
Section IX

Future emerging waterborne
zoonoses

Waterborne Zoonoses: Emerging pathogens and emerging patterns of infection

V.P.J. Gannon, C. Bolin, and C.L. Moe

30.1 INTRODUCTION

Sharma *et al.* (2003) have defined an emerging pathogen as “any new, re-emerging, or drug resistant infection whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the future.” This definition is derived from a report published by the Institute of Medicine of the National Academies in the USA in 1992 (Lederberg *et al.* 1992). Smolinski *et al.* (2003) of the Institute of Medicine have published

another work entitled *Microbial Threats to Health: Emergence, Detection and Response* to address current concerns associated with emerging pathogens.

Emerging pathogens are characterized by their increasing prevalence and have the potential to become endemic, epidemic, and even pandemic in nature. Clinical illness associated with these emerging pathogens, compared with other closely related pathogens, may be more severe, transmitted more rapidly or widely within populations, or more difficult to prevent or treat. Each of these features raises the awareness and attention paid to the emerging pathogen by members of the scientific, public health, regulatory, and political communities. The emerging pathogen may be a unique organism that is very distantly related to other known human pathogens (e.g., Ebola virus) or may simply represent a change in phenotype of a common organism, such as a new serotype, a new virulence attribute, or a different antimicrobial resistance pattern (e.g., multiple antibiotic-resistant *Mycobacterium tuberculosis* and *Salmonella enterica* serovar Typhimurium). However, the emergence of specific pathogens may also result from the recognition of a new subtype of a familiar pathogen that has long been responsible for specific human diseases but only recently associated with them by the scientific community (e.g., *Helicobacter pylori* and stomach ulcers). Increasing attention to and widespread ability to recognize and diagnose an infectious disease are often accompanied by an apparent increase in the isolation of the pathogen and prevalence of associated infections. This increase in diagnoses may simply be an artefact of increased awareness and the spread of new diagnostic or laboratory testing capabilities. However, the recognition of emerging infections as a result of increased surveillance may also indicate that earlier data had underestimated the prevalence of disease associated with a particular pathogen. Therefore, the “emergence of a pathogen” includes the element of recognition as well as the potential recent evolution of a unique pathogen.

Several authors in this book have highlighted the importance of ongoing surveillance activities in providing a better understanding of the impact of waterborne zoonoses and in the identification of emerging new pathogens and trends in waterborne diseases (e.g., chapters 5, 11, 12, and 19). Commitments are required from both developed and developing countries to build, sustain, and coordinate global epidemiological surveillance systems to monitor the emergence of waterborne zoonotic pathogens. In addition, laboratory studies are needed to support the identification and risk assessment of new pathogens as well as new phenotypic variants of pathogens traditionally responsible for waterborne zoonoses. For example, there has been much concern on the part of water authorities when oocysts of *Cryptosporidium parvum* have been identified in water samples. However, recent research suggests that there are considerable

differences in the risks to human health posed by closely related but distinct subtypes of *Cryptosporidium parvum*, and improved methods for identification and characterization of these organisms would be beneficial in the management of risks associated with these parasites (chapter 16).

The sudden appearance and true increases in the prevalence of zoonotic pathogens are thought to be the result of changes in pathogens, the environment, the animal reservoir, the human host, or the type or frequency of contact between animals and humans. In many cases, more than one of these factors may be involved in emergence of zoonoses.

30.2 EMERGING PATTERNS OF INFECTION

Water is often just one of several routes of transmission of the zoonotic pathogen, and the waterborne route is usually only clearly recognized in outbreak situations. Frequently sporadic disease and unrecognized outbreaks are the most common forms of infection with many zoonotic pathogens, and it is difficult to tease out the precise route of transmission, such as food, water, family contact, or animal contact, in these sporadic cases. With the increasing use of improved technology for the treatment of drinking-water supplies in large and medium-sized population centres, we would expect to see not only less waterborne disease associated with organisms susceptible to current water treatment processes, such as chlorination (e.g., *E. coli* O157:H7), but also less disease associated with organisms resistant to current water treatment processes (e.g., *Cryptosporidium parvum*). In spite of this optimism, authorities with responsibility for water treatment must continue to be vigilant and not complacent so that water treatment failures such as occurred in Walkerton, Ontario, Canada, in 2000 can be avoided (Hrudey *et al.* 2003). We would expect more sporadic disease associated with private wells, where there are problems with infrastructure, faulty equipment, and poor training, especially in rural areas where pathogens are abundant in animal reservoir populations. We would also expect to see more waterborne disease associated with the occupational and recreational use of water. Chapter 10 illustrates the shift away from transmission via drinking-water and the rise in recreational water-related waterborne zoonoses in the USA. Susceptible human populations (e.g., patients with human immunodeficiency virus) will experience more waterborne disease than the general population.

30.3 CAN WE PREDICT WHICH ZOOSES WILL EMERGE?

Emergence of disease is a complex process involving elements related to the pathogen, the host, and the environment. A number of such factors have been identified as important in the emergence of diseases, and many of these are also likely to be important in the emergence of zoonotic waterborne infections. Microbial adaptation and change, changes in climate, in the environment, and in land use patterns, animal husbandry and animal waste management practices (concentrated animal feeding operations), political policies that lead to a breakdown in public health measures, war and displacement of populations, international travel and commerce, changes in the susceptibility of human and animal hosts, and changes in human behaviour have been identified as posing risks for disease emergence. This book's authors were challenged to determine if it is possible to predict the emergence of zoonotic waterborne diseases based on an analysis of the pathogen, host, and environment, as summarized below.

30.3.1 Changes in zoonotic waterborne pathogens

While certain pathogens “emerge” as a result of improved laboratory testing and increased surveillance, there are many examples of situations where new phenotypes emerge and become more common. These new phenotypes may be associated with increasing frequency of known disease conditions (e.g., *Vibrio cholera* O139; Faruque and Mekalanos 2003) or may be responsible for entirely new disease syndromes (e.g., severe acute respiratory syndrome [SARS]). Several chapters in this book provide examples of the appearance of new waterborne pathogens. There are also other examples of situations where the pathogens have become more prevalent (e.g., noroviruses) or have an altered life cycle in a particular region (e.g., *Fasciola hepatica*). Changes in the phenotype of a pathogen are the result of genetic change or mutation. These mutations may consist of the simple alteration of the four-base code that makes up the sequence of nucleic acids (DNA and RNA) or the addition or deletion of nucleic acids. These alterations can have profound effects on gene expression, including the amount of specific proteins produced, under which circumstances they are produced, and also the characteristics of these proteins. Most mutations of genes lead to the death of the pathogen. However, changes to the genome may also result in altered proteins or patterns of gene expression that provide new ways for a microorganism to survive, flourish, and extract more energy and material from the environment. While genetic mutation is relatively common, the co-

occurrence of the right mutation and opportunities to exploit this mutation are much rarer.

When we think of emerging pathogens, we consider circumstances that set the stage for pathogen evolution and occupation of a new niche. These can be changes in selective pressures in the environment or simply a mutation resulting in a new phenotype that is better able to survive in the existing environment. Mutation rates not only are an intrinsic property of the organism but also can be specific to genetic loci within the organism. Mutation rates are low in essential or housekeeping genes responsible for cell homeostasis, metabolism, and reproduction. Mutation rates in pathogens appear to be higher for surface-exposed membrane, envelope, capsule, or coat proteins. Changes in these proteins may be selected for as a means of survival. These surface-related changes are thought to aid the pathogen in 1) escape from the host's immune system, 2) defence against predators, 3) advantage against competitors, 4) facilitating attachment to surfaces, including a new host, 5) allowing entry of beneficial substrates, and 6) promoting the exclusion or exit of toxic substances.

30.3.2 Repeated nucleic acid segments

DNA replication is template driven and independently occurs in the two complementary strands of DNA. During replication, slippage of the template DNA strand downstream would result in the same sequence being recopied into the new strand. Alternatively, hybridization of the template DNA to a site upstream in the new strand may cause the DNA sequence not to be copied. This process can result in the generation of nucleic acid repeats and tandem repeats. These repeat structures are thought to play an important role in the evolution of protozoa (Wickstead *et al.* 2003) and bacteria (van Belkum 1999). The number of bases that are repeated varies, and repeats can occur within structural genes or outside of genes. Repeats in DNA within genes result in repeating amino acid motifs in proteins. The altered domains in the proteins can result in a change in receptor affinity of the expressed protein, leading to altered transport systems and receptor affinity for attachment proteins and toxins.

30.3.3 Genetic exchange

Plasmids in bacteria are examples of self-replicating segments of extrachromosomal DNA. They can be passed not only vertically from generation to generation but also horizontally through a plasmid-encoded transfer process termed conjugation. Plasmids and other naked pieces of DNA in the environment can also be taken up by the cell through a process termed transformation. While these elements may exist as separate entities within the

cell, they also have the capacity to integrate into the chromosomal DNA by recombination; these elements may be extracted from the chromosome again through a process termed excision. In bacteria, a variety of virulence attributes are encoded by genes on plasmids. These include toxins, adhesins, and invasion-associated proteins. Other genes provide a competitive advantage to the bacteria, such as substrate utilization, production of biocins that kill other bacteria, and antibiotic resistance. These traits are often linked and tend to accumulate on plasmids; in this way, use of one antibiotic may select for bacteria with plasmids that have multiantibiotic resistance and other virulence attributes.

30.3.4 Mobile genetic elements and recombination

While some bacteriophages can be viewed as bacterial parasites, there are many with so-called “temperate” stages in their life cycle, where they integrate into the chromosome of the bacterium and depend on the bacterial replication for survival. Recent studies of bacterial chromosomes have identified numerous large segments of DNA that are likely of bacteriophage origin (e.g., Ohnishi *et al.* 2001). Some of these DNA segments are functional bacteriophages that can produce viable progeny and lyse the bacterium, liberating new phages to infect other hosts. However, many of the phage-derived segments in the chromosomes of the bacteria are not functional as bacteriophages and have lost many phage-related genes. Other large DNA segments derived from bacteriophages or plasmids have inserted or “hopped” into the chromosome by transposition (Schneider *et al.* 2002). These externally derived DNA segments are thought to be very important in the evolution of many bacterial pathogens and can encode unique genes or operons that work together and coordinate important enzymatic or structural processes. Presumably, they confer phenotypic attributes that allow these bacteria to be more successful than other competing bacteria. Some of these segments or genetic “islands” have been shown to be necessary for the agent to be a human or animal pathogen and are referred to as pathogenicity islands (Karaolis *et al.* 2001; Faruque and Mekalanos 2003; Morabito *et al.* 2003).

30.3.5 Changes in the environment

Changes in the environment can lead to increases in zoonotic disease by a variety of mechanisms. Changes in ecosystems and encroachment on habitat often initiate or increase contact between different species of animals. This contact can precipitate “jumping” of pathogens from one species to another (e.g., SARS) or may greatly enhance the transmission rate of some diseases. Global climatic change may increase the range of vector species, resulting in the

spread of an existing zoonotic disease to new areas. Climatic and natural events, such as flooding, storms, and fires, often precipitate outbreaks of disease associated with disruption and contamination of water treatment systems, displacement of persons and animals from their usual habitats, lack of sanitation, and enhanced spread of infectious agents. Outbreaks of leptospirosis that frequently follow flooding are good examples of the influence of climatic events and natural disasters on the emergence of zoonotic infectious disease.

30.3.6 Changes in the human or animal host

Human and animal hosts may become more susceptible to disease because of famine, malnutrition, chronic disease, and immunosuppression. Movements of humans and animals because of natural disasters, famine, conflict, etc. may result in immunologically naive populations entering areas with endemic infection. As the density of human and agricultural animal populations increases, often in close proximity, the risk of emerging infections increases due to concentrations of susceptible hosts, increased contamination of the environment because of improper waste disposal, and increased opportunities for transmission. Concentrations of mixed species of animals (including humans) in close proximity makes an event uncommon under normal circumstances — i.e., the jumping of a pathogen from one species to another — more probable and the consequences of that change in host range more serious. Changes in human behaviour are also thought to play an important role in emerging zoonoses. These changes include increased travel, increased recreational activities involving water and remote locales, and ecotourism.

30.4 WATERBORNE ZOOSES LIKELY TO EMERGE OR RE-EMERGE

While it is not possible to predict precisely, several agents were identified in this book as having the potential to represent significant risks for emergence or re-emergence. In addition, several agents were discussed because of their particular concern to public health officials (e.g., prions, SARS), but that seem unlikely to represent real risks of waterborne transmission.

30.4.1 Bacteria

The family Enterobacteriaceae contains a large number of human pathogens belonging to genera such as *Escherichia*, *Klebsiella*, *Salmonella*, *Shigella*, and *Yersinia*. Members of this family are known for infecting a wide variety of hosts and induce a variety of clinical syndromes associated with a large number of

groups, types, and serotypes (chapter 13; Iwobi *et al.* 2002; Liu *et al.* 2002; Schneider *et al.* 2002; Dobrindt *et al.* 2003). Considerable phenotypic plasticity also occurs within Vibrionaceae, Campylobacteriaceae, and Spirochetes, such as *Leptospira* (Karaolis *et al.* 2001; Boer *et al.* 2002; Zuerner and Huang 2002; Faruque and Mekalanos 2003). These groups represent important sources of emerging waterborne zoonotic pathogens. Fortunately, these bacterial agents are susceptible to chlorine and ultraviolet (UV) light. Infections associated with these organisms are likely to persist where there is little or no drinking-water treatment and for those involved in occupational or recreational use of contaminated water (Levett 2001; Hruday *et al.* 2003). Certain other specific bacterial pathogens, such as *Mycobacterium paratuberculosis* and other members of the *Mycobacterium avium* complex, are common in animal populations in certain regions and more resistant than other bacteria to heat and oxidation (Grant *et al.* 1996; Stabel *et al.* 1997; Falkinham 2003) and could pose a risk of waterborne illness, particularly in immunocompromised individuals. Organisms such as *Francisella tularensis* (Anda *et al.* 2001) and *Burkholderia pseudomallei* (Dance 2002) may emerge as naturally occurring waterborne pathogens or from intentional introduction. *Bacillus anthracis* spores may also pose a risk for waterborne infection. These spores are hardy and would require UV light inactivation (Beatty *et al.* 2003; Nicholson and Galeano 2003).

30.4.2 Parasites

The phylum Apicomplexa consists of a large number of obligate intracellular parasites, including the genera *Plasmodium*, *Toxoplasma*, *Cryptosporidium*, and *Cyclospora*. Members of the last three genera include waterborne zoonotic pathogens and form cysts that are resistant to chlorination (Marshall *et al.* 1997; Wright and Collins 1997). UV light and ozonation are effective in inactivating these pathogens, and microfiltration is effective in removing the cysts from water (Morita *et al.* 2002; Biswas *et al.* 2003; Hsu and Yeh 2003). Infections associated with these organisms are likely to persist where 1) there is a source of cysts infectious for humans, 2) there are failures in filtration processes, 3) there are significant precipitation events, and 4) sophisticated multi-step barriers for drinking-water and recreational water protection are not in place. The amitochondriate parasites *Giardia intestinalis* and *Entamoeba histolytica* offer similar challenges to water authorities (Wright and Collins 1997; Steiner *et al.* 1998).

30.4.3 Viruses

Enteric viruses have been detected in drinking-water and environmental waters in industrialized and developing countries (Deetz *et al.* 1984; Gratacap-Cavallier *et al.* 2000; Jothikumar *et al.* 2000; Borchardt *et al.* 2003). Traditional virology has long believed that viruses are quite host-specific, but there are numerous examples of new viral pathogens identified in humans that may have animal origins (e.g., SARS, hepatitis E virus [HEV], some strains of rotavirus A, some strains of noroviruses). All of these viruses are excreted in faeces, so it is possible that they may be transmitted by water contaminated by human or animal faeces.

SARS virus appears to be a new cause of human infections. HEV is newly recognized, but has been implicated retrospectively in hepatitis outbreaks dating back to the 1950s (Jothikumar *et al.* 1993). Recent studies suggest that there may be a close link between human HEV and swine HEV (chapter 15), and there is new evidence of interspecies transmission of HEV (Meng 2003). Some of these emerging pathogens may actually be new viruses that may have evolved through random genetic changes in the genomes of animal viruses that allowed them to bind and enter human host cells. RNA viruses (SARS, coronavirus, HEV, rotavirus A, and noroviruses) are known to have high mutation rates during replication in host cells. Some bovine and swine norovirus strains are genetically very similar to some human norovirus strains (van Der Poel *et al.* 2000), suggesting that they evolved from a common ancestor. Other new viral pathogens may have arisen through recombination events where an animal or human becomes co-infected with two different viruses that recombine during replication in the host cell. The new reassortant strain has portions of the genomes of both viruses. Animal rotaviruses have been detected in drinking-water, and other investigators have speculated on the role of water in the spread of animal strains to human populations and the emergence of reassortant strains (Gratacap-Cavallier *et al.* 2000). Bovine-human reassortant strains have been detected in infants in Bangladesh (Ward *et al.* 1996) and may possibly have been transmitted from humans to cows or vice versa via faecal contamination of water.

There is little information on the environmental persistence of these new viral pathogens and their removal or inactivation during water treatment. Most of our understanding of virus inactivation by water treatment processes comes from studies of human viruses (Hurst 1991). It is possible that some animal or reassortant strains may be more resistant than human viruses to water treatment and should be further investigated.

30.4.4 Prions

Bovine spongiform encephalitis (BSE) may have arisen spontaneously in cattle or originated from scrapie in sheep (Baylis *et al.* 2002; Manuelidis 2003; Smith 2003). Transmission of this transmissible spongiform encephalopathy (TSE) to cattle is thought to have initially occurred as a result of the feeding of scrapie-contaminated bovine or ovine meat and bone meal (MBM). Continued feeding of BSE-infected MBM is thought to have been responsible for the subsequent expansion of the BSE epidemic. Human consumption of BSE-infected beef products is thought to have been responsible for the variant Creutzfeldt-Jakob disease (vCJD) epidemic in humans that subsequently occurred in the United Kingdom. A ban on MBM feeding in cattle and other measures appear to have stopped the BSE epidemic in the United Kingdom and will presumably also stop the vCJD epidemic in humans in this region with time.

In contrast to BSE in cattle, scrapie in sheep and chronic wasting disease (CWD) in cervids appear to be readily transmitted horizontally to other animals. With the scrapie and CWD agents, infected animals are thought to contaminate the environment with infected tissues such as the placenta, and faecal excretion of these agents has also been postulated (Millar and Williams 2003). Subsequent persistence of scrapie and CWD agents in soil is thought to play an important role in animal-to-animal transmission of these TSEs. However, water is not considered a likely route of transmission for any of the TSE-related prion proteins, as they are very insoluble in water (Gale 2001). Various types of animal-derived prions, in addition to BSE, may be or may become infectious for humans. The most likely route of infection would be foodborne rather than waterborne. If environmental routes of horizontal transmission of scrapie and CWD infection posed a substantial risk for humans, we would expect to see CWD infections first in more closely related species that often share the same habitat (e.g., free-ranging cattle; Salman 2003), but this has not occurred. Epidemiological studies have thus far failed to show an association between consumption of potential sources of scrapie such as mutton and lamb or sources of CWD such as venison with CJD in humans (Davis *et al.* 2003).

However, as we have learned from the BSE and vCJD epidemics in cattle and humans, respectively, a species cross-over with these TSE agents may be just a matter of opportunity and time. Therefore, efforts on measures such as TSE eradication programmes must be made now to diminish the probability of such events in the future.

30.5 CONCLUSION

An increased understanding of the interplay between changes in pathogens, the environment, and the host and emergence of disease is developing based on thorough study by microbiologists, ecologists, infectious disease experts, geographers, and others. Progress in these areas is likely to continue, with an increasing emphasis on interdisciplinary teams of investigators, broad-based surveillance efforts, and enhanced cooperation between public health, animal health, and environmental health authorities. As new risks for waterborne zoonotic diseases are identified, authorities need to be able to respond in a scientifically based manner to address the threat where it occurs. This will require cooperation, sharing of information and resources, sound risk assessment, and multiple prevention and control strategies that are scalable and can be applied in low- and high-resource environments.

30.6 REFERENCES

- Anda, P., Segura del Pozo, J., Diaz Garcia, J.M., Escudero, R., Garcia Pena, F.J., Lopez Velasco, M.C., Sellek, R.E., Jimenez Chillaron, M.R., Sanchez Serrano, L.P. and Martinez Navarro, J.F. (2001) Waterborne outbreak of tularemia associated with crayfish fishing. *Emerg. Infect. Dis.* **7**(Suppl. 3), 575–582.
- Baylis, M., Houston, F., Kao, R.R., McLean, A.R., Hunter, N. and Gravenor, M.B. (2002) BSE — a wolf in sheep's clothing? *Trends Microbiol.* **10**(12), 563–570.
- Beatty, M.E., Ashford, D.A., Griffin, P.M., Tauxe, R.V. and Sobel, J. (2003) Gastrointestinal anthrax: review of the literature. *Arch. Intern. Med.* **163**(20), 2527–2531.
- Biswas, K., Craik, S., Smith, D.W. and Belosevic, M. (2003) Synergistic inactivation of *Cryptosporidium parvum* using ozone followed by free chlorine in natural water. *Water Res.* **37**(19), 4737–4747.
- Boer, P., Wagenaar, J.A., Achterberg, R.P., Putten, J.P., Schouls, L.M. and Duim, B. (2002) Generation of *Campylobacter jejuni* genetic diversity *in vivo*. *Mol. Microbiol.* **44**(2), 351–359.
- Borchardt, M.A., Bertz, P.D., Spencer, S.K. and Battigelli, D.A. (2003) Incidence of enteric viruses in groundwater from household wells in Wisconsin. *Appl. Environ. Microbiol.* **69**(2), 1172–1180.
- Dance, D.A. (2002) Melioidosis. *Curr. Opin. Infect. Dis.* **15**(2), 127–132.
- Davis, J.P., Kazmierczak, J., Wegner, M.D. and Wierzba, R. (2003) Fatal degenerative neurologic illnesses in men who participated in wild game feasts — Wisconsin, 2002. *Morbidity Mortality Weekly Rep.* **52**(7), 125–127.
- Deetz, T.R., Smith, E.M., Goyal, S.M., Gerba, C.P., Vollet, J.J., Tsai, L., Dupont, H.L. and Keswick, B.H. (1984) Occurrence of rotavirus and enterovirus in drinking and environmental waters in a developing nation. *Water Res.* **18**, 567–572.
- Dobrindt, U., Agerer, F., Michaelis, K., Janka, A., Buchrieser, C., Samuelson, M., Svanborg, C., Gottschalk, G., Karch, H. and Hacker, J. (2003) Analysis of genome plasticity in pathogenic and commensal *Escherichia coli* isolates by use of DNA arrays. *J. Bacteriol.* **185**(6), 1831–1840.

- Falkinham, J.O., III (2003) Factors influencing the chlorine susceptibility of *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*. *Appl. Environ. Microbiol.* **69**(9), 5685–5689.
- Faruque, S.M. and Mekalanos, J.J. (2003) Pathogenicity islands and phages in *Vibrio cholerae* evolution. *Trends Microbiol.* **11**(11), 505–510.
- Gale, P. (2001) Developments in microbiological risk assessment for drinking water. *J. Appl. Microbiol.* **91**(2), 191–205.
- Grant, I.R., Ball, H.J. and Rowe, M.T. (1996) Thermal inactivation of several *Mycobacterium* spp. in milk by pasteurization. *Lett. Appl. Microbiol.* **22**(3), 253–256.
- Gratacap-Cavallier, B., Genoulaz, O., Brengel-Pesce, K., Soule, H., Innocenti-Francillar, P., Bost, M., Goffi, L., Zmirou, D. and Seigneurin, J.M. (2000) Detection of human and animal rotavirus sequences in drinking water. *Appl. Environ. Microbiol.* **66**(6), 2690–2692.
- Hrudey, S.E., Payment, P., Huck, P.M., Gillham, R.W. and Hrudey, E.J. (2003) A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. *Water Sci. Technol.* **47**(3), 7–14.
- Hsu, B.M. and Yeh, H.H. (2003) Removal of *Giardia* and *Cryptosporidium* in drinking water treatment: a pilot-scale study. *Water Res.* **37**(5), 1111–1117.
- Hurst, C. (1991) Presence of enteric viruses in freshwater and their removal by conventional drinking water treatment processes. *Bull. W. H. O.* **69**, 113–119.
- Iwobi, A., Rakin, A., Garcia, E. and Heesemann, J. (2002) Representational difference analysis uncovers a novel IS10-like insertion element unique to pathogenic strains of *Yersinia enterocolitica*. *FEMS Microbiol. Lett.* **210**(2), 251–255.
- Jothikumar, N., Aparna, K., Kamatchiammal, S., Paulmurugan, R., Saravanadevi, S. and Khanna, P. (1993) Detection of hepatitis E virus in raw and treated wastewater with the polymerase chain reaction. *Appl. Environ. Microbiol.* **59**(8), 2558–2562.
- Jothikumar, N., Paulmurugan, R., Padmanabhan, P., Sundar, R.B., Kamatchiammal, S. and Rao, K.S. (2000) Duplex RT-PCR for simultaneous detection of hepatitis A and hepatitis E virus isolated from drinking water samples. *J. Environ. Monit.* **2**(6), 587–590.
- Karaolis, D.K., Lan, R., Kaper, J.B. and Reeves, P.R. (2001) Comparison of *Vibrio cholerae* pathogenicity islands in sixth and seventh pandemic strains. *Infect. Immun.* **69**(3), 1947–1952.
- Lederberg, J., Shope, R.E. and Oaks, S.C. (1992) *Emerging Infections. Microbial Threats to Health in the United States*. 312 pp., National Academy Press, Washington, DC.
- Levett, P.N. (2001) Leptospirosis. *Clin. Microbiol. Rev.* **14**(2), 296–326.
- Liu, G.R., Rahn, A., Liu, W.Q., Sanderson, K.E., Johnston, R.N. and Liu, S.L. (2002) The evolving genome of *Salmonella enterica* serovar Pullorum. *J. Bacteriol.* **184**(10), 2626–2633.
- Manuelidis, L. (2003) Transmissible encephalopathies, speculations and realities. *Viral Immunol.* **16**(2), 123–139.
- Marshall, M.M., Naumovitz, D., Ortega, Y. and Sterling, C.R. (1997) Waterborne protozoan pathogens. *Clin. Microbiol. Rev.* **10**(1), 67–85.
- Meng, X.J. (2003) Swine hepatitis E virus: cross-species infection and risk in xenotransplantation. *Curr. Top. Microbiol. Immunol.* **278**, 185–216.
- Millar, M.W. and Williams, E.S. (2003) Horizontal prion transmission in mule deer. *Nature* **425**, 35–36.

- Morabito, S., Tozzoli, R., Oswald, E. and Caprioli, A. (2003) A mosaic pathogenicity island made up of the locus of enterocyte effacement and a pathogenicity island of *Escherichia coli* O157:H7 is frequently present in attaching and effacing *E. coli*. *Infect. Immun.* **71**(6), 3343–3348.
- Morita, S., Namikoshi, A., Hirata, T., Oguma, K., Katayama, H., Ohgaki, S., Motoyama, N. and Fujiwara, M. (2002) Efficacy of UV irradiation in inactivating *Cryptosporidium parvum* oocysts. *Appl. Environ. Microbiol.* **68**(11), 5387–5393.
- Nicholson, W.L. and Galeano, B. (2003) UV resistance of *Bacillus anthracis* spores revisited: validation of *Bacillus subtilis* spores as UV surrogates for spores of *B. anthracis* Sterne. *Appl. Environ. Microbiol.* **69**(2), 1327–1330.
- Ohnishi, M., Kurokawa, K. and Hayashi, T. (2001) Diversification of *Escherichia coli* genomes: are bacteriophages the major contributors? *Trends Microbiol.* **9**(10), 481–485.
- Salman, M.D. (2003) Chronic wasting disease in deer and elk: scientific facts and findings. *J. Vet. Med. Sci.* **65**(7), 761–768.
- Schneider, D., Duperchy, E., Depeyrot, J., Coursange, E., Lenski, R. and Blot, M. (2002) Genomic comparisons among *Escherichia coli* strains B, K-12, and O157:H7 using IS elements as molecular markers. *BMC Microbiol.* **2**(1), 18.
- Sharma, S., Sachdeva, P. and Virdi, J.S. (2003) Emerging water-borne pathogens. *Appl. Microbiol. Biotechnol.* **61**(5–6), 424–428.
- Smith, P.G. (2003) The epidemics of bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease: current status and future prospects. *Bull. W. H. O.* **81**(2), 123–130.
- Smolinski, M.S., Hamburg, M.A. and Lederberg, J. (2003) *Microbial Threats to Health: Emergence, Detection, and Response*. 398 pp., National Academy Press, Washington, DC.
- Stabel, J.R., Steadham, E.M. and Bolin, C.A. (1997) Heat inactivation of *Mycobacterium paratuberculosis* in raw milk: are current pasteurization conditions effective? *Appl. Environ. Microbiol.* **63**(12), 4975–4977.
- Steiner, T.S., Thielman, N.M. and Guerrant, R.L. (1998) Protozoal agents: what are the dangers for the public water supply? *Annu. Rev. Med.* **48**, 329–340.
- van Belkum, A. (1999) Short sequence repeats in microbial pathogenesis and evolution. *Cell Mol. Life Sci.* **56**(9–10), 729–734.
- van Der Poel, W.H., Vinje, J., van Der Heide, R., Herrera, M.I., Vivo, A. and Koopmans, M.P. (2000) Norwalk-like calivirus genes in farm animals. *Emerg. Infect. Dis.* **6**(1), 36–41.
- Ward, R.L., Jin, Q., Nakagomi, O., Sander, D.S. and Gentsch, J.R. (1996) Isolation of a human rotavirus containing a bovine rotavirus VP4 gene that suppresses replication of other rotaviruses in coinfecting cells. *Arch. Virol.* **141**, 615–633.
- Wickstead, B., Ersfeld, K. and Gull, K. (2003) Repetitive elements in genomes of parasitic protozoa. *Microbiol. Mol. Biol. Rev.* **67**(3), 360–375.
- Wright, M.S. and Collins, P.A. (1997) Waterborne transmission of *Cryptosporidium*, *Cyclospora* and *Giardia*. *Clin. Lab. Sci.* **10**(5), 287–290.
- Zuerner, R.L. and Huang, W.M. (2002) Analysis of a *Leptospira interrogans* locus containing DNA replication genes and a new IS, IS1502. *FEMS Microbiol. Lett.* **215**(2), 175–182.