Assessing the importance of zoonotic waterborne pathogens

Kumar Govind Suresh, Gary A. Toranzos, Ronald Fayer, Veeranoot Nissaparton, Remigio Olveda, Nicholas Ashbolt and Victor Gannon

2.1 INTRODUCTION

Globally, over 1400 pathogenic microorganisms (bacterial, viral, parasitic and fungal) are thought to be associated with human illness (Cleaveland et al. 2001) and among these human pathogens, approximately 61% are considered to be of animal origin (Taylor et al. 2001). Only a handful of these zoonotic agents are, however, convincingly and consistently associated with waterborne disease in human populations around the world.

While many species of microorganisms associated with disease in humans can also be isolated from animal sources, this does not necessarily mean that human infections result from direct or indirect transmission of the pathogen from
animals to humans. Recent developments in genotyping and genome sequencing have allowed the ecological distinction of many subtypes of pathogenic microbes that may have different host preferences. Information generated by high-resolution typing has provided evidence which either supports or casts doubt on assertions that particular pathogen subtypes are truly zoonotic. Certain protozoan species such as *Cryptosporidium parvum* and *Giardia lamblia* (*duodenalis*) were previously all considered to be zoonotic. It is now recognized, however, that a limited number of genotypes or assemblages within these species complexes are indeed transmitted from animals to man (Fayer *et al.* 2010, Feng & Xiao 2011, Thompson & Smith 2011). Similarly, among species of bacterial and viral pathogens of animal origin, genetic subtypes or lineages differ significantly in the frequency with which they are associated with human disease and in the severity of this disease (Lan *et al.* 2009, Pavio *et al.* 2010, Sheppard *et al.* 2010, Teshale *et al.* 2010, Zhang *et al.* 2010, Medina *et al.* 2011).

Crossing of the species boundary and changes in the spectrum of hosts which can become infected appear to occur more readily with organisms such as influenza viruses (Medina & García-Sastre 2011). In other pathogenic microbial species, such host specificity mutations may have occurred thousands of years ago (Sheppard *et al.* 2010). Truly zoonotic pathogens are capable of infecting both human and animal hosts, but often the animal populations serve as reservoir and amplifying hosts. Co-evolution of the pathogen and the reservoir host may dampen the effects of infection on the animal and overt clinical disease may eventually be no longer evident (Karmali *et al.* 2010). Humans, by contrast, are often aberrant or “dead-end” hosts for these pathogens and may not play a significant role in pathogen maintenance or perpetuation. However, these pathogens can cause severe disease in humans. The risks of infection and the adverse health consequences are, as a rule, significantly greater for individuals whose immune status is somehow compromised than for healthy members of the community.

This immuno-compromised group includes otherwise healthy individuals whose immune systems have not yet matured or who have been compromised by infectious, physical or chemical agents, or inherited defects in the immune system; they include specific age and gender classes such as infants, the elderly, and pregnant women. A much greater number of opportunist agents are likely to be associated with sporadic infections in immuno-compromised individuals than outbreaks at the community level. Such outbreaks of, for example, waterborne disease do occur, however, as a consequence of intake of high concentrations of particular pathogens which overcome defence mechanisms of otherwise healthy individuals, and infections with particularly virulent pathogenic species or subtypes within a species.
Newly identified pathogens are of particular concern, as are those whose prevalence appears to be going through a rapid increase or whose geographical distribution is expanding significantly. Such processes have the potential for widespread and serious public health consequences. A pathogen or disease-causing agent is considered “emerging” when it makes its appearance in a new host population or when there is a significant increase in its prevalence in a given population (Cleaveland et al. 2007). Reperant (2010) suggests that emerging pathogens are the result of changes in and/or between the disease-affected host species and the reservoir population and/or vector species which act as a source of the pathogen. Examples of this would include changes in host or reservoir population densities and spatial distribution. While emerging waterborne pathogens are not the focus of this Chapter, the reader may wish to be aware of specific infectious agents that may be increasing in prominence.

2.2 RANKING ZOONOTIC PATHOGENS ASSOCIATED WITH WATERBORNE DISEASES

Public health agencies have used a variety of criteria to rank zoonotic pathogens (Cardoen et al. 2009, Haagsma et al. 2008, Havelaar et al. 2010, Craun et al. 2010) in response to the need to allocate limited public resources strategically to areas of prevention, diagnosis and treatment where the largest social and economic benefits can be realized. Along similar lines, Table 2.1 lists criteria to rank waterborne pathogens. This ranking considers

(1) Evidence that the pathogen is indeed zoonotic (i.e. genotypes of isolates from animals are highly related or identical to those found in humans),

(2) Waterborne transmission is known to be a significant route of infection for humans based on case-control studies (with an odds ratio >2.0) and/or based on molecular epidemiology,

(3) The frequency and severity of waterborne illness,

(4) The geographical distribution of waterborne illness associated with the pathogen,

(5) Evidence that the pathogen is “emerging”, that is increasing prevalence or geographic distribution, and,

(6) Resistance to water treatment and other remediation efforts.

Criteria 1 and 2 are the same as those used by Craun et al. (2010) in the classification of agents responsible for outbreaks associated with contaminated drinking-water in the United States.
Pathogens ranking 1 and 2 applying these criteria will be the focus of discussion in the remaining chapters. While those that ranked lower may be of significant importance in some parts of the world or under specific circumstances, epidemiological data suggests that the organisms in the first two categories pose the greatest global public health risks, and that control of these pathogens in animal populations, improved waste management, run-off controls and remediation, effective monitoring of water contamination, better water storage
Table 2.1 Criteria used to rank zoonotic pathogens and their association with waterborne diseases.

<table>
<thead>
<tr>
<th>Ranking by importance</th>
<th>Criteria description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>There is strong evidence that the organism is zoonotic (i.e. genotypes from animals are highly related or identical to those found in humans).</td>
</tr>
<tr>
<td></td>
<td>Waterborne transmission has been demonstrated to be a significant route of infection (i.e. case-control studies implicate water as the source of infection with an odds ratio &gt; 2.0).</td>
</tr>
<tr>
<td></td>
<td>Disease outbreaks occur in healthy humans.</td>
</tr>
<tr>
<td></td>
<td>Disease in humans results in serious illness and/or death.</td>
</tr>
<tr>
<td></td>
<td>The pathogen is global or nearly global in distribution or there is evidence that the prevalence of waterborne disease is increasing and there may be evidence of spread from one geographical region to another.</td>
</tr>
<tr>
<td></td>
<td>Agents are resistant to water treatment procedures or other remediation efforts.</td>
</tr>
<tr>
<td>2</td>
<td>As in 1, except that agents are inactivated by water treatment procedures such as chlorination.</td>
</tr>
<tr>
<td>3</td>
<td>As in 1, except that disease in humans associated with the agent is often endemic to specific regions of the world and/or in relation to certain activities for example occupational exposure.</td>
</tr>
<tr>
<td>4</td>
<td>As in 1, except that waterborne disease transmission only occurs rarely, or may or may not be an important route of infection or the evidence base for it has not been rigorously established.</td>
</tr>
</tbody>
</table>

and treatment infrastructure, effective therapies, effective policies and behaviour modification will have as a secondary effect the reduction of all waterborne diseases.

2.3 WATERBORNE PATHOGENS

2.3.1 Protozoa

*Cryptosporidium* species RANK 1 *Cryptosporidium*, an apicomplexan protozoan, is reported to infect humans in 106 countries and has been found in more than 150 mammalian species worldwide (Fayer 2008). Estimates of prevalence in humans vary greatly because reporting is not universally required, diagnostic methods
vary greatly and ill people in many countries have no access to health care or do not seek it. At least 325 water-associated outbreaks of parasitic protozoan disease have been reported; North America alone accounts for approximately 66% and, together, North America and Europe for 93% of all reports (Karanis et al. 2007). In 16 European countries, 7,960 cases of cryptosporidiosis were reported in 2005 (Semenza & Nichols 2007). In the USA, 3,505 cases were reported in 2003, 3,911 in 2004 and 8,269 in 2005 (Yoder & Beach 2007a). The greatest number of reported cases were children under ten years of age and adults 30–39 years of age, with a seasonal peak coinciding with the summer recreational water season, reflecting increased use of rivers, lakes, swimming pools and water parks (Yoder & Beach 2007b). Recreational waterborne outbreaks (n = 68) primarily associated with swimming pools and water parks have affected 4,592 persons in Australia, Canada, Japan, New Zealand, Spain, Sweden, England, Wales, and Scotland (Beach 2008). Another 68 similar recreational water outbreaks involving 14,679 persons were reported in the USA (Beach 2008). In Thailand, health risks have been associated with recreational exposure to urban canal water where Cryptosporidium and Giardia are estimated to cause ∼47% of diarrhoea cases (Diallo et al. 2008). In the latter study, in three canals receiving municipal, agricultural, and industrial wastewater there was a significant load of Cryptosporidium hominis, indicative of human, not animal sources. Likewise, the warm weather recreational use of water throughout the world is a temporal effect primarily associated with an anthroponotic cycle in swimming pools and other treated water venues.

Despite evidence of ubiquitous contamination of freshwater lakes and rivers with Cryptosporidium, only 12 outbreaks of cryptosporidiosis have been associated with recreational use of these waters in the USA and England, (Beach 2008). These include eight lakes, two rivers/streams and two hot springs. Numerous studies in the USA, Canada, Scotland, Ireland, Germany, Finland, Israel, Australia, Japan, the Chinese Province of Taiwan, and Hong Kong SAR have reported the presence and concentration of Cryptosporidium oocysts in surface waters destined to serve as a drinking-water source (Clancy & Hargy 2008). Of 325 water-associated outbreaks of parasitic protozoan disease documented worldwide, 23.7% were caused by Cryptosporidium spp. that either passed through filtered or unfiltered drinking-water systems, or contaminated water distribution systems in small and large community water systems (Karanis et al. 2007). Most of these studies did not use molecular methods to verify the pathogen species or genotypes and therefore it was not possible to determine whether the source was human or animal excreta.

The oocyst stage, excreted in faeces, is ubiquitous in the environment, is resistant to many disinfectants (including chlorine), remains infectious for months under moist...
Table 2.2  A listing of the waterborne zoonotic pathogens belonging to groups 1–4 based on the ranking criteria outlined in Table 2.1.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Severity of disease</th>
<th>Evidence for waterborne transmission</th>
<th>Killed by chlorination of water</th>
<th>Principal animal reservoirs</th>
<th>Distribution</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoan Cryptosporidium parvum</td>
<td>Outbreaks in healthy humans. Diarrhoea. Low case fatality</td>
<td>Yes</td>
<td>No</td>
<td>Cattle, other animals</td>
<td>Worldwide</td>
<td>1</td>
</tr>
<tr>
<td>C. hominis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protozoan Giardia duodenalis</td>
<td>Outbreaks in healthy humans. Diarrhoea. Low case fatality</td>
<td>Yes</td>
<td>No</td>
<td>Beavers, porcupines, dogs, other animals, cattle</td>
<td>Worldwide;</td>
<td>1</td>
</tr>
<tr>
<td>Bacteria Escherichia coli O157:H7</td>
<td>Outbreaks in healthy humans. Haemorrhagic colitis and hemolytic uremic syndrome, occasionally. Long-term systemic sequelae. Moderate case fatality</td>
<td>Yes</td>
<td>Yes</td>
<td>Cattle and other ruminants</td>
<td>Worldwide</td>
<td>2</td>
</tr>
<tr>
<td>Pathogen</td>
<td>Severity of disease</td>
<td>Evidence for waterborne transmission</td>
<td>Killed by chlorination of water</td>
<td>Principal animal reservoirs</td>
<td>Distribution</td>
<td>Rank</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------</td>
<td>---------------------------------------</td>
<td>---------------------------------</td>
<td>-----------------------------</td>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>Bacteria</td>
<td><em>Salmonella enterica</em> subspecies <em>enterica</em> (1,500 serovars)</td>
<td>Outbreaks in healthy humans. Diarrhoea. May result in septicemia. Moderate case fatality</td>
<td>Yes</td>
<td>Yes</td>
<td>Poultry, swine, cattle, horses, dogs, cats, wild mammals and birds, reptiles, amphibians</td>
<td>Worldwide</td>
</tr>
<tr>
<td>Bacteria</td>
<td><em>Leptospira interrogans</em> (200 serovars) in 23 serogroups</td>
<td>Endemic regions but outbreaks occur in healthy humans. May result in septicemia. Moderate case fatality</td>
<td>Yes</td>
<td>Yes</td>
<td>Domestic and wild animals, common in rodents, dogs</td>
<td>Worldwide</td>
</tr>
<tr>
<td>Bacteria</td>
<td><em>Francisella tularensis subsp holarctica</em></td>
<td>Endemic regions but outbreaks occur in healthy humans. Lymphadenitis, septicemia. Low case fatality.</td>
<td>Yes</td>
<td>Yes</td>
<td>Rodents</td>
<td>Europe and Asia</td>
</tr>
<tr>
<td>Helminth – Trematode</td>
<td>Schistosoma japonicum</td>
<td>Endemic. Fever. Chronic hepatosplenic disease, impaired physical and cognitive development</td>
<td>Yes</td>
<td>Yes</td>
<td>Cattle, buffalo, swine, dogs, cats, rodents</td>
<td>Southeast Asia, China, Philippines</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>---------------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Helminth – Trematode</td>
<td>Clonorchis sinensis (Chinese liver fluke)</td>
<td>Endemic. Cholangitis and biliary obstruction, poor digestion of fats, cholangio-carcinoma</td>
<td>Yes</td>
<td>Yes</td>
<td>Dogs, cats, swine, rats, wild animals</td>
<td>Asia 3</td>
</tr>
<tr>
<td>Helminth – Trematode</td>
<td>Fasciola hepatica</td>
<td>Endemic. Hepatic enlargement, jaundice, anemia, cholangitis, cholecystitis and cholelithiasis</td>
<td>Water-plant-humans</td>
<td>Yes</td>
<td>Cattle, sheep, other large ruminants (e.g. water buffalo)</td>
<td>Worldwide 3</td>
</tr>
<tr>
<td>Protozoan</td>
<td>Toxoplasma gondii</td>
<td>Outbreaks in pregnant women. Birth defects. Moderate case fatality</td>
<td>Yes, but uncommon</td>
<td>No</td>
<td>Mammals, especially cats, food animals, birds</td>
<td>Worldwide; common 2</td>
</tr>
<tr>
<td>Protozoan</td>
<td>Blastocystis hominis</td>
<td>Endemic and rare outbreaks, diarrhoea. Low case fatality</td>
<td>Yes</td>
<td>Yes</td>
<td>Various wild and domestic animals including birds, humans</td>
<td>Worldwide 3</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Severity of disease</th>
<th>Evidence for waterborne transmission</th>
<th>Killed by chlorination of water</th>
<th>Principal animal reservoirs</th>
<th>Distribution</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoan</td>
<td>Microsporidia</td>
<td>Unknown</td>
<td>No</td>
<td>Various wild and domestic animals, primates, rodents, psittacine birds</td>
<td>Worldwide</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Enterocytozoon bieneusis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Encephalitozoon cuniculi; E intestinalis; E hellem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endemic. Diarrhoea. Low case fatality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus</td>
<td>Hepatitis E virus (Genotypes 3 and 4)</td>
<td>Unknown</td>
<td>Yes</td>
<td>Swine</td>
<td>Probably worldwide</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Outbreaks in healthy humans. Systemic disease. Low case fatality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus</td>
<td>Rotavirus A</td>
<td>Unknown</td>
<td>Yes</td>
<td>pigs</td>
<td>Probably worldwide</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Outbreaks in healthy humans. Diarrhoea. Low case fatality.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
conditions and is responsible for transmission through person-to-person contact, contact with companion and farm animals, and ingestion with contaminated food, drinking-water and recreational water. Although Crypto. oocysts can be microscopically identified, they lack morphologic features for species identification; therefore, molecular tools are essential for species identification. Most species and genotypes of Crypto. appear host-specific or have a primary host and one or more less frequently infected host species. Of the current 20 species and approximately 60 genotypes of Crypto. that infect fish, amphibians, reptiles, birds and mammals nine species and seven genotypes are known to have infected humans (Plutzer & Karanis 2009). The two major species infecting humans are Crypto. hominis, transmitted from humans to humans, and Crypto. parvum, found primarily in pre-weaned (monogastric) ruminants, especially bovines. Crypto. meleagridis, described from avian and mammalian hosts, may also infect humans. Species causing rare infections include Crypto. andersoni, Crypto. baileyi, Crypto. canis, and Crypto. felis. The cervine genotype, found worldwide, is the only genotype with broad host range, found in wild and domestic ruminants, rodents, carnivores, and primates. It is the most common genotype found in rivers, streams and storm water and has been reported in over 20 human infections. Other rare infections include those caused by the monkey, pig, skunk, horse, rabbit and mouse genotypes. Most species and genotypes have been identified by SSU rRNA gene sequence data, although actin, HSP-70, and other genes are also used.

Crypto. hominis and Crypto. parvum subgenotypes have been defined by glycoprotein (GP) 60 gene sequence data, enabling more precise host (source) identification. Examination of this hypervariable locus and microsatellites has identified subgenotypes of Crypto. parvum found in animals, others found in both humans and animals, and still others found only in humans. Sequences of the GP60 gene strongly links Crypto. parvum from cattle with many of the same subgenotypes of Crypto. parvum found in sporadic human infections in Slovenia, Italy, Portugal, Spain, Ireland, Canada, the USA, Kuwait, Japan and Australia (Stantic-Pavlinic et al. 2003, Alves et al. 2003, Wu et al. 2003, Chalmers et al. 2005, Sulaiman et al. 2005, Trotz-Williams et al. 2006, Xiao et al. 2007, Thompson et al. 2007, Quilez et al.2008). Other mini- and micro-satellites used for subgenotyping also have identified human adapted strains of Crypto. parvum from humans and cattle (Mallon et al. 2003) and from persons reporting contact with animals (Hunter et al. 2007). The same subgenotype found in humans and cattle in Portugal also was detected at sampling sites from which water is supplied to the city of Lisbon (Alves et al. 2006).

Cattle are the major animal source of Crypto. parvum oocysts; infection in cattle is age-related. The prevalence of cryptosporidiosis in pre-weaned dairy calves (1–8
weeks of age), post-weaned calves (3–12 months of age) and heifers (12–24 months of age) was 45.8, 18.5, and 2.2%, respectively (Santin et al. 2008). The cumulative prevalence for *Crypto. parvum*, *Crypto. bovis*, *Crypto. ryanae* and *Crypto. andersoni* was 100%, 80%, 60% and 3.3%, respectively. *Crypto. parvum* constituted 97% of infections in pre-weaned calves but only 4% and 0% of infections in post-weaned calves and in heifers, respectively. In the USA the incidence in dairy cattle was not seasonal whereas in India cryptosporidiosis in cattle due to *Crypto. parvum* was most prevalent in the monsoon months (37.3%), with calves below 15 days of age mostly affected (45.1%) (Paul et al. 2008). Calves can excrete >10⁹ *Crypto. parvum* oocysts during a week of infection.

Three *Crypto. parvum* isolates (two from calves, one from a horse) were investigated in healthy adult volunteers (Okhuysen et al. 1999). The ID50 differed among isolates: 87 and 1042 oocysts from the calf isolates, and nine oocysts from the horse isolate. Symptoms and duration of infection can vary but diarrhoea and abdominal pain lasting about a week are common for immunologically healthy persons although asymptomatic infections have also been reported. Susceptibility to a wide range of species and genotypes, chronic diarrhoea, extraintestinal infection sites, dehydration, malnutrition/malabsorption, and even death may occur in immuno-compromised individuals.

**Giardia species RANK 1** *Giardia duodenalis*, is found worldwide and causes an estimated 2.8 × 10⁸ cases of giardiasis annually (Lane & Lloyd 2002). In Asia, Africa and Latin America there were an estimated 2.0 × 10⁸ cases with 0.5 × 10⁶ new cases reported each year. In developed countries *Giardia* is the most common intestinal parasite reported from humans. Of the 325 outbreaks of water-associated parasitic disease reported from North America and Europe, *G. duodenalis* and *Crypto. parvum* accounted for 132 and 165, respectively (Karanis et al. 2007). In the USA 20,084, 20,962 and 20,075 cases were reported for 2003, 2004 and 2005, respectively (Yoder & Beach 2007b). As for *Crypto.*., the greatest number of cases in the USA was reported in children under ten years of age and for adults 30–39 years of age with a seasonal peak in age-related cases coinciding with the summer recreational water season, possibly reflecting increased use of rivers, lakes, swimming pools and water parks. In Thailand, the highest risk for diarrhoea was *G. duodenalis* assemblages A and B from accidental ingestion of water when swimming in urban canals during the rainy season, particularly in the most polluted section, downstream of a large wholesale market (Diallo et al. 2008).

*G. duodenalis*, the species found in mammals, also appears in the literature under the names *G. intestinalis* and *G. lamblia.* It exists as a complex of seven morphologically indistinguishable assemblages or genotypes identified by the
letters A through G based on genetic differences. Only assemblages A and B have been detected in human infections and these vary geographically in prevalence. Assemblages (C–G) appear host-specific with C and D found in canids (dogs, wolves, coyotes) and cats, E in ruminants (cattle, sheep, goats, water buffalo, mouflons) and pigs, F in cats, and G in rats. The genes most often used for identification are β-giardin, SSU rRNA, glutamate dehydrogenase, and the triphosphate isomerase loci (Caccio & Ryan, 2008). The genome of isolate WB of assemblage A, subgroup A1 and isolate GS of assemblage B have been completely sequenced and are genetically so different that they most likely are distinct species (Franzen et al. 2009). Animals including livestock, companion animals and aquatic mammals (beavers and muskrats) have been considered potential sources of human infection but direct evidence is lacking. In studies employing genotyped specimens or reference strains of known genotype, assemblage A1 cysts were transmitted to dogs and assemblage B cysts from a Gambian pouched rat were transmitted to a human volunteer. Most studies attempting to understand the potential for zoonotic transmission of Giardia have identified assemblages A and B in animals. Although A1 is generally found in animals, and A2 mostly in humans, A2 and other A genotypes have been found in cattle, horses and dogs. Assemblage B has been found in cattle, dogs, cats, beavers, foxes and monkeys. To demonstrate zoonotic potential, more information is needed on the hosts of subtypes of A and B because interpretation based on existing loci is problematic, especially when different subtypes are identified using sequences of different genes. Experimental infection of humans and animals with the same isolate would confirm zoonotic capability.

Point prevalence surveys of dairy cattle in Australia, Canada, the Netherlands and the USA have reported that Assemblage E is predominant and lower levels of Assemblage A are present. A longitudinal study in the USA found giardiasis in dairy cattle from one week through 24 months of age, a prevalence peak at 4–8 weeks of age, and 100% and 70% acquired assemblages E and A, respectively. Infections after seven weeks of age when all calves had become infected, were due to the inability to clear initial infection or to reinfection. Assemblage B was reported in cattle in Italy, Canada, New Zealand and Portugal, two lambs were associated with an outbreak of giardiasis in sheep in Italy (Aloisio et al. 2006) and one healthy sheep in Spain (Castro-Hermida et al. 2007).

Infection can follow ingestion with as few as 10 cysts (Rendtorff 1954). Symptoms including diarrhoea, flatulence, greasy stools, abdominal discomfort, nausea and dehydration may begin about a week after ingestion of cysts and last two weeks or longer. Asymptomatic infections also have been reported. Chronic infections, usually in immuno-compromised persons, can result in prolonged
symptoms with severe dehydration, malabsorption, weight loss, and possibly mortality.

*Toxoplasma gondii* RANK 3 Toxoplasmosis is caused by the protozoan *T. gondii*; it is one of the most common parasitic infections worldwide. It is an economically important cause of disease in animals and produces a variety of clinical presentations in humans.

*T. gondii* is an obligate intracellular parasite with felids the only definitive hosts. The life cycle is complex. There are three infectious stages of *T. gondii*. Tachyzoites, crescent to oval shape, are seen in the active infection and are transmitted through the placenta from mother to fetus, by blood transfusion, or by organ transplantation. Tissue cysts, containing thousands of bradyzoites, are transmitted to persons or animals that eat infected meats or organs. They are associated with latent infection, and are reactivated in persons who lose their immunity. The oocyst stage, excreted only in domestic or wild cat faeces, is the most environmentally hardy form of *T. gondii*. It is ubiquitous in nature, is highly resistant to disinfectants and environmental influences, and plays a key role in the transmission through the faecal-oral route. Oocysts in environmental samples are detected by means of conventional parasite concentration methods including traditional mouse bioassays and by microscopy. PCR, a favoured molecular technique, has the potential that not only provides the sensitive and specific detection of *T. gondii* oocysts in water (Kourenti *et al.* 2004) but also reduces the detection time from weeks to one to two days.

Water reservoirs have been implicated as a source of toxoplasmosis outbreaks for more than two decades. In 1979, the first waterborne outbreak occurred in Panama; 39 soldiers who drank unfiltered, iodine treated water from streams possibly contaminated by jungle cats became infected. No other identifiable common sources of exposure were found (Benenson *et al.* 1982). In 1995 up to 7,718 persons became infected with toxoplasmosis from a municipal water supply in British Columbia, Canada (Bowie *et al.* 1997) that used unfiltered and chloraminated surface water. The likely source of contamination was cougar and/or domestic cats faeces (Aramini *et al.* 1999). In Santa Isabel do Ivaí, Brazil, waterborne toxoplasmosis was thought to be responsible for an outbreak involving 155 persons served by an underground tank reservoir delivering unfiltered water contaminated with faeces from cats that lived on top of the site (de Moura *et al.* 2006). Another outbreak reported in the same year occurred in Coimbatore, India, where 178 cases of toxoplasmosis were linked to a municipal water supply contaminated by heavy rainfall in catchment areas infested with domestic and wild cats (Palanisamy *et al.* 2006). In endemic toxoplasmosis, a high *Toxoplasma* prevalence related to drinking unfiltered water was found in Brazilian communities (Bahia-Oliveira *et al.* 2003), and in rural Guatemalan
children (Jones et al. 2005). In addition, in a Polish farm population, there was a positive correlation between drinking unboiled well water and the presence of *T. gondii* antibodies (Sroka et al. 2006). Based on these reports, it is possible that consumption of inadequately treated water or accidental drinking of recreational water from streams, lakes, ponds or wells explains human infection.

A small percentage of affected humans and animals develop symptomatic toxoplasmosis. It is not well understood whether the severity of disease depends on parasite genotypes, infection load, immune status, a combination of these or other factors. Severe cases of toxoplasmosis reported in humans were epidemiologically linked to ingestion of *T. gondii* oocysts from water (Benenson et al. 1982, Bowie et al. 1997, de Moura et al. 2006). Three clonal lineages of *T. gondii* strain types I, II and III (Howe & Sibley 1995) may be associated with human symptomatic toxoplasmosis. It has been demonstrated that type II isolates predominate in congenital (Nowakowska et al. 2006) and immuno-compromised patients (Lindström et al. 2006). In French Guiana and Suriname, *T. gondii* strains with atypical genotypes have been isolated from severe cases of toxoplasmosis in immuno-competent patients (Demar et al. 2007). Cases of severe or acquired ocular toxoplasmosis are more likely to be due to types I or recombinant genotypes (Grigg et al. 2001). In animals, mouse-virulent strains with atypical genotypes have been found in asymptomatic chickens and cats from Brazil (Pena et al. 2008). Two new genotypes (Types A and X) of *T. gondii* have been found as causes of meningoencephalitis and death in sea otters from North America (Sundar et al. 2008).

Domestic cats bury their faeces in soft and moist soil, which provides a high possibility of widespread environmental contamination. Cats can shed one million oocysts per gram of faeces (Schares et al. 2008) over a period of one to three weeks. Oocysts in faeces survived outdoor in Texas (6–36°C), uncovered, for 46 days, covered for 334 days (Yilmaz & Hopkins, 1972) and outdoors in soil buried at the depth of 3–9 cm in Kansas for 18 months (Frenkel et al. 1975). Oocysts in seawater (15 ppt NaCl) kept at 4°C, had a long-term survival of 24 months and were infectious to mice (Lindsay & Dubey 2009). Cats (domestic/wild) and other felines are highly exposed: in the USA, *T. gondii* antibodies were found among wild captive felids in zoos: 27.3% in cheetahs, 50% in African lynx, 54.5% in African lions, 28.8% in Amur tigers, 25% in fishing cats, 50% in pumas and 35.7% in snow leopards (de Camps et al. 2008). The seroprevalence of antibodies to *T. gondii* in livestock was found to be 68.7% in pigs (Dubey et al. 2008), 30% in goats, 35% in sheep (Sharif et al. 2007), 2.4% in cattle (Sharma et al. 2008), 38.1% in horses (Ghazy et al. 2007), 59.5% in turkeys, 47.2% in chickens and 50% in ducks (El-Massry et al. 2000). In coastal marine mammals, recent findings on prevalence of *T. gondii*...
antibodies were found in up to 100% of sea otters, 50% of seals, 100% of dolphins, and 61.1% in sea lions (Dubey et al. 2003). These epidemiological surveys are a good indicator of the extensive environmental contamination with *T. gondii* oocysts through infected raw chicken meat, raw beef, bird, free ranging domestic cats, and other mechanical vectors including flies, cockroaches, dung beetles, and earthworm.

*Toxoplasma* is a cosmopolitan parasite in humans. Seroprevalence rates vary according to geographical distribution, sample size and diagnostic methods. Based on epidemiological surveillance in general populations, approximately 30% in USA and the United Kingdom, 50–80% in Europe, 30% in Asia, 60% in South Africa, and 70% in South America are seropositive for *Toxoplasma* infection. The prevalence of human toxoplasmosis appears to be higher in less developed countries, in humid environment and plains, in adults and in persons in close contact with soil and animals. In the last two decades, water contamination with *T. gondii* has been implicated as a source of endemic toxoplasmosis (Carme et al. 2009) with outbreaks at both small (Palanisamy et al. 2006) and large (Bowie et al. 1997) scale worldwide. Acute infection in a previously uninfected pregnant woman can lead to a wide spectrum of clinical disease in congenitally infected children. Mild disease may consist of slightly diminished vision whereas severe cases may have eye, brain, and other organ involvement in the infant. Acute infection can also lead to ocular lesions and some loss of vision. Encephalitis, as a result of the reactivation of chronic *T. gondii* infection, is the most severe form of toxoplasmosis in immuno-suppressed patients and is a major cause of death in AIDS patients (Nissapatorn et al. 2004).

*Blastocystis* species RANK 3 *Blastocystis* is an emerging pathogen whose life cycle involves polymorphic stages including vacuolar, granular, amoebic and cystic forms (Zierdt 1991, Tan & Suresh 2006). *Blastocystis* infection causes diarrhoea, bloating of the stomach and other gastrointestinal symptoms with recent studies showing the existence of pathogenic and nonpathogenic “strains” (Tan et al. 2008). The organism has been shown to be present in a wide range of both captive and farm animals including birds (Boreham & Stenzel 1993, Abe et al. 2002). The prevalence of infection in animal workers is higher (44%) than in the normal population (17%) (Suresh et al. 2001), suggesting that close proximity with animals may facilitate transmission (Rajah Salim et al. 1999).

*Blastocystis* subtypes from humans and animals have been shown to have low host-specificity, comprising isolates from humans and various animal hosts. A number of studies provide evidence for zoonotic transmission of the parasite and cross-transmissibility among heterogeneous hosts (Abe 2004, Noël et al. 2005,
Yan et al. 2007, Rivera 2008). Human isolates of subtypes 4 and 7 were shown to be capable of infecting both chickens and rats (Iguchi et al. 2007). Parkar et al. (2007) demonstrated for the first time molecular-based evidence supporting the zoonotic potential of Blastocystis in dogs, possums and primates in a natural setting.

Polymerase Chain Reaction (PCR)-based genotype classification using known sequence-tagged site (STS) primers showed that out of 92 isolates from mammals and birds roughly two-thirds (67.4%) were identical with human Blastocystis hominis isolates (Yoshikawa et al. 2004) and 31.8% (7/22) of isolates from cattle and pigs (Abe et al. 2003a) and 12 isolates from primates (Abe et al. 2003b) examined were zoonotic genotypes of B. hominis. In another study, partial ssu rDNA of Blastocystis isolates from a human, a pig, and a horse were shown to belong to a common subgroup. Blastocystis isolated from a pig and a horse in the same study was shown to be monophyletic and have 92 to 94% identity with B. hominis (Thathaisong et al. 2003). Blastocystis isolated from chicken were also shown to be zoonotic using arbitrary primer PCR (Yoshikawa et al. 1996).

A recent study in Spain showed subtypes of Blastocystis obtained from symptomatic patients were similar to B. ratti from rats (Domínguez–Márquez et al. 2009). The pathogenic potential of human strains of Blastocystis in rats was evidenced by significant up regulation of the expression of interferon-γ, interleukin (IL)-12, and tumor necrosis factor alpha, but not IL-6 or granulocyte-macrophage colony-stimulating factor, in the caecal mucosa at two and/or three weeks post-infection (Iguchi et al. 2009). The first demonstration that cysteine proteases of B. ratti WR1, a zoonotic isolate, could activate IL-8 gene expression in human colonic epithelial cells further supports evidence for a zoonotic role for Blastocystis isolated from rats (Puthia et al. 2008).

Out of the 325 water associated outbreaks of parasitic protozoan disease reported, North American and European outbreaks accounted for 93% of all reports and nearly two-third of outbreaks occurred in North America. Two of these outbreaks were related to Blastocystis (Kourenti et al. 2007).

A survey of intestinal parasites among soldiers in Thailand demonstrated 21.9% of stools positive for Blastocystis; parasite incidence was statistically associated with the quality of drinking-water (Taamasri et al. 2000). Further evidence of waterborne transmission of cysts of Blastocystis was provided by a study where 334/904 stool samples from personnel in another Thai army camp were found to be positive for Blastocystis (Leelayoova et al. 2004). In the study, soldiers that consumed unboiled water were found to be more commonly infected with this protozoon (Tuncay et al. 2008; Kitvatanachai et al. 2008). Even more compelling evidence emerged from a subsequent study, where 18.9% of the 675
stool samples from school children in Thailand found positive for *Blastocystis* had subtypes similar to those found in the water samples, strongly suggesting that waterborne transmission had taken place (Leelayoova et al. 2008).

Another study implicating water to be a mode of transmission collected information through a detailed questionnaire given to 60 patients diagnosed with *B. hominis*, living in two localities of the Girardot municipality in Aragua State, Venezuela. It revealed that the affected age group was under ten years of age and drank water from lid-covered storage containers (Serna et al. 2005). Whether these water containers were contaminated is unknown. In a survey of potable water in Egypt, 1% of 840 samples were found to be positive for *Blastocystis* (Elshazly 2007). Viable cysts have been demonstrated in sewage effluent in Pakistan (Zaman et al. 1994) and in Scottish and Malaysian sewage and effluents (Suresh et al. 2005). Recently, viable cysts of *Blastocystis* have been isolated from recreational waters for the first time (Suresh et al. 2010).

In summary, *Blastocystis* is widespread in animals (including birds) and in human populations and there is increasing evidence, based on modern molecular typing methods, that many subtypes of *Blastocystis* can infect a number of host species including humans. The 3–4 µm cysts produced by the organism are robust and appear to be readily transmitted through water. Epidemiological evidence suggests that boiling and perhaps other treatment of drinking-water are required to decrease exposure to the pathogen. Due to these factors they feature among the most common intestinal parasites detected in human stool surveys carried out in developing countries in Asia and Latin America. Recent advances in the sensitivity of detection methodologies such as the in vitro culture method usually used to detect the organism in stools (Suresh & Smith 2004) will greatly facilitate stool and water surveys to detect the parasite and allow a better assessment of the extent of the zoonotic waterborne disease associated with this pathogen.

**Schistosoma species RANK 3** Schistosomiasis remains a major public health problem in countries where the disease is endemic. There are four species of blood fluke of the genus *Schistosoma* that parasitize humans namely, *S. mansoni*, *S. haematobium*, *S. japonicum* and *S. mekongi*. Globally, it is estimated that more than 200 million individuals are infected with schistosomes and around 800 million more are at risk of infection (Steinmann et al. 2006).

Humans are infected when they come in contact with water contaminated with the infective stage of the parasite. Contamination of water starts when eggs of the parasite from faeces of an infected host reach the water. These eggs give rise to miracidia which infect the snail intermediate host. The infective form of the parasite, cercariae, develop and are released from the snail. Cercariae can survive for 24 hours in the water at room temperature and can be carried more than 100
meters from the site of release from the snail. Types of bodies of water that may be contaminated include: open wells, springs, streams, irrigation canals and other hydraulic structures in irrigation schemes, rivers, impoundments, reservoirs and lakes. Risky behaviours that predispose individuals to schistosome infection include the use of bath and laundry water from a contaminated source, crossing affected streams, irrigation canals and rivers, agricultural activities, and fishing and swimming in infested rivers. It should be stressed that in addition to these risks of infection, there are also behaviours of schistosome-infected individuals that put others at risk such as urinating and/or defecating in fields, in or near water bodies, and lack of proper sanitation in general.

The disease is characterized by an acute phase usually occurring 2–12 weeks after cercarial skin penetration followed by the development of debilitating disease which can persist for years if left untreated. Symptoms include inflammatory and granulomatous reactions around the sites where eggs are deposited in the host’s tissues. The most commonly affected organs are the liver and intestines (by *S. mansoni*, *S. japonicum* and *S. mekongi*) and the genito-urinary tract (by *S. haematobium*).

Among the four species, *S. japonicum* is unique because it is the only species in which zoonotic transmission is considered important. In the People’s Republic of China and in the Philippines, around 60 million individuals are at risk and an estimated one million people are infected (Blas et al. 2004, Zhou et al. 2007). While a small focus of transmission persists on the island of Sulawesi in Indonesia (Izhar et al. 2002), the disease has been eliminated in Japan (Tanaka & Tsuji 1997). Humans and animals are a significant sources of these parasites. Animals that contribute significantly to the transmission cycle include cattle, water buffalo, pigs, goats, dogs and wild rats (Mao 1948; Lung et al. 1958, Maegraith 1958, Pesigan et al. 1958, Dumag et al. 1981, Fernandez et al. 1982, Zheng et al. 1989, Wu et al. 1992, Chen 1993, Brindley et al. 1995, Wan et al. 1998, McGarvey et al. 1999). Infections are transmitted naturally between man and animals with the infection being maintained by all of these species (Nelson 1975). Prevalence studies in animals have shown that cattle and buffalo are the most commonly infected animals in China. This finding is supported by studies on the spatial distribution of animal faeces in endemic areas of China showing that cattle dung contributes substantially to the transmission of *S. japonicum* in that country. While equivalent studies have not been done in the Philippines, studies do provide indications of the relative contribution of specific animals to *S. japonicum* transmission (Carabin et al. 2005). For example, Table 2.3 below presents a summary of findings from a study based on surveys in 50 barangays (villages) in Dagami, Leyte, the Philippines in 1979 (Dumag et al. 1981).
The remarkably high percentage of cercariae from field rats in this 1979 Leyte study suggests that they could be an important source of the pathogen for other animals and humans, despite the lower hatchability of the *Schistosoma* eggs. Recently, a parasitological survey across 50 villages in the province of Samar in the Philippines found a mean prevalence of 14.9% among dogs, the highest detected among all domesticated animal species sampled, with prevalence rates reaching up to 86.3% in some villages (Carabin *et al*. 2005).

Studies on the genetic characterization of parasite samples from these study areas showed a lack of genetic differentiation between parasite isolates from different definitive host species suggesting high levels of parasite gene-flow between host species, and thus also a high frequency of *S. japonicum* transmission among these species, particularly between dogs and humans. Dogs could thus potentially be an important zoonotic reservoir of *S. japonicum* in the Philippines province of Samar. This finding is in contrast to what has been found in marshland regions of China where parasite genotypes from humans have been demonstrated to cluster with cattle isolates and are distinct from isolates from other domesticated animals such as dogs, cats, pigs and goats (Jia-Gang *et al*. 2001). These findings suggest that there are different transmission patterns/roles for animal reservoir hosts in these two countries. It appears that cattle are more important in the transmission in China than in the Philippines.

In the Philippines, recent studies have shown a low prevalence of *S. japonicum* infection in carabaos (water buffaloes; Carabin *et al*. 2005). The low prevalence rates found in carabaos may just be a result of the test used in the stool examination because repeat examination of faeces from these animals by a PCR assay showed positive results for up to 25% of the animals (Wu *et al*. 2010). Positive PCR results are equivalent to an average of 5 eggs per gram.

**Table 2.3** Contribution of definitive hosts to transmission in 50 villages in Dagami, Leyte in 1979 (adapted from Table I in Dumag *et al*. 1981).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dogs</th>
<th>Pigs</th>
<th>Carabao</th>
<th>Rats</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Est Total Pop</td>
<td>1756</td>
<td>2672</td>
<td>1424</td>
<td>1,408,806</td>
<td>20,121</td>
</tr>
<tr>
<td>Prevalence % in sample</td>
<td>7.7%</td>
<td>4.2%</td>
<td>0.07%</td>
<td>73.7%</td>
<td>18.5%</td>
</tr>
<tr>
<td>Mean epg/dl</td>
<td>1747.8</td>
<td>1367.2</td>
<td>9513.7</td>
<td>12.7</td>
<td>11.2</td>
</tr>
<tr>
<td>Hatchability %</td>
<td>19.5%</td>
<td>30.8%</td>
<td>29.6%</td>
<td>10.7%</td>
<td>42.4%</td>
</tr>
<tr>
<td>Est. pop inf.</td>
<td>135</td>
<td>112</td>
<td>1</td>
<td>1,038,572</td>
<td>3723</td>
</tr>
<tr>
<td>Est # cerd ind-</td>
<td>339.9</td>
<td>421.6</td>
<td>2814.1</td>
<td>1.36</td>
<td>4.8</td>
</tr>
<tr>
<td>Est % Cerceriot</td>
<td>3.0%</td>
<td>3.1%</td>
<td>0.02%</td>
<td>92.6%</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

The remarkably high percentage of cercariae from field rats in this 1979 Leyte study suggests that they could be an important source of the pathogen for other animals and humans, despite the lower hatchability of the *Schistosoma* eggs. Recently, a parasitological survey across 50 villages in the province of Samar in the Philippines found a mean prevalence of 14.9% among dogs, the highest detected among all domesticated animal species sampled, with prevalence rates reaching up to 86.3% in some villages (Carabin *et al*. 2005).

Studies on the genetic characterization of parasite samples from these study areas showed a lack of genetic differentiation between parasite isolates from different definitive host species suggesting high levels of parasite gene-flow between host species, and thus also a high frequency of *S. japonicum* transmission among these species, particularly between dogs and humans. Dogs could thus potentially be an important zoonotic reservoir of *S. japonicum* in the Philippines province of Samar. This finding is in contrast to what has been found in marshland regions of China where parasite genotypes from humans have been demonstrated to cluster with cattle isolates and are distinct from isolates from other domesticated animals such as dogs, cats, pigs and goats (Jia-Gang *et al*. 2001). These findings suggest that there are different transmission patterns/roles for animal reservoir hosts in these two countries. It appears that cattle are more important in the transmission in China than in the Philippines.

In the Philippines, recent studies have shown a low prevalence of *S. japonicum* infection in carabaos (water buffaloes; Carabin *et al*. 2005). The low prevalence rates found in carabaos may just be a result of the test used in the stool examination because repeat examination of faeces from these animals by a PCR assay showed positive results for up to 25% of the animals (Wu *et al*. 2010). Positive PCR results are equivalent to an average of 5 eggs per gram.
Infection is very light but it is important to note that a carabao produces 50–60 kg of stool per day (Wu et al. 2010) and there are around 1 million carabaos (used in rice farming) in the endemic areas of the country. This finding suggests that carabaos are also important in the transmission of *S. japonicum* in the Philippines.

The fact that animal reservoir hosts are considered to play an integral role in transmission of *S. japonicum* to one another and humans, has led to new strategies for control of *S. japonicum* infections. Aside from chemotherapy in humans, basic and applied research is being directed at the use of chemotherapy and vaccination in animals to eliminate the reservoir for human transmission (Wan et al. 1998, McGarvey et al. 1999, Shi et al. 1998; McManus et al. 1998, Lin et al. 1998, Nara et al. 1998, Zhou et al. 1998).

In the Philippines, dogs are also a potential target of control programmes (Rudge et al. 2008). Dogs are owned by a high proportion of households in rural communities and are usually permitted to roam freely, often entering or feeding in other households as they scavenge for food. Such behaviours might be expected to facilitate environmental contamination by *S. japonicum*-infected dogs in areas where there is an overlap with human activities. Furthermore, census data from study villages in the Philippines, show a mean number of 104.9 dogs per village, which is almost three times that of carabao (36.2/village) and somewhat greater than the number of cats (90.4/village) (Rudge et al. 2008).

Wild rats should also be a target of control programmes to reduce contamination of water bodies with *S. japonicum* but designing control measures for feral animals such as rats would be much more difficult to implement than those for domestic animals such as cattle and dogs.

It is important to point out that the intermediate host of *S. japonicum* is amphibious and therefore simple environmental management of canals is not a solution; rather interruption of the zoonotic cycle must also include animal waste management in order to have a significant impact on transmission of this important pathogen to humans.

### 2.3.2 Bacterial pathogens

**E. coli O157:H7 and other enterohemorrhagic E. coli RANK 2** Most strains of the bacteria *E. coli* are thought to be harmless commensals which reside in the gastrointestinal tract of warm-blooded animals. Its widespread occurrence in faeces and the ease with which it can be cultured in the laboratory has led to its use as indicator of faecal contamination of water and food. While most members of the species are non-pathogenic, others belong to “pathogroups” that are
associated with intestinal and extra-intestinal diseases in both humans and animals (Donnenberg & Whittam 2001). Most *E. coli* pathogroups that cause enteric disease in humans are host species-specific and are important agents of waterborne disease in children and adults in parts of the world where the infrastructure and services necessary for adequate treatment of drinking-water and sewage are rudimentary. In contrast to these human-restricted *E. coli* pathogroups, members of the enterohemorrhagic *E. coli* (EHEC) pathogroup are zoonotic pathogens. Although *E. coli* O157:H7 has been isolated from a wide variety of other animals sources, including feral and domestic pigs, dogs, horses, raccoons, starlings, gulls, geese and flies (Renter & Sargeant 2002, Pedersen & Clark 2007), most outbreaks of infection have been linked to ruminants. It remains unclear whether other animal species are significant pathogen sources or simply act as passive carriers.

EHEC produce one or more antigenic type of potent bipartite protein toxins termed Shiga toxins (Stxs) (Gyles 2007; Karch et al. 2009; Karmali et al. 2010; Mohawk & O’Brien 2011; Tam et al. 2008b). The toxins are thought to bind to specific glycolipid receptors on host cells and become internalised. Once in the cytoplasm, the toxin A subunit cleaves the host ribosomal RNA at a specific site. As this ribosomal RNA is essential for protein synthesis this action eventually leads to cell death. During EHEC infection, Stxs are absorbed into the blood stream and damage endothelial cells lining the small vessels of organs such as the intestine, kidney, pancreas and brain. Stxs not only cause cell death but also the release of mediators of inflammation from endothelial cells which promote clot formation in the small blood vessels. This formation of microthrombi causes platelet depletion, haemolysis and prevents blood flow, resulting in ischemic damage to vital organs. Damage to the colon is manifest as haemorrhagic colitis and to the kidney as the sometimes fatal haemolytic uremic syndrome (HUS).

In addition to Stx production, EHEC typically possess a large plasmid which encodes a number of virulence-related genes including a special haemolysin (Lim et al. 2010) and also specific chromosomal regions known as pathogenicity islands that are thought to contribute to bacterial virulence (Hayashi et al. 2001, Perna et al. 2001). One of the best characterized of these is the locus of enterocyte attachment and effacement which encodes for a specific bacterial attachment factor called intimin and proteins which form a so-called type III secretion system which acts like a molecular syringe and injects effector proteins from a number of different pathogenicity islands into the eukaryotic cell (Coombes et al. 2008, Kaper et al. 1997, Ogura et al. 2007, Tobe et al. 2006). These effector proteins participate in bacterial binding to the microvilli of entocytes and also change the cell’s morphology and physiology.
Of the many different serotypes of Stx-producing \textit{E. coli}, EHEC O157:H7 is the serotype most frequently associated with human disease outbreaks and life threatening manifestations such as HUS. This is reflected in the EHEC classification developed by Karmali et al. (2003). In this “seropathotype” scheme \textit{E. coli} O157:H7 is the sole member of highest risk group, seropathotype A. EHEC belonging to seropathotype B include O serogroups 26, 45, 103, 111, 121 and 145, strains that are less frequently associated with HC and HUS; members of seropathotypes C and D are even less frequently associated with human disease and members of seropathotype E have never been associated with clinical disease. The disease burden associated with the secondary EHEC serotypes has been underestimated in the opinion of many and as a result they may have attracted much more attention recently (Brooks et al. 2005). This has culminated in regulatory changes in the USA, expanding the list of EHEC considered to be food contaminants to include members of seropathotype B. Foods containing these pathogens are now subject to recall and their importation is prohibited.

Not all Stx-producing \textit{E. coli} have been associated with human disease, however, and even within \textit{E. coli} O157:H7 differences exist among genetic lineages in the frequency and severity of human disease they cause (Kim et al. 2001, Zhang et al. 2007, Zhang et al. 2010). Among the three genetic lineages recognized, lineage II is primarily bovine-associated and is infrequently associated with human disease, whereas lineages I and I/II are the most frequently associated with human illness. Lineage I/II contains members of a so-called “hypervirulent clade” associated with higher levels of hospitalization and HUS than other \textit{E. coli} O157:H7 genetic groups (Manning et al. 2008).

Most human \textit{E. coli} O157:H7 and other EHEC infections can be traced to food and water contaminated with bovine faeces containing these organisms (Rangel et al. 2005). Consumption of undercooked ground beef has been implicated in many outbreaks and is also associated with an increased risk of sporadic EHEC infections. In \textit{E. coli} O157:H7 outbreaks other foods such as unpasteurized dairy products and fruit juices, and fresh produce such as sprouts, spinach and lettuce have been shown to be the sources (Cooley et al. 2007). Those outbreaks associated with the consumption of fresh produce have been associated with soil and/or water contaminated with ruminant manure. The ability of this organism not only to proliferate in the ruminant gastrointestinal tract, but also to survive in manure, soil and sediments in water for long periods, to enter plant tissues, and to form biofilms resistant to physical and chemical agents (Maule 2000; Niemira & Cooke 2010, Wang et al. 2011) is thought to be responsible for fresh produce being a source of human infections. This is further exacerbated by its low infectious dose (Tuttle et al.1999, Hara-Kudo & Takatori 2011).
E. coli O157:H7 is able to survive for longer periods in sediments than in flowing water. It is thought that biological agents such as bactivorous protozoa decrease free living or planktonic E. coli O157 numbers and biofilm formation in sediments increases its long-term survival (Wang & Doyle 1998; Ravva et al. 2010). Despite this, large waterborne disease outbreaks have been associated with the organism. One of the largest of these occurred in Swaziland where cattle manure was thought to be the source of more than 40,000 cases of waterborne infection with the organism (Effler et al. 2001). Another large waterborne outbreak occurred in May of 2000 in Walkerton, Ontario, Canada when heavy rains lead to the contamination of a municipal well with cattle manure (Garg et al. 2006). This event, coupled with a failure of the town chlorination system, led to 2500 cases of illness and seven deaths in this small community. In addition to the acute effects of infection with the organism such as haemorrhagic colitis and HUS, residents of Walkerton have been shown to have suffered from long-term sequelae following infection such as higher rates of irritable bowel syndrome, hypertension, renal impairment and cardiovascular disease (Clark et al. 2010). In the USA, 10 waterborne outbreaks associated with EHEC E. coli O157:H7 and O145:NM were reported between 1971–2006 (Craun et al. 2010). In the Scotland, water consumed by vacationers was reported to be associated with an E. coli O157:H7 outbreak (Licence et al. 2001). In Ireland, a case control study pointed to a private well water source as being the cause of an E. coli O157 outbreak in a child care facility (Mannex et al. 2007). Most if not all E. coli O157:H7 outbreaks related to drinking-water occur with water derived from small systems such as private wells where there is no chlorination or there has been a chlorination failure (Craun et al. 2010; Smith et al. 2006). In a number of these outbreaks and in sporadic cases high resolution molecular typing methods such as pulsed-field gel electrophoresis and comparative genomic fingerprinting (Laing et al. 2008) have verified the close relationship between isolates from cattle, water and humans and provided very strong evidence for the waterborne transmission of E. coli O157 (Bruce et al. 2003, Effler et al. 2001, Mannix et al. 2007, Jokinen et al. 2011).

The prevalence of EHEC infections varies significantly from country to country and among regions within countries (<1 to >20 cases per 100,000 population members). While some of this difference can be attributed to differences in surveillance and diagnostic abilities among countries, differences in rates of infection also appear to be related to cattle density, the level of beef consumption and cultural practices which promote consumption of uncooked or undercooked beef, e.g. HUS rates and beef consumption per capita in Argentina are amongst the highest in the world (Rivas et al. 2008). Regional differences in
EHEC prevalence within countries appears to be associated with cattle densities (Michel et al. 1999) and may reflect greater occupational exposure, animal contact, raw milk consumption as well as drinking-water and recreational water exposure.

Young children and the elderly are the most susceptible to severe infections (Karmali et al. 2010). Epidemiological evidence suggests that as few as 2 CFU of *E. coli* O157 are capable of causing illness (Tuttle et al. 1999, Hara-Kudo & Takatori 2011).

**Salmonella spp RANK 2** *Salm. enterica* is the cause of gastrointestinal and systemic illness in human populations around the world. Although there are six different subspecies of the organism, most human infections are associated with *Salm. enterica* subspecies *enterica* which includes more than 1500 different serovars of the organism (Littrup et al. 2010). The widespread occurrence of illness and the high levels of morbidity and mortality associated with the organism have made it the target of control programmes around the world.

A distinction must be made between salmonellosis associated with human host-restricted typhoid *Salmonella* serovars (Typhi, Paratyphi A, Paratyphi B, and Paratyphi C) (TS) and salmonellosis associated with non-host restricted nontyphoid *Salmonella* serovars (NTS). Following infections with TS serovars there is an incubation period from 5–9 days which is followed by fever lasting for approximately three weeks. During this period the organism becomes disseminated throughout the body and can be isolated from the liver, blood, spleen, bone marrow, lymph nodes and gallbladder. In infections with TS serovars, antimicrobial treatment may be necessary to ensure recovery (Thaver et al. 2009). NTS infections, by contrast, are characterized by a short incubation period (<3 days), a sudden onset of fever and symptoms of gastrointestinal illness including abdominal pain, nausea, vomiting and diarrhoea. In infections with NTS serovars, symptoms in healthy adults usually last less than 10 days and antimicrobial treatment is not recommended (Hohmann 2001). In infections associated with both TS and NTS serovars the organisms are thought to preferentially invade M-cells covering lymphoid follicles in the Peyer’s patches in the ileum. Following invasion of the Peyer’s patches the organisms are taken up by dendritic cells in the lamina propria and monocytes in the mesenteric lymph nodes (Tam et al. 2008a). In infections with TS serovars the gastrointestinal phase of illness is followed by systemic dissemination of the organism and the accompanying persistent fever. NTS infections, by contrast, are typically limited to the gastrointestinal tract and are only severe, disseminated and prolonged in children, pregnant women, the elderly and immuno-compromised adults (Hohmann 2001). TS serovars are thought to be able to overcome immune defence mechanisms of immuno-competent adults.
whereas NTS serovars are not (Raffatellu et al. 2011). Following systemic dissemination of TS, certain of the recovered individuals have been shown to be asymptomatic carriers of the organism and continue to shed it in their faeces. The source of the organism often can be traced to a persistent infection in the gall bladder. While NTS are associated with significant morbidity and mortality in livestock, recovered animals also frequently act as asymptomatic carriers of the organism. In animals, the organism has been shown to persist in lymph nodes and is shed in the faeces following stressful events such as transport and lairage (Mannion et al. 2010). These stressful events likely cause alternations in the host immune status. The intestinal microflora has also been shown to protect animals from Salmonella colonization (Endt et al. 2010) and perturbations in the microflora brought about by antimicrobials have been shown to significantly influence the severity of Salmonella-associated disease in mice (Ferreira et al. 2011, Sekirov et al. 2008).

In the developing world, human-to-human transmission of Salmonella is common. Food and water contaminated with human faeces are the main sources of infection. Human host-restricted Salmonella TS serovars are the most common causes of human illness in these parts of the world. Typhoid fever caused an estimated 21.6 million episodes of illness and 216,500 deaths throughout the world in 2000 and paratyphoid fever an estimated 5.4 million episodes of illness (Kothari et al. 2008). However, the disease burden appears to vary considerably between continents, between countries, and between regions within specific countries. The average prevalence estimate in Africa (50 cases per 100,000) is much lower than in parts of Asia where prevalence rates can be as high as 274 per 100,000. In many developed countries, typhoid fever was a significant cause of illness until measures such as increased levels of hygiene in food preparation, protection of source water, and drinking-water and wastewater treatment procedures were implemented. Today, imported cases (travellers) from developing countries are the main foci of outbreaks in developed countries with these human-restricted serovars (Basnyat et al. 2005). Some success has been achieved in controlling typhoid fever in the developing world with the use of antimicrobials and through vaccination (Crump & Mintz 2010). However, the organisms have responded to these selective pressures through the development of resistance to antimicrobials and serovar switching in response to vaccination, that is a decrease in Salmonella serovar Typhi infections and an increase in Salmonella serovar Paratyphi infections are seen in some regions. Interestingly, NTS serovar Typhimurium strains have also recently emerged in Africa which appear to be human host-restricted and are resistant to a number of antimicrobials (Gordon et al. 2010). These Salmonella Typhimurium strains cause high morbidity and mortality in children and HIV-infected
adults. Interestingly, host-restriction appears to be accompanied by a reduction in genome size for both *Salm. typhi* and serovar Typhimurium (Kingsley *et al.* 2009).

In the developed world, most autochtonous outbreaks of salmonellosis are foodborne and of animal origin (Majowicz *et al.* 2010). In contrast to the highly host-specific serovars associated with human enteric fevers, many NTS serovars appear to have a broad host range and are frequently associated with human infections. Other NTS serovars, however, appear to be relatively host-restricted and are rarely the cause of human disease (e.g. NTS serovar Gallinarum in poultry). In developed countries, significant efforts have been made to reduce risks associated with the organism throughout the entire food chain, from farm gate to the consumer’s plate. As mentioned above, waterborne transmission of *Salmonella* has been decreased significantly (but certainly not prevented) by chlorination and other modern source water and waste water treatment procedures.

In the United States, among the 479 foodborne disease outbreaks of known etiology reported in 2008, *Salmonella* accounted for 23% of outbreaks and 31% of illnesses (MMWR, 2011). According to the European Food Safety Authority there were over 100,000 human cases of salmonellosis reported in 2009 among the member states; the economic loss associated with this burden of disease was estimated at 3 billion Euros (EFSA, 2011). These figures only reflect cases and outbreaks which were identified by health authorities, however. The true burden of disease is thought to be much higher. Scallan *et al.* (2011) have recently estimated that there may be as many as 1.2 million episodes of illness associated with NTS each year in the United States resulting in more than 23,000 hospitalizations and 450 deaths and a recent global estimate suggests an annual rate of NTS-related illness of 93.8 million episodes with 155,000 deaths – 80.3 million episodes being foodborne (Majowicz *et al.* 2010).

In the EU control efforts have focused on *Salmonella* Enteriditis and Typhimurium which together account for 75% of human cases. *Salmonella* Enteriditis is acquired from poultry and eggs, while *Salmonella* Typhimurium infections largely come from eating contaminated pork, beef and poultry. In United States, *Salmonella* serovars associated with human disease appear to be somewhat more diverse with *Salmonella* Enteriditis, Typhimurium, Newport and Heidelberg serovars representing 45% of the isolates from humans and together with 16 other serovars make up 70% of the human *Salmonella* isolates. Hara-Kudo & Takatori (2011) recently reported the infectious dose for NTS to have been as low as 89 CFU in one outbreak with exposures as high as $14 \times 10^9$ CFU in another. Predictably, higher exposures were associated with higher rates of infection (up to 100%). However, differences in virulence among serovars are also likely to influence the infectious dose and infection rates.
Between 1971 and 2006, *Salmonella* was identified in the USA as the only pathogen in 20 outbreaks associated with drinking-water which represented 3,588 cases; seven deaths were attributable to *Salmonella* serovar Typhimurium outbreaks (Craun et al. 2010). Interestingly, only five of the outbreaks were associated with the human-restricted pathogen *Salmonella* serovar Typhi. In Canada, between 1974 and 2001 *Salmonella* was associated with 16 waterborne disease outbreaks. In Australia, *Salmonella* was associated with five drinking-water associated outbreaks between 2001–2007 (Dale et al. 2010).

**Campylobacter spp RANK 2** Campy. spp. are the most common cause of bacterial gastroenteritis worldwide (Friedman et al. 2004, Silva et al. 2011). The genus *Campylobacter* is comprised of approximately 21 species and eight subspecies (Debruyne et al. 2010). At least twelve of these species are associated with human illness; however, the vast majority of infections (80–90%) are associated with *Campy. jejuni*. *Campy. coli* is the second most common species associated with campylobacteriosis and other species such as *Campy. upsaliensis* and *Campy. lari* are occasionally associated with gastrointestinal illness in humans (Friedman et al. 2004, Humphrey et al. 2007).

Subspecies of *Campy. fetus* have long been recognized as a cause of abortion and infertility in sheep and cattle (Silva et al. 2011); however, the role of *Campy. jejuni* in gastroenteritis in humans was only established in the 1970s after selective cultural techniques were devised to isolate the organism from stools (Engberg et al. 2000). The organism is a small spiral-shaped Gram-negative bacterium which possesses a single flagellum. Its small size has been exploited in selective isolation of the organism in the laboratory. Passage of liquid samples through a 0.45 µm filter excludes most other bacteria and allows isolation of the campylobacters on solid media. The genome of the organism is also small (1.6–1.7 Mb) and has a low GC content (Lefébure et al. 2010; Biggs et al. 2011). Further, the organism metabolizes few carbohydrate substrates and most strains are microaerophilic and cannot grow in an atmosphere with more than 10% oxygen. Most strains grow best at a temperature of 42°C and are highly susceptible to desiccation. Resistance to the fluoroquinolone antibiotics is very common among *Campy. jejuni* isolates and is thought at least in part to be related to their use for growth promotion in animal feeds (Cody et al. 2010, Smith & Fratamico 2010).

Campylobacteriosis is usually a self-limiting gastroenteritis with symptoms ranging from loose stools to profuse watery diarrhoea and occasionally stools that contain blood, mucus or pus (Kirkpatrick & Tribble 2011). However, in certain individuals such as the very young and immuno-compromised adults, a more persistent diarrhoea with prolonged excretion of the organism may ensue and there is greater chance of the infection recurring following subsequent
exposures to the organism. Recently, higher rates of the irritable bowel syndrome have also been reported among patients who have recovered from Campylobacter infections (Thabane et al. 2010).

Following ingestion, the organism passes through the stomach before entering the intestinal tract. In the intestine, the bacteria are thought to penetrate the mucus layer covering the epithelial cells and attach to their surfaces (Crushell et al. 2004, Young et al. 2007, Zilbauer et al. 2008, Dasti et al. 2010). Invasion of the intestinal epithelial cells follows and results in an inflammatory response, fluid loss and diarrhoea. In addition to the gastrointestinal effects, the pathogen may also enter the blood stream and provoke an inflammatory response in several other organ systems. In children, there may be vomiting and abdominal pain and signs of systemic illness such as fever and headaches. Abortion and premature birth have also been reported following infections with the pathogen.

The organism is also thought to employ mechanisms such as molecular mimicry to avoid both the innate and acquired immune system. Campy. jejuni surface lipo-oligosaccharides have been shown to be antigenically similar to the gangliosides present on the surface of host cells. This mimicry results in either a failure of the host to mount an effective immune response or the production of autoantibodies against gangliosides on host cell surfaces. The binding of these antibodies to host cell gangliosides precipitates an inflammatory response and is thought to result in conditions such as reactive arthritis and the Guillain-Barré syndrome (GBS) (Crushell et al. 2004, Hardy et al. 2011). GBS is an acute progressive neuropathy which is characterized by an ascending paralysis involving the peripheral nerves of the body and the facial nerve (Hughes & Cornblath 2005, Kuwabara, 2004, Uzoigwe, 2005). Approximately one in every 1000 cases of campylobacteriosis results in the GBS. Specific Campylobacter serotypes and genotypes appear to be more commonly associated with GBS than others, suggesting that certain surface lipo-oligosaccharides are more likely to evoke an autoimmune response than others (Hardy et al. 2011). The Miller-Fischer syndrome is a variant of GBS which is characterized by ophthalmoplegia, ataxia and areflexia. Reiter’s Syndrome is another long-term outcome of Campylobacter infections and is characterized by asymmetric arthritis, urethritis and ophthalmitis (Crushell et al. 2004).

While certain genotypes of Campy. jejuni appear to be more frequently associated with gastrointestinal disease in humans than others, it has been very difficult to determine if this has simply been the result of greater human exposure to specific genotypes or if specific genotypes of the organism are in fact more virulent. Factors such as capsular polysaccharide, lipo-oligosaccharides attached to the outer membrane, a plasmid encoded-type IV secretion system, the
flagellum, adhesins, and toxins are thought to play a role in colonization, invasion of epithelial cells, and in activation or evasion of the host innate and acquired immune system (Crushell et al. 2004, Young et al. 2007, Zilbauer et al. 2008). However, many of these possible virulence factors appear to be widely distributed among Campy. jejuni strains (Zhang et al. 2010).

Campy. jejuni can be isolated from the faeces of a large number of wild and domestic animal species. In most animal species, the organism appears to be a commensal in the gastrointestinal tract and clinical disease is not commonly observed (Altekruse et al. 1994, Silva et al. 2011). There is a high prevalence of Campylobacter colonization among birds and their intestines are thought to be ideal incubators for the organism.

While most Campylobacter infections are sporadic, outbreaks of illness have been associated with the consumption of contaminated raw beef liver, raw milk and untreated drinking-water (Robinson 1981, Garg et al. 2006, Hara-Kudo & Takatori 2011). Experimental infections in humans have shown the infectious dose to be as low as 500 organisms (Black et al. 1988). This value is close to an estimated 360 organisms that were consumed in a raw beef liver-associated outbreak in Japan (Hara-Kudo & Takatori 2011). The reason for the sporadic nature of most Campylobacter infections is unknown; however, it may be explained by variable levels of exposure coupled with differences in susceptibility in the human population and/or differences in virulence among Campy. jejuni strains.

Campy. jejuni can also be readily isolated from retail poultry and case-control studies have established that consumption and/or handling of undercooked poultry is the most significant risk factor for human campylobacteriosis (Mead et al. 1999, Friedman et al. 2004). Further, recent studies which have compared the genotypes of C. jejuni isolated from human, animal and environmental sources using multiple locus sequence typing (MLST) have also concluded that chickens are the most important source of human infections (Lévesque et al. 2008, Sheppard et al. 2009, Oporto et al. 2011). Interestingly, these MLST studies have also shown that ruminants are an important secondary source of the organism. Cattle and sheep are known to shed the organism in their faeces and it can be readily isolated from liver and offal samples; however, its prevalence rate in retail beef and mutton is relatively low compared with rates in raw poultry (Kramer et al. 2000, Wong et al. 2007, Ogden et al. 2009). The route of infection from ruminant sources, therefore, likely includes consumption of raw milk (Teunis et al. 2005), animal contact and drinking untreated water (Belongia et al. 2003, Friedman et al. 2004, Humphrey et al. 2007).

Green et al. (2006) in Manitoba, Canada, first reported that young children (<4 years of age) living in close proximity to high densities of livestock were at a much
greater risk of *Campylobacter* infections than their urban dwelling counterparts. Recent studies from Scotland, Germany and New Zealand have supported these findings (Fitzenberger *et al.* 2010, Strachan *et al.* 2009, Spencer *et al.* 2011). Strachan *et al.* (2009) also reported that children 5–14 yrs of age which are at greatest risk of campylobacteriosis in urban centres were predominantly infected with poultry-related MLST types, while younger children (<4 yrs) were are at the greatest risk in rural areas and were infected predominantly by ruminant MLST types. These findings suggest that many *Campylobacter* infections are likely to be acquired from sources such as raw milk and the environment by children in rural areas.

Seasonal variation has been noted in the prevalence of campylobacteriosis in temperate regions in both the southern and northern hemispheres. However, in contrast to the single seasonal summer peaks observed for *Salmonella* and *E. coli* O157:H7 infections, *Campylobacter* infections show two warm season peaks, one in the late spring-early summer and another in the late summer-early fall (Stanley *et al.* 1998). The reason for these two seasonal peaks in *Campylobacter* infections are unknown but could be related to factors such as changes in fly density, increased environmental survival of the pathogen or increased exposure to the organism associated with outdoor recreational and cooking activities.

*Campylobacteriosis* was the most common zoonotic disease reported in the European Union in 2007, with more than 200,000 cases of *Campylobacter* infections from 24 Member States and an average infection rate of 45.2 per 100,000 inhabitants (Silva *et al.* 2011). In the USA, the prevalence of campylobacteriosis is lower (estimated at 13 cases per 100,000 inhabitants); however, it is thought that many cases go undiagnosed and estimates run up to over 2 million episodes of illness, 13,000 hospitalizations and about one hundred deaths per year related to infections with this pathogen. New Zealand (Sears *et al.* 2011, Spencer *et al.* 2011) reported some of the highest average annual rates of *Campylobacter* infections in the developed world (353.8 cases per 100,000 inhabitants from 2002–2006). However, the authorities appear to have succeeded in reducing the prevalence to 161.5 cases per 100,000 inhabitants through efforts aimed at reducing the levels of poultry contamination.

In the developing world the rates of infection are much higher than in the industrialized world. The age of onset is younger (<3 years) and the disease is frequently more severe and can lead to dehydration and death. While *Campylobacter* infection rates are also greater in children than in adults in developed countries, in developing countries this difference can be much more pronounced, with estimates ranging from 40,000 to 60,000 cases per 100,000 among children <5 years of age compared to approximately 90 per 100,000 for
adults (Coker et al. 2002). Many children have multiple bouts of campylobacteriosis before their third birthday. *Campylobacter* spp. are also the most common bacterial agents associated with traveler’s diarrhoea (de la Cabada Bauche et al. 2011).

While waterborne outbreaks of campylobacteriosis are less common than those associated with *C. parvum*, *G. duodenalis* and *E. coli* O157:H7, they do occur. Outbreaks of campylobacteriosis are typically associated with the absence or deficiencies in the chlorination of drinking water (Hrudey & Hrudey 2007, Said et al. 2003). A large outbreak of waterborne disease in Walkerton, Ontario, Canada resulting in seven deaths and over 2,300 cases of gastro-intestinal illness was associated with infections by both *E. coli* O157:H7 and *C. jejuni* (Auld et al. 2004; Garg et al. 2006). In this outbreak, intense rainfall is thought to have washed excreta from a dairy farm into a well which supplied water to the town. This contamination event coupled with a failure in the water chlorination system is thought to have caused the outbreak. Similar but smaller outbreaks associated with drinking-water have been reported in other regions of Canada, Norway, Finland and Sweden (Jakopanec et al. 2008, Schönberg-Norio et al. 2004, Schuster et al. 2005). Craun et al. (2010) reported that in the USA *Campylobacter* spp. (mostly *C. jejuni*) was associated with 19 drinking water disease outbreaks (5,565 cases) as the sole pathogen and in another six outbreaks where more than one pathogen was involved between 1971 and 2006. Finally, campylobacteriosis has also been associated with recreational use of water (Kärenlampi et al. 2007, Schönberg-Norio et al. 2004).

**Leptospirosis RANK 2.** Leptosporosis has a world-wide distribution (McBride et al. 2005) and it has been estimated that there are more than 500,000 human cases of the illness each year with an approximate 10% case-fatality rate (McBride et al. 2005).

Leptospires are spirochetes and members of the genus have until recently been placed into two species, with pathogenic members identified as *L. interrogans* and non-pathogenic saprophytics assigned to the species *L. biflexa*. There are more than 300 serovars of the organism and recent evidence, based on genome comparisons of the organisms, suggests that there are as many as 20 different species of the organism (Cerqueira & Picardeau 2009). Leptospirosis in domestic animals is a significant cause of economic loss. It is associated with abortions, stillbirths and loss of milk production and is sometimes fatal in livestock.

The organism enters the blood stream of human and animal hosts after crossing mucous membranes or broken skin. After the pathogen enters the blood stream it invades and causes damage to endothelial cells lining small vessels in the liver, lung, kidney and placenta. Following infection a serogroup-specific immune response ensues which results in clearance of the organism from the blood
stream. However, the organism may persist in several “immunologically privileged” sites such as the renal tubules, brain, eye and genital tract. Once established in the renal tubules and/or genital tracts of animals, pathogenic leptospires can be passed in the urine and placental fluids, for periods from a few days to several weeks. Chronically infected animals act as the reservoir for the organism and act as the source of infection for herd mates and other animals. Specific serovars tend to be maintained in specific host animal species reservoirs; however, there are overlaps and shifts in serovar occurrence among different domestic and wild animals.

Leptospirosis in humans is almost always derived either directly or indirectly from animal sources (McBride et al. 2005). While many domestic and wildlife species can be the source of human infections, rodents are considered the most important reservoir host worldwide. Individuals in certain occupations are at a high risk for developing leptospirosis; these include abattoir workers, veterinarians, and sugar cane and rice farmers (Acha & Szyfres 1987; Heath & Johnson 1994). Recently, leptospirosis has also been associated with recreational activities, travel and adventure tourism (McBride et al. 2005). Typical symptoms of leptospirosis in humans include fever, jaundice and renal failure; however, the specific manifestations of disease are highly serovar-dependent. A study of one waterborne outbreak associated with recreational water showed that there was an increased likelihood of developing leptospirosis in individuals with the human leukocyte antigen-DQ6, strongly suggesting that there is a genetically based difference in susceptibility to the infection among humans (Lingappa et al. 2004).

Leptospirosis is endemic in animal and human populations in many tropical and subtropical developing nations. McBride et al. (2005) noted that leptospirosis is a disease of urban slum dwellers and the rural poor and that Brazil, India and China have a high incidence of human leptospirosis. *Leptospira* was responsible for an epidemic of severe pulmonary haemorrhagic syndrome in a rural community in Nicaragua in 1995 (Trevejo et al. 1998). The outbreak is thought to have been caused by flood waters contaminated with urine from infected dogs.

In industrialized countries leptospirosis is usually sporadic; however, epidemics have also been reported. Human infections with *Leptospira* are uncommon in the continental USA, but are more frequent in the Hawaiian Islands where about 300 cases were reported from 1999 to 2006. This is a clear case where the ecology of the pathogen has to be taken into consideration. The tropical/subtropical climates are more likely to lead to environmental growth of this pathogen. Puerto Rico and Florida are likely to be afflicted by this pathogen. Preliminary studies in Puerto Rico have demonstrated the presence of *Leptospira* spp in fresh water lakes (Toranzos, unpublished data), and there are sporadic human cases reported.
Although human infections are confined mostly to direct contact with infected animals, contaminated water has also been associated with numerous outbreaks in the USA (Levett 2001, Fuortes & Nettleman 1994). Cattle, pigs, rats, and dogs have all been suspected sources of various waterborne outbreaks (Levett et al. 2001).

The organism survives best in freshwaters and moist terrestrial environments at temperatures above 10°C. Therefore, leptospirosis is most common in temperate climates in spring and fall and in tropical climates during the rainy season. Changes in management practices have shifted the serovar distribution among many domestic animals; as shown in some cases by decreasing exposure to wildlife (e.g. in confined animal feeding operations) and in other cases through serovar-specific vaccination.

*Francisella tularensis* subsp *holarctica* RANK 3 *Francisella tularensis* subsp *tularensis* infections are most commonly acquired by contact with infected wild mammals such as rabbits and deer and also indirectly through an arthropod vectors such as ticks; however, waterborne illness associated with this subspecies is rare (Petersen & Molins, 2010). In contrast, scattered waterborne disease outbreaks have recently been reported in Turkey, Georgia, Norway and several other parts of Europe and Asia associated with *F. tularensis* subsp *holarctica*. Drinking of unchlorinated water contaminated by infected water rats and voles are thought to have been the source of the pathogen. *F. tularensis* subsp *holarctica* is associated with a much milder form of human illness than subsp. *tularensis*.

Antimicrobial resistant bacteria RANK 2 Antimicrobials are not only used therapeutically to treat and as a prophylactic to prevent animal diseases, but they are also used extensively at low doses for growth promotion in livestock production all over the world. This long-term administration of antimicrobials to animals has led to the evolution of bacteria that are not only resistant to single antimicrobial agents but often to multiple antibiotics. These antimicrobial resistance (AMR) determinants vary considerably in their mechanisms of action. Mutations in the gene encoding the bacterial target protein for example, DNA gyrase mutants, enzymes which chemically modify and inactivate antimicrobials, mechanisms which prevent entry of antimicrobials into cells and those that promote the active removal of the antimicrobial from the cell. Multiple AMR determinants, frequently reside adjacent to each other as part of a gene cluster on transmissible genetic elements such as plasmids, transposons and frequently as part of smaller elements termed integrons. Antimicrobial resistance and in particular AMR bacteria are of increasing concern in public health particularly where resistance has developed to antimicrobial agents used to treat nosocomial (hospital-associated) infections. Certain organisms are of particular concern because few treatments remain for pathogens such as
methicillin-resistant *Staphylococcus aureus* (MRSA) and bacteria with extended-spectrum beta-lactamase activity that are resistant to all penicillin and cephalosporin based antibiotics (Kadlec et al. 2009, Van den Eede et al. 2009).

AMR bacteria from animals are, therefore, of serious concern as a potential source of antimicrobial-resistant determinants that may spread to humans through food and through the water supply (Collignon et al. 2009). The concern is not only about the transmission of animal pathogens such as multiple AMR *Salmonella* Typhimurium to humans but also the transmission of AMR determinants from animal-specific pathogens to related human-specific pathogens. It is clearly important to improve the management of the use of antimicrobials in livestock production, so that cross-resistance to antimicrobials used in human medicine is prevented, and, in so doing, preserve the benefits of these antimicrobials as a future resource for use in treating human infections.

The World Health Organization has developed and applied criteria to rank antimicrobials according to their relative importance in human medicine. Clinicians, regulatory agencies, policy makers, and other stakeholders are encouraged to use this ranking when developing risk management strategies for the use of antimicrobials in food production animals (Collignon et al. 2009). Based on this ranking, the antimicrobials of most concern when used in animal production are the fluoroquinolones, macrolides, and third- and fourth-generation cephalosporins.

By way of example of the potential problem arising from antibiotic use in animal production, consider one of the leading zoonotic waterborne pathogenic genera, *Campylobacter*, which causes gastroenteritis in humans. Campylobacters are increasingly resistant to antibiotics, especially fluoroquinolones and macrolides, which are the most frequently used antimicrobials for the treatment of campylobacteriosis (Bostan et al. 2009, Luangtongkum et al. 2009). For example, in the Canadian swine industry, over 60% of *Campylobacter* spp. are resistant to two or more antimicrobial classes (e.g. 71% are resistant to clindamycin, azithromycin, and erythromycin; Rosengren et al. 2009). By contrast, the odds of resistance to a quinolone were nine times higher in *Campylobacter* from herds with beta-lactam exposure in grow-finish pigs compared with those with no exposure. Such extreme clustering demonstrates the potential for herd and, in other studies, flock-level interventions to influence antimicrobial resistance (Rosengren et al. 2009, Schwaiger et al. 2009).

In addition to managing antimicrobial resistance in livestock production, manure management can also play an important role. Inactivation of AMR pathogens normally occurs during waste storage and can be accelerated by thermal treatments such as composting; however, some feel that resistance genes may persist and be passed onto other microbiota which survive heat treatment.
processes (Chénier & Juteau 2009, Sharma et al. 2009). Thermotolerant, naturally-persistent (spore-forming) pathogens, such as tetracycline and macrolide resistant *Clostridium perfringens* have been shown to transfer their resistance genes to bacteria, such as *Enterococcus faecalis* (Soge et al. 2009). Hence, antibiotic-resistant environmental *C. perfringens* also appear capable of acting as reservoirs for antibiotic resistance genes.

As mentioned above, AMR encoding integrons are central players in the worldwide problem of antibiotic resistance, because they can capture and express diverse resistance genes, and are often embedded in plasmids and transposons, which facilitate their lateral transfer into a wide range of pathogens (Gillings et al. 2008). Gillings et al. have shown that class 1 integrons are present in the chromosomes of nonpathogenic soil and freshwater Betaproteobacteria, and that lateral transfer between animal commensal bacteria and pathogens is inevitable. Sulfonamide-resistant pathogens, including *Shigella flexneri*, *Aerococcus* spp., and *Acinetobacter baumannii*, have also been identified in slurry-amended soil and soil leachates, suggesting a potential environmental reservoir (Byrne-Bailey et al. 2009). Further, Byrne-Bailey et al. (2009) have recently demonstrated sulfonamide resistance outside members of the family *Enterobacteriaceae* and reported this resistance determinant to be common in soil bacteria. Clearly the role of water in the transport, transmission and maintenance of AMR determinants and AMR resistant pathogens requires further study.

### 2.3.3 Viruses

**Enteric viruses** RANK 4 Viruses generally have a narrow host-range and most animal viruses do not infect humans and vice-versa. Animal and human viruses that are closely related such as calciviruses, enteroviruses, coronaviruses, picoranviruses, influenza and rotaviruses have the potential to cross species boundaries and cause zoonotic disease. However, the role of water in the transmission of many of these zoonotic viruses is either thought to be minor or is unknown. For example, while there is convincing evidence that lineages 3 and 4 of Hepatitis E virus can be acquired by humans from pork and contact with swine, disease associated with this agent tends to be sporadic. Waterborne transmission of these Hepatitis E lineages has not been convincingly demonstrated (Pavio et al. 2010, Rutjes et al. 2009, Teshale et al. 2010).

### 2.4 CONCLUSIONS

Recent advances in population-based molecular genotyping have helped to differentiate zoonotic waterborne pathogens (i.e. those that originate in animal
populations and cause infection in humans) from closely related agents that are either human or animal host-specific and are not zoonotic. While outbreak data are helpful in assessing the frequency and severity of waterborne disease associated with specific zoonotic waterborne pathogens, it is thought that as much as 90% of illness associated with specific agents is sporadic. Most sporadic illness is under-diagnosed and therefore under-reported even in developed countries. Further, certain pathogens such as *Campylobacter*, while highly prevalent, are rarely associated with outbreaks. In the assessment of the severity of illness, not only short-term morbidity and mortality must be considered but also the long-term sequelae of these infections. Hypertension, diabetes, renal insufficiency, central neurological defects, polyneuritis, inflammatory bowel disease and other chronic conditions, have been shown to occur post infection with certain of these pathogens. These chronic conditions can significantly compromise the long-term health of individuals. The highest risk of infections with waterborne zoonotic pathogens occurs in immunologically naive or compromised members of the population such as children and the elderly, infection rates are highest in rural regions with high animal densities and in regions where water treatment is poor or nonexistent. Occupational and recreational exposure to water contaminated with animal excreta has also been shown to be an important route of infection. In addition to the spread of infectious agents, the spread of genetic determinants associated with increased virulence and resistance to biocides such as antibiotics and their transfer from pathogens and nonpathogens in animal and their environment to human pathogens via animal excreta and indirectly through water to humans is also a concern. However, further studies are needed to determine the persistence of these determinants in animal excreta and water and the conditions in these media such as biofilms which facilitate the transfer of these determinants to human pathogens. Improved and at the same time economically feasible intervention strategies are required to prevent the transmission of zoonotic waterborne pathogens and specific virulence and AMR genetic determinants through water.

**REFERENCES**


Jokinen, C., Edge, T. A., Ho, S., Koning, W., Laing, C., Mauro, W., Medeiros, D., Miller, J., Robertson, W., Taboada, E., Thomas, J. E., Topp, E., Ziebell, K. and Gannon, V. P. J. (2011). Molecular subtypes of *Campylobacter* spp., *Salmonella enterica*, and


Assessing the importance of zoonotic waterborne pathogens


Ogura, Y., Ooka, T., Asadulghani, Terajima, J., Nougayrède, J.-P., Kurokawa, K., Tashiro, K., Tobe, T., Nakayama, K., Kuhara, S., Oswald, E., Watanabe, H. and Hayashi, T.


