathogens, the environment, the animal reservoir, the human host, or the type or frequency of contact between animals and humans. In many cases, more than one of these factors may be involved in emergence of zoonoses.

30.2 EMERGING PATTERNS OF INFECTION

Water is often just one of several routes of transmission of the zoonotic pathogen, and the waterborne route is usually only clearly recognized in outbreak situations. Frequently sporadic disease and unrecognized outbreaks are the most common forms of infection with many zoonotic pathogens, and it is difficult to tease out the precise route of transmission, such as food, water, family contact, or animal contact, in these sporadic cases. With the increasing use of improved technology for the treatment of drinking-water supplies in large and medium-sized population centres, we would expect to see not only less waterborne disease associated with organisms susceptible to current water treatment processes, such as chlorination (e.g., *E. coli* O157:H7), but also less disease associated with organisms resistant to current water treatment processes (e.g., *Cryptosporidium parvum*). In spite of this optimism, authorities with responsibility for water treatment must continue to be vigilant and not complacent so that water treatment failures such as occurred in Walkerton, Ontario, Canada, in 2000 can be avoided (Hrudey *et al*. 2003). We would expect more sporadic disease associated with private wells, where there are problems with infrastructure, faulty equipment, and poor training, especially in rural areas where pathogens are abundant in animal reservoir populations. We would also expect to see more waterborne disease associated with the occupational and recreational use of water. Chapter 10 illustrates the shift away from transmission via drinking-water and the rise in recreational water-related waterborne zoonoses in the USA. Susceptible human populations (e.g., patients with human immunodeficiency virus) will experience more waterborne disease than the general population.

30.3 CAN WE PREDICT WHICH ZOONOSES WILL EMERGE?

Emergence of disease is a complex process involving elements related to the pathogen, the host, and the environment. A number of such factors have been identified as important in the emergence of diseases, and many of these are also likely to be important in the emergence of zoonotic waterborne infections. Microbial adaptation and change, changes in climate, in the environment, and in land use patterns, animal husbandry and animal waste management practices (concentrated animal feeding operations), political policies that lead to a breakdown in public health measures, war and displacement of populations, international travel and commerce, changes in the susceptibility of human and animal hosts, and changes in human behaviour have been identified as posing risks for disease emergence. This book's authors were challenged to determine if it is possible to predict the emergence of zoonotic waterborne diseases based on an analysis of the pathogen, host, and environment, as summarized below.

30.3.1 Changes in zoonotic waterborne pathogens

While certain pathogens "emerge" as a result of improved laboratory testing and increased surveillance, there are many examples of situations where new phenotypes emerge and become more common. These new phenotypes may be associated with increasing frequency of known disease conditions (e.g., *Vibrio cholera* O139; Faruque and Mekalanos 2003) or may be responsible for entirely new disease syndromes (e.g., severe acute respiratory syndrome [SARS]). Several chapters in this book provide examples of the appearance of new waterborne pathogens. There are also other examples of situations where the pathogens have become more prevalent (e.g., noroviruses) or have an altered life cycle in a particular region (e.g., *Fasciola hepatica*). Changes in the phenotype of a pathogen are the result of genetic change or mutation. These mutations may consist of the simple alteration of the four-base code that makes up the sequence of nucleic acids (DNA and RNA) or the addition or deletion of nucleic acids. These alterations can have profound effects on gene expression, including the amount of specific proteins produced, under which circumstances they are produced, and also the characteristics of these proteins. Most mutations of genes lead to the death of the pathogen. However, changes to the genome may also result in altered proteins or patterns of gene expression that provide new ways for a microorganism to survive, flourish, and extract more energy and material from the environment. While genetic mutation is relatively common, the co-

occurrence of the right mutation and opportunities to exploit this mutation are much rarer.

When we think of emerging pathogens, we consider circumstances that set the stage for pathogen evolution and occupation of a new niche. These can be changes in selective pressures in the environment or simply a mutation resulting in a new phenotype that is better able to survive in the existing environment. Mutation rates not only are an intrinsic property of the organism but also can be specific to genetic loci within the organism. Mutation rates are low in essential or housekeeping genes responsible for cell homeostasis, metabolism, and reproduction. Mutation rates in pathogens appear to be higher for surface-exposed membrane, envelope, capsule, or coat proteins. Changes in these proteins may be selected for as a means of survival. These surface-related changes are thought to aid the pathogen in 1) escape from the host's immune system, 2) defence against predators, 3) advantage against competitors, 4) facilitating attachment to surfaces, including a new host, 5) allowing entry of beneficial substrates, and 6) promoting the exclusion or exit of toxic substances.

30.3.2 Repeated nucleic acid segments

DNA replication is template driven and independently occurs in the two complementary strands of DNA. During replication, slippage of the template DNA strand downstream would result in the same sequence being recopied into the new strand. Alternatively, hybridization of the template DNA to a site upstream in the new strand may cause the DNA sequence not to be copied. This process can result in the generation of nucleic acid repeats and tandem repeats. These repeat structures are thought to play an important role in the evolution of protozoa (Wickstead *et al*. 2003) and bacteria (van Belkum 1999). The number of bases that are repeated varies, and repeats can occur within structural genes or outside of genes. Repeats in DNA within genes result in repeating amino acid motifs in proteins. The altered domains in the proteins can result in a change in receptor affinity of the expressed protein, leading to altered transport systems and receptor affinity for attachment proteins and toxins.

30.3.3 Genetic exchange

Plasmids in bacteria are examples of self-replicating segments of extrachromosomal DNA. They can be passed not only vertically from generation to generation but also horizontally through a plasmid-encoded transfer process termed conjugation. Plasmids and other naked pieces of DNA in the environment can also be taken up by the cell through a process termed transformation. While these elements may exist as separate entities within the

cell, they also have the capacity to integrate into the chromosomal DNA by recombination; these elements may be extracted from the chromosome again through a process termed excision. In bacteria, a variety of virulence attributes are encoded by genes on plasmids. These include toxins, adhesins, and invasion-associated proteins. Other genes provide a competitive advantage to the bacteria, such as substrate utilization, production of biocins that kill other bacteria, and antibiotic resistance. These traits are often linked and tend to accumulate on plasmids; in this way, use of one antibiotic may select for bacteria with plasmids that have multiantibiotic resistance and other virulence attributes.

30.3.4 Mobile genetic elements and recombination

While some bacteriophages can be viewed as bacterial parasites, there are many with so-called "temperate" stages in their life cycle, where they integrate into the chromosome of the bacterium and depend on the bacterial replication for survival. Recent studies of bacterial chromosomes have identified numerous large segments of DNA that are likely of bacteriophage origin (e.g., Ohnishi *et al*. 2001). Some of these DNA segments are functional bacteriophages that can produce viable progeny and lyse the bacterium, liberating new phages to infect other hosts. However, many of the phage-derived segments in the chromosomes of the bacteria are not functional as bacteriophages and have lost many phage-related genes. Other large DNA segments derived from bacteriophages or plasmids have inserted or "hopped" into the chromosome by transposition (Schneider *et al*. 2002). These externally derived DNA segments are thought to be very important in the evolution of many bacterial pathogens and can encode unique genes or operons that work together and coordinate important enzymatic or structural processes. Presumably, they confer phenotypic attributes that allow these bacteria to be more successful than other competing bacteria. Some of these segments or genetic "islands" have been shown to be necessary for the agent to be a human or animal pathogen and are referred to as pathogenicity islands (Karaolis *et al*. 2001; Faruque and Mekalanos 2003; Morabito *et al*. 2003).

30.3.5 Changes in the environment

Changes in the environment can lead to increases in zoonotic disease by a variety of mechanisms. Changes in ecosystems and encroachment on habitat often initiate or increase contact between different species of animals. This contact can precipitate "jumping" of pathogens from one species to another (e.g., SARS) or may greatly enhance the transmission rate of some diseases. Global climatic change may increase the range of vector species, resulting in the

spread of an existing zoonotic disease to new areas. Climatic and natural events, such as flooding, storms, and fires, often precipitate outbreaks of disease associated with disruption and contamination of water treatment systems, displacement of persons and animals from their usual habitats, lack of sanitation, and enhanced spread of infectious agents. Outbreaks of leptospirosis that frequently follow flooding are good examples of the influence of climatic events and natural disasters on the emergence of zoonotic infectious disease.

30.3.6 Changes in the human or animal host

Human and animal hosts may become more susceptible to disease because of famine, malnutrition, chronic disease, and immunosuppression. Movements of humans and animals because of natural disasters, famine, conflict, etc. may result in immunologically naive populations entering areas with endemic infection. As the density of human and agricultural animal populations increases, often in close proximity, the risk of emerging infections increases due to concentrations of susceptible hosts, increased contamination of the environment because of improper waste disposal, and increased opportunities for transmission. Concentrations of mixed species of animals (including humans) in close proximity makes an event uncommon under normal circumstances — i.e., the jumping of a pathogen from one species to another — more probable and the consequences of that change in host range more serious. Changes in human behaviour are also thought to play an important role in emerging zoonoses. These changes include increased travel, increased recreational activities involving water and remote locales, and ecotourism.

30.4 WATERBORNE ZOONOSES LIKELY TO EMERGE OR RE-EMERGE

While it is not possible to predict precisely, several agents were identified in this book as having the potential to represent significant risks for emergence or re-emergence. In addition, several agents were discussed because of their particular concern to public health officials (e.g., prions, SARS), but that seem unlikely to represent real risks of waterborne transmission.

30.4.1 Bacteria

The family Enterobacteriaceae contains a large number of human pathogens belonging to genera such as *Escherichia*, *Klebsiella*, *Salmonella*, *Shigella*, and *Yersinia*. Members of this family are known for infecting a wide variety of hosts and induce a variety of clinical syndromes associated with a large number of

groups, types, and serotypes (chapter 13; Iwobi *et al*. 2002; Liu *et al*. 2002; Schneider *et al*. 2002; Dobrindt *et al*. 2003). Considerable phenotypic plasticity also occurs within Vibrionaceae, Campylobacteriaceae, and Spirochetes, such as *Leptospira* (Karaolis *et al*. 2001; Boer *et al*. 2002; Zuerner and Huang 2002; Faruque and Mekalanos 2003). These groups represent important sources of emerging waterborne zoonotic pathogens. Fortunately, these bacterial agents are susceptible to chlorine and ultraviolet (UV) light. Infections associated with these organisms are likely to persist where there is little or no drinking-water treatment and for those involved in occupational or recreational use of contaminated water (Levett 2001; Hrudey *et al*. 2003). Certain other specific bacterial pathogens, such as *Mycobacterium paratuberculosis* and other members of the *Mycobacterium avium* complex, are common in animal populations in certain regions and more resistant than other bacteria to heat and oxidation (Grant *et al*. 1996; Stabel *et al*. 1997; Falkinham 2003) and could pose a risk of waterborne illness, particularly in immunocompromised individuals. Organisms such as *Francisella tularensis* (Anda *et al*. 2001) and *Burkholderia pseudomallei* (Dance 2002) may emerge as naturally occurring waterborne pathogens or from intentional introduction. *Bacillus anthracis* spores may also pose a risk for waterborne infection. These spores are hardy and would require UV light inactivation (Beatty *et al*. 2003; Nicholson and Galeano 2003).

30.4.2 Parasites

The phylum Apicomplexa consists of a large number of obligate intracellular parasites, including the genera *Plasmodium*, *Toxoplasma*, *Cryptosporidium*, and *Cyclospora*. Members of the last three genera include waterborne zoonotic pathogens and form cysts that are resistant to chlorination (Marshall *et al*. 1997; Wright and Collins 1997). UV light and ozonation are effective in inactivating these pathogens, and microfiltration is effective in removing the cysts from water (Morita *et al*. 2002; Biswas *et al*. 2003; Hsu and Yeh 2003). Infections associated with these organisms are likely to persist where 1) there is a source of cysts infectious for humans, 2) there are failures in filtration processes, 3) there are significant precipitation events, and 4) sophisticated multi-step barriers for drinking-water and recreational water protection are not in place. The amitochondriate parasites *Giardia intestinalis* and *Entamoeba histolytica* offer similar challenges to water authorities (Wright and Collins 1997; Steiner *et al*. 1998).

30.4.3 Viruses

Enteric viruses have been detected in drinking-water and environmental waters in industrialized and developing countries (Deetz *et al*. 1984; Gratacap-Cavallier *et al*. 2000; Jothikumar *et al*. 2000; Borchardt *et al*. 2003). Traditional virology has long believed that viruses are quite host-specific, but there are numerous examples of new viral pathogens identified in humans that may have animal origins (e.g., SARS, hepatitis E virus [HEV], some strains of rotavirus A, some strains of noroviruses). All of these viruses are excreted in faeces, so it is possible that they may be transmitted by water contaminated by human or animal faeces.

SARS virus appears to be a new cause of human infections. HEV is newly recognized, but has been implicated retrospectively in hepatitis outbreaks dating back to the 1950s (Jothikumar *et al*. 1993). Recent studies suggest that there may be a close link between human HEV and swine HEV (chapter 15), and there is new evidence of interspecies transmission of HEV (Meng 2003). Some of these emerging pathogens may actually be new viruses that may have evolved through random genetic changes in the genomes of animal viruses that allowed them to bind and enter human host cells. RNA viruses (SARS, coronavirus, HEV, rotavirus A, and noroviruses) are known to have high mutation rates during replication in host cells. Some bovine and swine norovirus strains are genetically very similar to some human norovirus strains (van Der Poel *et al*. 2000), suggesting that they evolved from a common ancestor. Other new viral pathogens may have arisen through recombination events where an animal or human becomes co-infected with two different viruses that recombine during replication in the host cell. The new reassortant strain has portions of the genomes of both viruses. Animal rotaviruses have been detected in drinking-water, and other investigators have speculated on the role of water in the spread of animal strains to human populations and the emergence of reassortant strains (Gratacap-Cavallier *et al*. 2000). Bovine–human reassortant strains have been detected in infants in Bangladesh (Ward *et al*. 1996) and may possibly have been transmitted from humans to cows or vice versa via faecal contamination of water.

There is little information on the environmental persistence of these new viral pathogens and their removal or inactivation during water treatment. Most of our understanding of virus inactivation by water treatment processes comes from studies of human viruses (Hurst 1991). It is possible that some animal or reassortant strains may be more resistant than human viruses to water treatment and should be further investigated.

30.4.4 Prions

Bovine spongiform encephalitis (BSE) may have arisen spontaneously in cattle or originated from scrapie in sheep (Baylis *et al*. 2002; Manuelidis 2003; Smith 2003). Transmission of this transmissible spongiform encephalopathy (TSE) to cattle is thought to have initially occurred as a result of the feeding of scrapie-contaminated bovine or ovine meat and bone meal (MBM). Continued feeding of BSE-infected MBM is thought to have been responsible for the subsequent expansion of the BSE epidemic. Human consumption of BSE-infected beef products is thought to have been responsible for the variant Creutzfeldt-Jakob disease (vCJD) epidemic in humans that subsequently occurred in the United Kingdom. A ban on MBM feeding in cattle and other measures appear to have stopped the BSE epidemic in the United Kingdom and will presumably also stop the vCJD epidemic in humans in this region with time.

In contrast to BSE in cattle, scrapie in sheep and chronic wasting disease (CWD) in cervids appear to be readily transmitted horizontally to other animals. With the scrapie and CWD agents, infected animals are thought to contaminate the environment with infected tissues such as the placenta, and faecal excretion of these agents has also been postulated (Millar and Williams 2003). Subsequent persistence of scrapie and CWD agents in soil is thought to play an important role in animal-to-animal transmission of these TSEs. However, water is not considered a likely route of transmission for any of the TSE-related prion proteins, as they are very insoluble in water (Gale 2001). Various types of animal-derived prions, in addition to BSE, may be or may become infectious for humans. The most likely route of infection would be foodborne rather than waterborne. If environmental routes of horizontal transmission of scrapie and CWD infection posed a substantial risk for humans, we would expect to see CWD infections first in more closely related species that often share the same habitat (e.g., free-ranging cattle; Salman 2003), but this has not occurred. Epidemiological studies have thus far failed to show an association between consumption of potential sources of scrapie such as mutton and lamb or sources of CWD such as venison with CJD in humans (Davis *et al.* 2003).

However, as we have learned from the BSE and vCJD epidemics in cattle and humans, respectively, a species cross-over with these TSE agents may be just a matter of opportunity and time. Therefore, efforts on measures such as TSE eradication programmes must be made now to diminish the probability of such events in the future.

30.5 CONCLUSION

An increased understanding of the interplay between changes in pathogens, the environment, and the host and emergence of disease is developing based on thorough study by microbiologists, ecologists, infectious disease experts, geographers, and others. Progress in these areas is likely to continue, with an increasing emphasis on interdisciplinary teams of investigators, broad-based surveillance efforts, and enhanced cooperation between public health, animal health, and environmental health authorities. As new risks for waterborne zoonotic diseases are identified, authorities need to be able to respond in a scientifically based manner to address the threat where it occurs. This will require cooperation, sharing of information and resources, sound risk assessment, and multiple prevention and control strategies that are scalable and can be applied in low- and high-resource environments.

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