
Section V

Categories of waterborne disease organisms

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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,

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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 cayentanensis* 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.

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

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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 <i>E. coli</i>	VTEC or STEC	<i>E. coli</i> that produce verocytotoxin (Shiga toxin) VT1 and/or VT2	Diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome (HUS)
Enterotoxigenic <i>E. coli</i>	ETEC	<i>E. coli</i> that produce enterotoxins that are heat stable (STh, STp) and/or heat labile (LT)	Acute watery diarrhoea
Attaching and effacing <i>E. coli</i>	A/EEC	<i>E. coli</i> that attach to and efface the microvilli of enterocytes, but do not produce high levels of verocytotoxin	Acute or persistent diarrhoea
Enteropathogenic <i>E. coli</i>	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
Enteraggregative <i>E. coli</i>	EAggEC	<i>E. coli</i> that exhibit a pattern of aggregative adherence to tissue culture	Acute watery, often protracted diarrhoea
Diffuse adherent <i>E. coli</i>	DAEC	<i>E. coli</i> that exhibit a pattern of diffuse adherence to tissue culture	Acute or persistent diarrhoea
Enteroinvasive <i>E. coli</i>	EIEC	<i>E. coli</i> that share virulence determinants with <i>Shigella</i> 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 EAaggEC 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.

EPEC is a leading cause of diarrhoeal disease in humans and domestic animals. EPEC is, however, not a zoonosis. Waterborne transmission of EPEC 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 EAaggEC 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 <i>et al.</i> 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 <i>et al.</i> 1992
Oregon, USA	1991	O157:H7	<i>Shigella sonnei</i>	Prolonged outbreak from lake water. The unusually long duration supported the notion that <i>E. coli</i> O157 survive well in lake water and have a low infectious dose.	Faecal contamination from bathers	21 with <i>E. coli</i> O157 and 38 with <i>S. sonnei</i>	Keene <i>et al.</i> 1994
Scotland	1992	O157:H7		Children playing in a paddling pool	Human faecal contamination	1 case ill, 5 carriers, 1 case of HUS	Brewster <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 1996
New York State, USA	1994	O157:H7		Lake water at a country park	Faecal contamination from bathers or animals	12 persons ill	Ackman <i>et al.</i> 1997
Scotland	1995	O157:H7	<i>Campylo-bacter jejuni</i>	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 <i>et al.</i> 1999
Finland	1997	O157:H7		Freshwater lake	Human faecal contamination	5 primary and 8 secondary cases	Paunio <i>et al.</i> 1999
USA	1998	O157:H7		Improperly chlorinated swimming pool	Faecal contamination from bathers	18 persons ill	Friedman <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 2001
New York State, USA	1999	O157:H7	<i>Campylobacter jejuni</i>	Drinking water supply at Washington County Fair. At least one shallow well was contaminated with <i>E. coli</i> 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 <i>et al.</i> 2003
Connecticut, USA	1999	O121:H19		Freshwater lake	Transient local contamination from a sick child	11 persons ill, 3 cases of HUS	McCarthy <i>et al.</i> 2001
Washington, USA	1999	O157:H7		Freshwater lake	Faeces from ducks		Samadpour <i>et al.</i> 2002
California, USA	1999	O157:NM		Freshwater lake	Faeces from humans, cattle, or deer	7 ill persons	Feldman <i>et al.</i> 2002
Ontario, Canada	2000	O157:H7	<i>Campylobacter jejuni</i>	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; Hrudney <i>et al.</i> 2003
Japan	2001	O26:H11		Secondary (untreated) water source	Faeces from wild animals	1 person ill, 5 other carriers	Hoshina <i>et al.</i> 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

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 °C, at pH 4–8, and at water activities above 0.93 (Baird-Parker 1991). Under special conditions, they may proliferate below 4 °C (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
<i>S. enterica</i> subsp.	
<i>enterica</i>	1478
<i>salamae</i>	498
<i>arizonae</i>	94
<i>diarizonae</i>	327
<i>houtenae</i>	71
<i>indica</i>	12
<i>S. bongori</i>	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.

14.6.1.4 Pigs

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 oropharyngeal 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 $-5\text{ }^{\circ}\text{C}$, is capable of growth at temperatures up to $44\text{ }^{\circ}\text{C}$ (optimum range of $22\text{--}28\text{ }^{\circ}\text{C}$), 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

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 ZOOSES

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 (Karenyi *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 (Karenyi *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

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 paromomycin 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 $10 \times 12 \mu\text{m}$ 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 Ivaí, 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 $2565 \times g$ ranged from 35.8 to 82.5%, and recovery by flocculation with aluminium or iron sulfate ranged from $35.9\% \pm 12.3$ to $100.3\% \pm 26.9$ (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 takes 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 log₁₀ for *Giardia* and 4 log₁₀ 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-log₁₀ 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.

16.7 REFERENCES

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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.

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 coprophagic.

Table 17.1. Reports of *Cyclospora cayetanensis* in water

Location	Type of water	References (based primarily on Ortega <i>et al.</i> 1998)
Chicago, USA	Water reservoir	Anon 1991; Huang <i>et al.</i> 1995
Nepal	Untreated water	Hoge <i>et al.</i> 1993
Chicago, USA	Lake water	Wurtz 1994
Nepal	River/municipal water	Rabold <i>et al.</i> 1994
Utah, USA	Farm water	Hale <i>et al.</i> 1994
New York, USA	Water cooler	Carter <i>et al.</i> 1996
Massachusetts, USA	Well water	Ooi <i>et al.</i> 1995
Peru	Wastewater	Sturbaum <i>et al.</i> 1998
Nepal	Sewage water	Sherchand and Cross 2001
Vietnam	Tap water	Cam <i>et al.</i> 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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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

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; Kùchle *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. matthei*, 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*, *Austrotilharzia 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

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) autochthonous, 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* spp. 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. swinhoi* 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 olitor* (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% *Senecio* 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. (Cyanofitas), 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*, *Petroselinum 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.

19.11 REFERENCES

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Leptospirosis and other potential zoonoses in water

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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.

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 (<i>n</i>)	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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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/cm²) 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. bienersi*, 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. bienersi* (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) (Petruccioli *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
Encephalomyocarditis	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 <i>et al.</i> 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 <i>et al.</i> 2000
Influenza A	Yes, between birds and mammals	Pigs may serve as an intermediate 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.

20.7 REFERENCES

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