The excreta (faeces and urine) of mammals and birds are widespread across planet Earth and frequently contaminate water that is used for bathing and recreation, that is treated and distributed for human consumption, and that is used to irrigate crops.

The risk that such contamination represents to human health is inadequately understood. It is widely assumed that faeces of animals represents a lesser risk to human health than human faeces of because of the 'species barrier' and especially the species-specificity of most viruses. This book points to a rational and systematic way of assessing the risks and to cost-effective approaches to manage them. The information presented is expected to have important consequences for prioritisation of preventive and remedial interventions.

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Summary Statement[1]

The hazards to human health represented by zoonotic pathogens in animal excreta remain poorly understood and inadequately addressed in the literature. These hazards present special challenges for authorities charged with maintaining the quality of surface waters used for recreation and as sources of drinking-water. Current water quality standards in most countries focus on control of human faecal contamination and do not reflect risk posed by faecal contamination from animal sources. Few studies have attempted to examine the relationship between swimming-associated health impacts and the quality of water contaminated by animals or birds, and that the evidence base is lacking to determine whether or not this type of exposure may result in excess illness. Furthermore, we do not know if the current regulatory response, with its focus on contamination by human excreta, is appropriate for animal or bird-contaminated waters. Human faeces are frequently treated and may be disinfected before they are discharged into surface waters, whereas non-human faecal contamination is commonly neither treated nor disinfected. Moreover, the human derived pathogens are frequently viruses, whereas zoonotic pathogens are primarily bacteria and protozoans. A limited number of studies in a range of contexts have shown that their relative relationship to faecal indicator organisms varies widely. These key differences complicate the application of standards derived from studies on the health impacts of human faeces to waters contaminated by animals or birds.

[1] This summary was prepared by Al Dufour, Jamie Bartram and Robert Bos. The summary reflects significant highlights from the chapters in this book and ideas that were discussed at the workshop on Animal; Waste, Water Quality and Human Health conducted at the University of North Carolina, Chapel Hill in October 2009.

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The variability in the occurrence of zoonotic pathogens in waters used for recreation or as a source of drinking-water adds to the complexities of developing water quality standards for surface waters. Unlike pathogenic viruses, which typically occur in human populations with a consistent frequency, the zoonotic pathogens found in animal and bird populations tend to occur sporadically and/or seasonally. This characteristic of zoonotic pathogens in animal and bird faeces makes the establishment of the relationship between water quality and health difficult.

These issues limit our ability to develop guidelines and also monitoring requirements for determining and maintaining the safety of waters contaminated by domestic animals and birds. At the same time, pressures including increasing numbers of humans and livestock and changes in their spatial distribution and related interface create an imperative to act to assess and manage risks in order to protect health. These limitations will, however, not be remedied in the near future. Therefore, innovation is required on various fronts in order to protect the public from potential exposures to zoonotic pathogens that may occur in waters contaminated with livestock faecal waste and to reduce the associated health risks.

Two protozoan parasites (of the genera *Cryptosporidium* and *Giardia*) and three bacteria (EHEC with *E. coli* O157 as the most important representative, and of the genera *Salmonella* and *Campylobacter*), all cosmopolitan in their distribution, meet the criteria to warrant further attention: they are important pathogens in humans, they are known to be carried by animals and discharged in their faeces, and they have been isolated from surface waters. The parasites and bacteria differ in that the former do not replicate outside of their hosts.

This book collects relevant information, in connection with these five pathogens, on the scope of domestic animal and bird faeces discharged into the environment, the fate and transport of the faecal wastes (and the pathogens they may contain) that have been discharged into the environment, human exposure to the faecal wastes, potential health effects associated with those exposures and interventions that will limit human exposures to livestock waste. It also addresses the monitoring, detection and management related to these phenomena.

Participants in the expert meeting that contributed to the development of this volume agreed on twelve key conclusions from their deliberations:

- Although there are a large number of zoonotic pathogens that affect humans, five are known to cause illness around the world with high-frequency:, *Cryptosporidium*, *Giardia*, *Campylobacter*, *Salmonella* and *E. coli* O157. Efforts to control these pathogens are likely to be effective in controlling other related zoonotic pathogens whether known, as-yet-unrecognized or emergent.
- Domestic animals such as, poultry, cattle, sheep and pigs generate 85% of the world's animal faecal waste, proportionally a far greater amount than

the contribution by the human population. The faecal production rate and contribution to the environment of these animals can be as high as 2.62×10^{13} Kg/Year*.

- Limiting zoonotic pathogen-shedding in farm or production facilities for domestic animals should be accomplished by preventing illness in livestock, through minimizing exposure to pathogens, by increasing immunity, by manipulation of the animal gastrointestinal tract microbial ecology and by managing (including treating) animal waste to reduce the release of zoonotic pathogens into the environment.
- The carrier status of animals, particular for the protozoan parasites, appears to peak in very young animals, while in the entire animal population the phenomenon of "super-shedders" provides a possible handle on reducing overall pathogen loads in the environment, provided reliable and cost-effective techniques are developed for timely detection of super-shedders.
- Rivers and streams deliver faecal wastes (and the zoonotic pathogens they may contain) to surface water bodies used for recreation, commercial shellfish harvesting and as sources of drinking-water. The transport of faecal material and the fate of zoonotic pathogens in a catchment is not understood with a great degree of certainty. Modeling is the greatest source of the information in this area and has the potential to be further developed.
- In the context of animal husbandry four control points exist: minimizing exposure of livestock to pathogens, increasing reservoir host immunity and resistance, manipulation of the microbial ecology of the host's gastro-intestinal tract and treatment of animal wastes to reduce zoonotic pathogens. Raising pathogen-free animal herds or flocks as well as treatment of animal waste through composting and anaerobic digestion at high temperatures have proved feasible and effective but, at a considerable cost. Animal vaccination and manipulating the host animal's intestinal flora have met with mixed success.
- The most effective management practices in catchments are at the farm or production facility. Their overall purpose is to reduce pathogen transfer from their sources to a watercourse and they should be evaluated and optimized to that end. Manures can be intercepted and stored in ponds, contaminated water can undergo on-farm treatment and constructed farm wetlands can be used to reduce pathogen load. Bridging and fencing can be used to control livestock near streams. Managing the spatial distribution of animals can avoid the build-up of high concentrations of contamination in sensitive areas.

* http://faostat.fao.org

- Direct human exposure to zoonotic pathogens can be limited by restricting use of affected beaches or other resources through regulation or through public health education regarding exposure to nonhuman sources of faecal contamination. Effective regulation and monitoring may be hindered by sectoral, jurisdictional and geographical boundaries.
- Sanitary surveys and modeling for source attribution have important contributions to make towards the evaluation and categorization of principal sources of faecal pollution.
- Faecal source identification methods should be used to measure potential risk associated with exposure to human and animal pathogens and to target interventions effectively. They merit further research and development.
- Comparative risk assessment can be used to compare risk from various sources in order to make informed decisions about what health or economic benefits might be realized by taking appropriate actions to protect water resources
- Economic evaluations are more frequently being required so that the greatest benefit might be realized from actions taken to improve the safety of recreational and other waters.

The paucity of information in the literature on human health effects associated with exposure to recreational waters contaminated with animal and bird faeces, combined with the limitations outlined above, leads to the conclusion that current standards and control measures may not be appropriate for maintaining the safety of recreational water contaminated with non-human faeces. The implication is that other means of protecting the health of swimmers and other water users must be considered. The approach suggested in this book is to better define the risk posed by animal and bird faecal contamination and provide effective means to manage them.

Information that defines the scope of faecal discharge by domestic animals and the estimated carriage of zoonotic pathogens in their faeces, their transport in the environment and the potential exposure of humans to animal wastes is available in some detail. Intervention and prevention approaches for reducing exposures to animal excreta include prevention of animal infections, intervention at the source, and control measures at exposure locations such as bathing beaches. Of the methods for identifying sources of animal faecal contamination, currently comparative risk assessment provides the most reliable tool for ranking risk and making judgments about where significant economic benefits might be realized. Methods and procedures for benefit: cost and health effectiveness: cost analysis exist to support decision making taking economic benefits into account.

1

Introduction

Jamie Bartram

The excreta (faeces and urine) of mammals and birds are widespread across planet Earth (Figure 1.1) and frequently contaminate water used for bathing and recreation, for treatment and distribution for human consumption, and for irrigating crops.

The risk that such contamination represents to human health is inadequately understood. It is widely assumed that animal faeces represents a lesser risk to human health than human faeces, because of the "species barrier" and especially the species-specificity of most viruses. This assumption has had important consequences for the selection and prioritization of remedial interventions. For example, studies on the impact of faecally-contaminated coastal "waters on the health of bathers" often report symptoms that are consistent with viral aetiology. Species-specificity among viruses indicates an association with human faeces and a priority focus on reducing sewage pollution where this is occurs.

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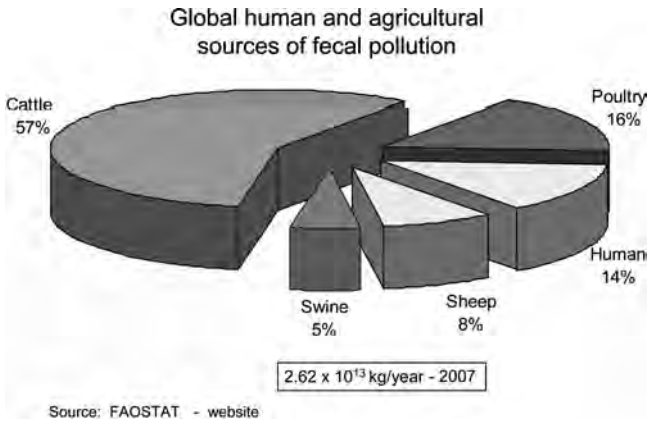


Figure 1.1 Global human and agricultural sources of faecal pollution FAO (2007).

There is at least some cause for concern about contamination of waters with animal excreta, as animal to human waterborne transmission has been documented for several pathogens. Waterborne transmission of *E. coli* O157 has been repeatedly documented and has been associated with outbreaks, including cases of haemolytic uremic syndrome (HUS), especially through drinking-water and to a lesser extent recreational water use. In one outbreak in Swaziland cattle manure was thought to be the source of more than 40, 000 cases of waterborne infection with the organism (Effler *et al.* 2001). One study (Wilson *et al.* 2008) concluded that 96.6% of human clinical infections with *Campylobacter jejuni* in Lancashire, UK could be attributed to farm livestock. Animal reservoir hosts play an important role in the transmission of *Schistosoma japonicum* in both China and the Philippines, with a high level of transmission between species, although evidence suggests that different animal species are important in the two countries (see Chapter 2 and a case study in Chapter 4).

There may be "win-win" opportunities with net economic benefits in better regulation of animal excreta. These arise from the potential to reduce human disease, improve animal health and recover the energy and nutrient value embedded in this resource – which is largely treated as waste at present. These benefits are most readily achievable in more concentrated operations such as large animal feedlots.

Many of the tools that have been used in assessing, managing and regulating risks to human health from contamination of water with human faeces are not applicable, or require adaptation for application, to the control of contamination by animal

faeces. As examples: the relationship between the measurements of faecal indicator bacteria that have been used to index the health risk from faecal contamination are derived for sewage-polluted waters and unlikely to be applicable to waters where faecal pollution is significantly non-human in origin. Similarly, while the discipline of microbial risk assessment is advancing rapidly, data to support its application to organisms of zoonotic origin are limited; lack of adequate exposure measures effectively precludes prospective epidemiological studies.

Much of the management and regulatory experience that has been accrued from the control of human excreta is also directly transferable to management of animal waste. Livestock sources are mostly diffuse (e.g. fresh faeces or stored manures applied to land), though some point sources occur (cattle feedlots, manure heaps, etc.) and have variable characteristics, depending on local conditions.

These challenges and opportunities are compounded by differences between correct and incorrect risk perceptions among the general public, professionals and regulators alike, that are likely to be substantive.

During the consultations that led to the preparation of this publication examples were encountered of both over-confidence in the protection afforded by the "species barrier" and of concern driven by alarmist description of risk. For example, the suggestion that gastroenteritis among swimmers and animal non-point source contaminated water were "not associated" (Calderon *et al.* 1991) has been disputed (McBride 1993) and indeed in a subsequent review USEPA concluded

> "*Thus, water bodies with substantial animal inputs can result in potential human health risks on par with those that result from human faecal inputs.*" *(EPA, 2011 lines 1605–1607).*

One anecdote described a country in which responsibility for control of campylobacteriosis was transferred from the Health to the Food Safety authority, whose policy makers then asserted that *Campylobacter* could not be waterborne, *because it is foodborne.* Of course it is both, and to exclude the waterborne component would inadequately protect public health (see for example McBride *et al.* (2011)).

1.1 PROBLEM DESCRIPTION

There is a mismatch between present regulatory approaches and the needs of effective health protection against the potential risks from water-related zoonotic hazards. There is no specific guidance available on the assessment of risks arising from contamination of waters by animal faeces; nor on the development of health criteria for waters contaminated by animal faeces. This initiative is therefore driven by two pressures: regulatory and risk (including perceived risk).

This will be the second book in the series on "Emerging issues in water and infectious disease" to deal with issues related to zoonoses; in part in recognition of the fact that the majority, around 75%, of emerging and re-emerging pathogens are zoonotic. The first book, "Water-borne Zoonoses" (Cotruvo *et al.* 2004), which complements this volume, focused on three questions:

- The nature of waterborne zoonotic disease threats;
- Identification of new disease candidates; and,
- Adequacy of existing control measures.

In contrast this volume focuses on:

- The adequacy of the evidence base for policy;
- The appropriateness and effectiveness of present regulatory responses; and,
- Opportunities for effective low-cost regulation and management of actual and potential health risks.

For the purposes of assessment, management and regulation, the issue consists of three principal components: the *source* (animal excreta), its *transport* (i.e. transfer to, survival in and movement through watercourses); and resulting *human exposure*. All three can be the object of interventions.

This report, therefore, reflects on understanding what are the assessment, managerial and regulatory challenges, the adequacy of present regulatory approaches, and the characteristics of a better system.

For bathing waters the established approach was, for many years, retrospective compliance testing on an annual basis. However the 1999 "Annapolis Protocol" (WHO, 1999), associated World Health Organization's Guidelines (WHO 2003; 2006) and their implementation in associated developments (EU, New Zealand 2003) show a strong shift towards prevention and real-time support for informed decision-making by members of the public. These documents also recognize, however, that health risks from zoonotic sources in absolute terms or as compared with faecal indicators are inadequately understood and they are cautious in interpreting the associated risks, except in assuming that the risk presented by animal excreta is less than that from human excreta.

For drinking-water the shift to preventive management is more advanced, with detectable changes in regulation and/or practice since the publication of the third edition of WHO's Guidelines for Drinking-water Quality which recommended a "Framework for Drinking-water Safety" and associated "Water Safety Plans" (WHO 2004, reconfirmed in the fourth edition, WHO 2011). However, problems remain of inadequate understanding of zoonotic risks and the inadequacy of faecal bacteria as indicators of risk.

In the case of wastewater use in agriculture, preventive management approaches have been long-advocated to control the risks associated with introducing human excreta, and also effluents and sludge derived from sewage processing, into food production systems (WHO 1973; WHO 1989 and, more recently, with a multi-barrier approach, WHO, 2006b). In deriving these guidelines no account has been taken, however, of potential hazards in mixed wastes where human and animal excreta are both present; and there have been no substantive efforts targeted specifically on animal waste.

All of these routes of human exposure (recreational water use, drinking-water consumption and food produce grown with animal excreta inputs) have the potential to transmit a range of hazards including pathogens (micro-organisms capable of causing disease in humans) and toxic chemicals, including heavy metals and pharmaceuticals, including drugs, antibiotics and their residues. Antibiotics and chemicals used in animal care are of concern but are not separately addressed here. Available evidence suggests that the risks to human health are overwhelmingly dominated by microbial pathogens, and interrupting their transmission is therefore the focus of this book.

1.2 CHALLENGES

Available evidence suggests that risk associated with animal excreta is likely to be episodic in nature. This may arise because of sporadic load (e.g. migrating bird flocks) or sporadic transmission (e.g. mobilization of material on the ground surface or from water sediments following rainfall). Further complexity is added by the extreme spatial and temporal variability arising from factors such as seasonal weather patterns, livestock operations management (e.g. washing out of cattle sheds, seasonal grazing) as well as cycles of calving and associated microbial colonization and shedding including the phenomenon of "super-shedders" among herds and flocks. In New Zealand for example, at times of low river flow, *Campylobacter* isolates are primarily of ovine origin while strains of bovine origin dominate at times of high river flow.

Several studies have quantified the relative impacts of sewage and animal derived fluxes of microbial pollution to bathing waters (Stapleton *et al.* 2008, 2010; Kay *et al.* 2009; 2010; 2012). In an early study of rural catchments, Crowther *et al.* (2002) report on two such studies in the United Kingdom. Both catchments were livestock farming areas and the streams draining these areas were considered "pristine" in ecological terms. In both areas, bathing water quality non-compliance occurred after rainfall events. Studies revealed that this non-compliance was caused by microbial pollution from "normal" farming activities. Sewerage was not a significant cause of non-compliance during dry weather

conditions when anthropogenic load dominated. A similar pattern was reported by Stapleton *et al.* (2010). Again, livestock-derived, microbial flux dominated during periods of rainfall and improvements to water quality during this period from investments in sewerage systems were imperceptible. In this case, water quality was key to microbial compliance of adjacent shellfish-harvesting waters.

Many of the species of pathogens of concern are circulating naturally within one animal species or the other or among humans, and there may be significant strain or species specificity. Thus, simple and conventional classification and categorization of microbes may be misleading; and criteria based on aggregate microbial groupings may significantly under- or over-estimate risk. Similarly a single water body may be contaminated by faeces from different animal species and this contamination may contain strains of the same micro-organism from different host species. These factors all add complexity to the assessment of risk and to the management and regulation of water contamination with animal excreta.

These complexities highlight some of the demands on risk assessment practice. Neither available tools nor data can adequately respond to the associated challenges. Most available data, for example on the effectiveness of interventions, relate to faecal indicator bacteria (such as *E coli,* thermotolerant coliforms or intestinal enterococci) and not to pathogens. These faecal indicator bacteria are imperfect measures or indicators of pathogen die-off. Nevertheless, systematic monitoring of zoonotic pathogens in the environment is rare. Much risk assessment practice focuses primarily on steady state conditions but, in fact, zoonotic risk is likely to be very episodic and variable. Similarly, most risk assessment is based on impacts on numbers of individuals, not dynamically on populations (disease dynamics) and there is increasing recognition of the inadequacy of such approaches for the above reasons.

These complexities also point to challenges for effective regulation. For example, present regulatory approaches may permit sporadic failure, especially when associated with extreme events. However, targeting regulation on health risk would mean recognizing that such episodes may represent the periods of greatest risk that should, in fact, be targeted by regulation. A further challenge in assembling an effective portfolio of regulatory measures is that its elements may be many and diverse. Distinct components may be direct (for example, targeted on agricultural practices or land use planning) or indirect (for example, food standards requiring irrigation water quality standards or best practice in manure use in agriculture). Securing a consistent approach that is effective and not onerous or costly across the multiple potential legal instruments that may apply in any given jurisdiction will not be easy. Similarly, while there is extensive experience with regulating *point sources* of pollution in many

jurisdictions, regulation targeting microbial *movement* is relatively new and lessons are still being learned; and there is little substantive experience with regulation targeting human exposure.

At the interface of narrowly-defined regulation and wider policy, and including arrangements for inter-sectoral coordination, are the differing perspectives of the individual facility or farm and the collective impact of many such facilities or farms on a single catchment, including its multiple and diverse uses and the value and utility of such uses – extending into the associated coastal zone. This also implies an understanding of overall microbial load and of associated health risks (Kay *et al.* 2004).

Benefit-cost analysis (BCA) is one means to pursue regulatory efficiency, by demanding that both costs and benefits be described and to the extent possible quantified; and using the evidence derived to compare alternative responses and to reflect on means to minimize cost and maximize benefits of adopted approaches (Hahn & Tetlock 2008). Application of BCA to livestock excreta management for health protection has not been attempted in large part because benefits are poorly understood. For example, many interventions will operate on a number of pathogens and not a single agent. The outbreak of waterborne disease in Walkerton, Ontario, Canada that resulted in seven deaths and over 2300 cases of gastrointestinal illness was associated with infections by both *E. coli* O157:H7 and *Campylobacter jejuni*. Intense rainfall is thought to have washed cattle manure into a well which served as the water source for the town (Auld, Klaassen & Geast 2001). The nature of benefits is also evolving as greater attention is given to energy and nutrient recovery; and as the appreciation of the value of the recovered resources increases, so the benefit component of BCA expands accordingly.

1.3 OPPORTUNITIES

There may be opportunities to look for alternative solutions applicable to animal excreta as compared to those associated with the monitoring, management and regulation of human faecal contamination.

Direct targeting of specific pathogens has received little support in monitoring of human faecal contamination, in part because of the large number of potential pathogens, both known and unknown. However, since the number of credible zoonotic pathogens from any given animal species may be more limited, the role of direct pathogen monitoring may merit re-appraisal.

High-intensity production systems presently account for three quarters of chickens, two thirds of milk, half of eggs, and a third of pigs (FAO 2009). More intensive operations (such as the zero-grazing option: cattle managed exclusively

in sheds) may provide opportunities to increase management controls. Overall, livestock production is intensifying with larger facilities accounting for an increasing proportion of total production. In developed nations such as the USA consolidation of the livestock industry is likely to maintain this trend while in less-developed nations intensive and traditional systems co-exist. High-intensity operations provide more achievable opportunities for recovery of energy and nutrient resources.

Developments in scientific understanding (including the emerging ability to predict health benefits), the increasing global attention to preventive management and accruing experience with its regulation in many countries combine to provide an opportunity to advance significantly the control of pathogens derived from animal excreta and of the associated human health risks.

There are diverse management options (interventions) available that provide approaches that may be directed at sources of contamination (such as stock management, vaccination, test-and-slaughter, quarantining, control of feed and water quality and on-site waste storage and/or treatment); transmission (such as barrier strips, retention ponds, constructed wetlands, timing of land applications and restriction of direct access to water courses by animals); and human exposure (such as drinking-water treatment and provision of public awareness-raising and information on recreational water quality).

Approaches to preventive management have been analysed and advocated for diverse exposure routes (Fewtrell & Bartram, 2001). Examples of their application include the multiple-barrier principle, Hazard Assessment Critical Control Point (HACCP) analysis, applied in food safety, the Annapolis Protocol (applicable to recreational waters); Water Safety Plans (WSPs), applied to drinking-water supply and long-established approaches to regulation of use of wastewater and excreta in agriculture (now evolving into sanitation safety planning). These approaches, especially if combined with recent developments in microbial source attribution and alternative indicators of faecal contamination, may provide opportunities to optimize health protection, contain costs and understand the relative contributions of different compartments to health protection.

By providing multiple opportunities to intervene, including different interventions at the levels of source, transmission and exposure, it is possible to evaluate preventive approaches that are amenable to comparative analysis and to benefit:cost analysis in particular. Experience has shown that "smarter" regulation can increase public health protection without significant increase in cost (see Chapter 11). Tools such as BCA enable the aggregation of multiple benefits and in doing so may point to the greater value of "upstream" interventions, the benefits of which accrue to multiple "downstream" uses.

Health impact assessment (HIA, Quigley *et al.* 2006) has proven effective in supporting this integrated perspective capturing diverse types and locations of impact and pointing to a range of possible interventions by actors from different sectors as part of a public health management plan.[1]

One positive development has been the development and validation of catchment models, although these have focused on describing the movement of faecal indicator bacteria, rather than specific pathogens or classes of pathogens, in river and coastal systems. Early papers in this area have centred on generation of microbial export coefficients for different land use types and sewage treatment (Kay *et al.* 2008a,b), but more process-based modelling is required to provide management information on the efficacy of field-scale intervention strategies.

1.4 DRIVING FORCES AND FUTURE PERSPECTIVES

Available information suggests that the underlying issue confronted by this volume is, and is likely to remain, of ongoing or increasing concern.

Water-related disease remains an issue of major global public health concern, with the water-sanitation-hygiene risk complex globally accounting for around 10% of the global burden of disease (Pruess *et al.* 2008). This disease burden is heavily skewed towards the developing world, and least-developed countries in particular. The largest single disease outcome contributing to this burden of disease is infectious diarrhoea (accounting for 65% of the total), including its direct impact on nutritional status and indirect impact on diseases associated with malnutrition. Humphrey (2009) suggests that tropical enteropathy may be an under-appreciated adverse health outcome with pervasive impacts on child development arising from excessive exposure to faecal bacteria. The contribution of organisms of immediate or recent zoonotic origin to infectious diarrhoea and to tropical enteropathy is unknown. However, given the overall importance of faecal contamination to this large fraction of the global burden of disease, even a small fraction of this total would be of potential public health significance.

The phenomenon of true *emergence of "new" pathogens* from zoonotic sources (e.g. SARS, H1N1) is itself beyond the scope of this book but will place increasing pressure on entities responsible for public health and will continue to draw attention to the importance of zoonoses to public health in general.

[1] see: http://www.iaia.org/publicdocuments/special-publications/SP5.pdf

Intensification of livestock production places larger numbers of animals in closer proximity to one another, bringing with it an increased likelihood of true pathogen emergence and also of development and transmission of antibiotic resistance.

Increasing numbers and proportions of *more vulnerable human population groups* (notably the elderly and immuno-compromised) will provide opportunities for different pathogens to attain prominence and points to continuing public health relevance.

Population growth and increasing affluence are associated with increased demand for meat protein. Domestic animals already outnumber humans by a factor of four (FAO 2009). Satisfaction of demand will be through *increased livestock production and intensification,* aggravating the challenges of their excreta management that are already significant in high-, middle- and low income settings.

Extension and proliferation of human settlements will bring human populations into greater immediate proximity to livestock concentrations with greater *opportunities for pathogen transmission* from livestock to humans, with water a medium of prominent importance.

Predicted shortfalls in fertilizer availability will encourage *use of animal manures on agricultural land*. The economic value of nutrients contained in manure may contribute to encouraging source management (containment and close-to-source treatment).

In parallel, increasing concern for the "carbon footprint" of economic activities and the *energy value of animal excreta* will increase the incentive for their containment and productive management and this will replace their previous consideration as waste products.

Policy attention to *climate change mitigation* is likely to be sustained and will bring with it attention to greenhouse gas emissions from livestock – it has been estimated that livestock produce between 8 and 18 percent of anthropogenic carbon dioxide (Steinfeld *et al.* 2006). It is unclear whether this will impact excreta management but it is likely to place pressure on good practices in the industry and the potential clearly exists for animal manure to replace fertilizers with a greater carbon footprint in production and distribution or with finite natural reserves.

Even short-term predicted *variability in climate* will lead to increased frequency of dry and wet events in many regions (Howard *et al.* 2010). While impacts may increase or reduce pathogen transport to water courses, including groundwater, specific concern relates to failures in containment of animal wastes and in their mobilization and dispersal in response to increased or more intense rainfall.

1.5 SCOPE OF THIS BOOK

The subject of zoonoses of potential concern for waterborne transmission has a large scope. During the development of this book it was decided to focus on those zoonoses for which there is a credible prospect of regulatory action. This led to a focus on infectious agents from animals that are under human management (both commercial and subsistence livestock), safe management of wastes produced by which might reasonably be required or encouraged. Thus, the focus is on livestock, rather than feral or wild animals. Such a distinction is to some extent artificial and some pathogens may derive from both livestock and wild animals. For example both *Giardia duodenalis* and *Cryptosporidium parvum* infect the intestinal tract of a range of host animals including humans, dogs, cats, cattle, sheep, horses and rodents among others; while the infectivity to humans of strains from different animal sources appears to vary (e.g. *S. japonicum*).

Furthermore, a pragmatic decision was made to exclude domestic pets such as dogs and cats from consideration as the interventions that would contribute to the control of their excreta are distinct. While fish farming may be included under a definition of intensified livestock production, fish excreta were also excluded from consideration.

Finally, this book looks at the assessment, regulation and management of existing hazards. It does not address the issue of true "emergence" that is natural propagation of an entirely new pathogen of human public health significance. Such emergence occurs across viruses, bacteria, protozoa and other pathogens; it is, however, especially frequent among viruses. Examples include Severe Acute Respiratory Syndrome (SARS), that arose from a 27 base pair deletion in an animal corona virus (likely to have previously sporadically infected humans with less severe consequences); this mutation rendered it highly pathogenic for humans. The periodic emergence of novel strains of influenza viruses from recombination provides a further example. True emergence has, presumably, also happened at some point in the past with Hepatitis E virus (HEV). It is noteworthy that around 75% of emerging pathogens are of zoonotic origin.

Notwithstanding the exclusion of true emergence, history suggests that resilience can be embedded into public health systems through recognizing the value of planning for the unexpected.

Thus prevention of faecal contamination of recreational waters and drinking-water sources will impact beneficially on the control of a wide range of known and potentially unknown and yet-to-emerge pathogens; and multiple-barrier treatments that employ multiple and different processes are more likely to be effective against an unrecognized or emergent agent than those which rely on a single process of removal or inactivation.

1.6 STRUCTURE OF THIS BOOK

This book is structured according to the major issues discussed and as summarized in Figure 1.2:

- This introduction summarizes the rationale, context, objectives, scope and structure of the book.
- Chapter 2 summarises the state of knowledge with regard to specific, credible waterborne pathogens. In terms of the further development of the book it importantly introduces the idea of a limited number of priority pathogens that are used to assess and calibrate interventions in the remainder of the book.
- Chapters 3–8 then explore the major components of source (load), transport and (human) exposure:
  - Chapter 3 looks at sources and load as a composite issue, including state of knowledge and the adequacy of available data and tools in assessment and monitoring. Chapter 4 explores current knowledge of interventions to reduce load including specifically those that may be the targets of regulation and issues associated with such regulation.
  - Chapter 5 looks at transport as an issue, including state of knowledge and the adequacy of available data and tools in assessment and monitoring. Chapter 6 explores current knowledge of interventions to manage transport including specifically those that may be the targets of regulation and issues associated with such regulation.
  - Chapter 7 looks at human exposure, including the state of knowledge and the adequacy of available data and tools in assessment and monitoring. Chapter 8 explores current knowledge of interventions close to the point of exposure, including specifically those that may be the targets of regulation and issues associated with such regulation.
- Following introduction of the critical issues of load transport and exposure, three chapters are dedicated to exploring the data and tools used, associated state of knowledge and the adequacy of information generated by them to inform both regulation and management.
  - Chapter 9: Indicators, Sanitary Surveys and Source Attribution Techniques.
  - Chapter 10: Comparative Risk Analysis.
  - Chapter 11 contains a review of epidemiological studies on swimmer health effects associated with potential exposure to zoonotic pathogens in bathing beach water.
  - Chapter 12 looks at economic evaluation – the assessment of risks, costs and effectiveness of interventions and costs and impact of regulatory

alternatives, using a specific example from bathing water in the Netherlands which is relevant to a wide range of similar hazards and their control.

- A summary statement serves as an "executive summary" for the work as a whole and highlights critical implications and recommended actions for different target groups. In convention with other books in this series it summarizes the conclusion of those experts that participated in the process of development of the book as a whole.

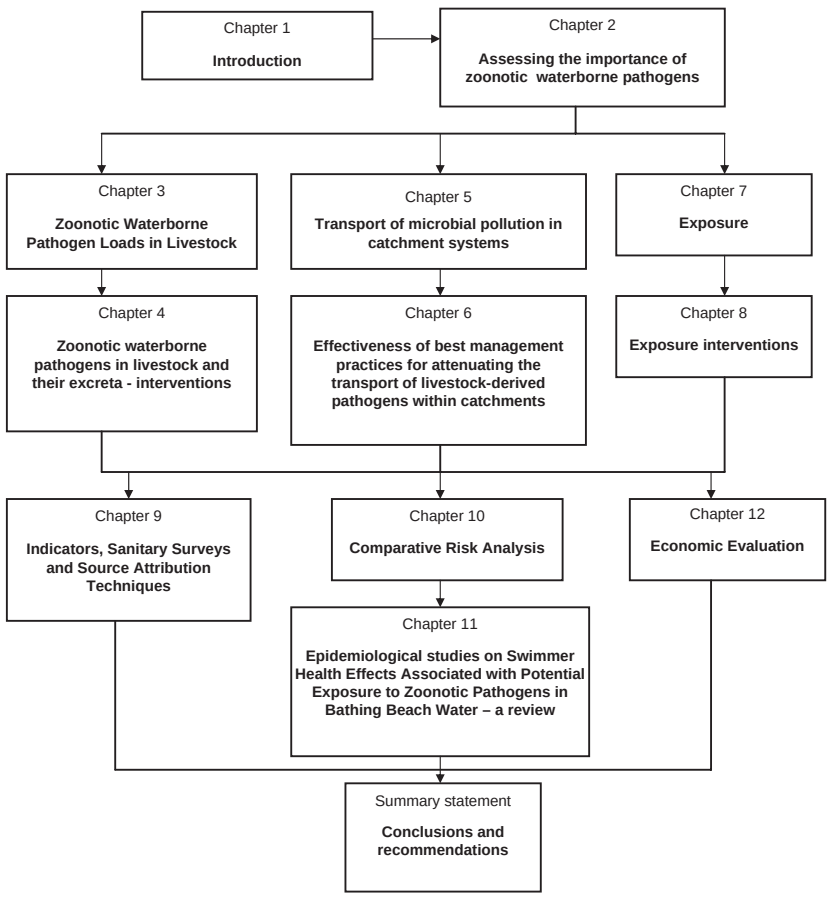


Figure 1.2 Structure and chapters of this book.

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2

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

© 2012 World Health Organization (WHO). *Animal Waste, Water Quality and Human Health*. Edited by Al Dufour, Jamie Bartram, Robert Bos and Victor Gannon. ISBN: 9781780401232. Published by IWA Publishing, London, UK.

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.

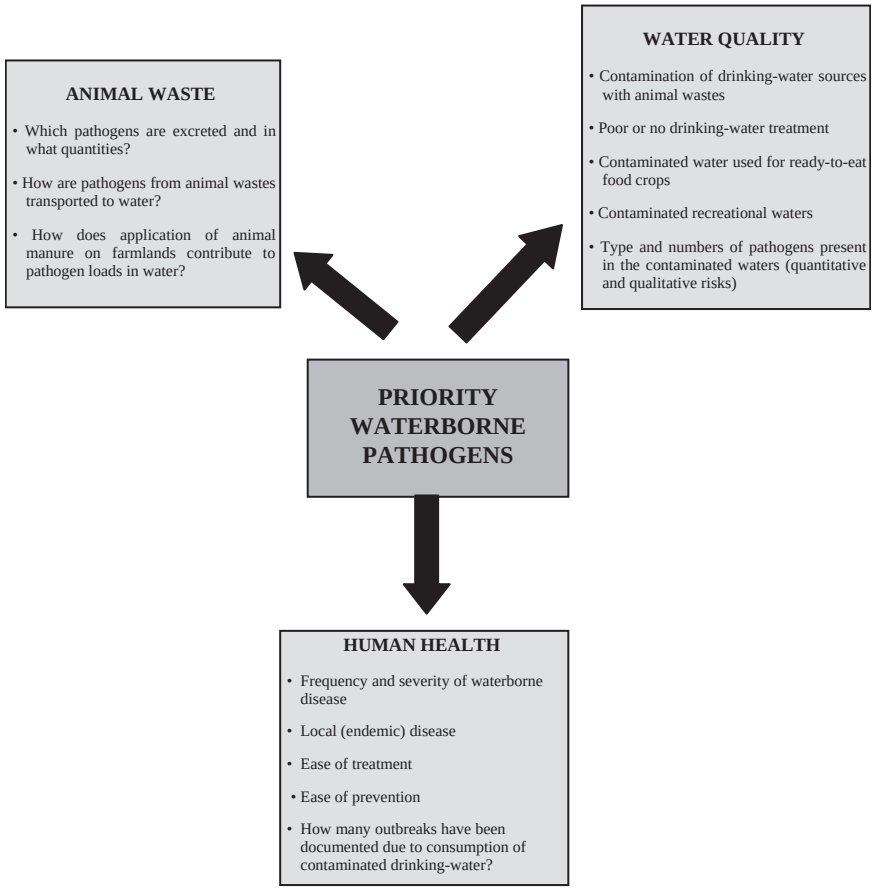


Figure 2.1 Questions to facilitate the interrelation of seemingly diverse subjects such as animal waste, water quality and human health.

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.

Ranking by importance	Criteria description
1	There is strong evidence that the organism is zoonotic (i.e. genotypes from animals are highly related or identical to those found in humans). 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 >2.0). Disease outbreaks occur in healthy humans. Disease in humans results in serious illness and/or death. 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. Agents are resistant to water treatment procedures or other remediation efforts.
2	As in 1, except that agents are inactivated by water treatment procedures such as chlorination.
3	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.
4	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.

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 excretia.

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.

	Pathogen	Severity of disease	Evidence for waterborne transmission	Killed by chlorination of water	Principal animal reservoirs	Distribution	Rank
Protozoan	*Cryptosporidium parvum* *C. hominis*	Outbreaks in healthy humans. Diarrhoea. Low case fatality	Yes	No	Cattle, other animals	Worldwide	1
Protozoan	*Giardia duodenalis*	Outbreaks in healthy humans. Diarrhoea. Low case fatality	Yes	No	Beavers, porcupines, dogs, other animals, cattle	Worldwide;	1
Bacteria	*Escherichia coli* O157:H7	Outbreaks in healthy humans. Haemorrhagic colitis and hemoylitic uremic syndrome, occassionally. Long-term systemic sequela. Moderate case fatality	Yes	Yes	Cattle and other ruminants	Worldwide	2
Bacteria	*Campylobacter jejuni*	Outbreaks occur in healthy humans. Bloody diarrhoea. Usually self – limiting. Long-term systemic sequelae. Low case fatality	Yes	Yes	Cattle, swine, poultry, dogs, cats, wild birds	Worldwide	2

(*Continued*)

Table 2.2 (*Continued*)

	Pathogen	Severity of disease	Evidence for waterborne transmission	Killed by chlorination of water	Principal animal reservoirs	Distribution	Rank
Bacteria	*Salmonella enterica* subspecies *enterica* (1,500 serovars)	Outbreaks in healthy humans. Diarrhoea. May result in septicemia. Moderate case fatality	Yes	Yes	Poultry, swine, cattle, horses, dogs, cats, wild mammals and birds, reptiles, amphibians	Worldwide	2
Bacteria	*Leptospira interrogans* (200 serovars) in 23 serogroups	Endemic regions but outbreaks occur in healthy humans. May result in septicaemia. Moderate case fatality	Yes	Yes	Domestic and wild animals, common in rodents, dogs	Worldwide	2
Bacteria	*Francisella tularensis* subsp *holarcticas*	Endemic regions but outbreaks occur in healthy humans. Lymphadenitis, septicaemia. Low case fatality.	Yes	Yes	Rodents	Europe and Asia	3

Helminth – trematode	*Schistosoma japonicum*	Endemic. Fever. Chronic hepatosplenic disease, impaired physical and cognitive development	Yes	Yes	Cattle, buffalo, swine, dogs, cats, rodents	Southeast Asia, China, Philippines	3
Helminth – Trematode	*Clonorchis sinensis* (Chinese liver fluke)	Endemic. Cholangitis and biliary obstruction, poor digestion of fats, cholangio-carcinoma	Yes	Yes	Dogs, cats, swine, rats, wild animals	Asia	3
Helminth – Trematode	*Fasciola hepatica*	Endemic. Hepatic enlargement, jaundice, anemia cholangitis, cholecystitis and cholelithiasis	Water-plant-humans	Yes	Cattle, sheep, other large ruminants (e. g. water buffalo)	Worldwide	3
Protozoan	*Toxoplasma gondii*	Outbreaks in pregnant women. Birth defects. Moderate case fatality	Yes, but uncommon	No	Mammals, especially cats, food animals, birds	Worldwide; common	2
Protozoan	*Blastocystis hominus*	Endemic and rare outbreaks. Diarrhoea. Low case fatality	Yes	Yes	Various wild and domestic animals including birds, humans	Worldwide	3

(*Continued*)

Table 2.2 (*Continued*)

	Pathogen	Severity of disease	Evidence for waterborne transmission	Killed by chlorination of water	Principal animal reservoirs	Distribution	Rank
Protozoan	Microsporidia Enterocytozoon bieneusis Encephalitozoon cuniculi; E intestinalis; E hellem	Endemic. Diarrhoea. Low case fatality	Unknown	No	Various wild and domestic animals, primates, rodents, psittacine birds	Worldwide	4
Virus	Hepatitis E virus (Genotypes 3 and 4)	Outbreaks in healthy humans. Systemic disease. Low case fatality	Unknown	Yes	Swine	Probably worldwide	4
Virus	Rotavirus A	Outbreaks in healthy humans. Diarrhoea. Low case fatality.	Unknown	Yes	pigs	Probably worldwide	4

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 $>10^9$ *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 2.8×10^8 cases of giardiasis annually (Lane & Lloyd 2002). In Asia, Africa and Latin America there were an estimated 2.0×10^8 cases with 0.5×10^6 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 Ivai, 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 *Blastocytis* 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).

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).

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

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 *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 *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 *E. coli* have been associated with human disease, however, and even within *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 *E. coli* O157:H7 genetic groups (Manning *et al.* 2008).

Most human *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 *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 Walkteron 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 practises 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 (Litrup *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 14×10^9 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 ophthalitis (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, Schőnberg-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).

***Leptospira* 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 *holarcticas* 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 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.

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3

Zoonotic waterborne pathogen loads in livestock

Edward R. Atwill, Xunde Li, Delia Grace and Victor Gannon

3.1 INTRODUCTION

3.1.1 Objectives

This chapter provides an overview on the prevalence and environmental load of waterborne zoonotic pathogens of public health importance shed in the excreta of livestock. Chapter 2 presents the five zoonotic pathogens that will be reviewed in this book: *Cryptosporidium parvum*, *Giardia duodenalis*, *Escherichia coli* O157:H7, *Salmonella*, and *Campylobacter*. These protozoa and bacteria are important disease-causing agents and satisfy the three criteria agreed to be classified as priority one waterborne zoonotic pathogens associated with livestock populations: capacity to induce clinical illness in susceptible humans,

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capability to be transmitted to humans through water, and prevalence in a biological reservoir host including one or more livestock species. A biological reservoir is defined as a host (in the context of this book: a livestock species) that allows the pathogen to amplify or reproduce and not merely pass through.

Information on the prevalence, intensity and environmental load of these five pathogens is presented along with methodological concerns on how we draw inferences from these measures of occurrence and the human health risk they represent. Important aspects of the ecology and epidemiology of these five pathogens in livestock populations are also discussed in as much as they may be useful in the development of intervention strategies to limit their prevalence in livestock and to reduce the risks of waterborne transmission to humans.

3.1.2 Livestock status and trends in developed and developing countries

At the beginning of the 21st century, the volume and pace of expansion of the global livestock population is unprecedented. As a consequence, environmental loads with livestock faeces are also unprecedented and this faecal material potentially contains the agents of waterborne zoonoses. The problem of livestock-associated zoonoses starts with livestock populations, and we will briefly review the current status of, and major trends in, these populations. In 2007, the world population of domestic animals was estimated at 24 billion, out-numbering humans by nearly four to one (Table 3.1). The dramatic growth in animal husbandry is driven by the growth in the world population and the rise in incomes in many emerging countries especially in Asia and South America: the so-called "Livestock Revolution" (Delgado *et al.* 1999). Livestock production systems, both intensive and extensive, are present on a large percentage of earth's surface area; 26% of the earth's terrestrial surface is pasture and 33% of global arable land is used to grow animal feed (FAO 1996). Although livestock trade is growing, more than 90% of livestock products are consumed in the countries where they are produced so the problem of livestock faecal load is essentially domestic. The lack of water treatment facilities and widespread use of untreated wastewater in poor countries suggest waterborne zoonoses may be a greater problem there than they are in high-income countries.

The livestock sector is dichotomized into high-intensity production systems in developed countries and parts of Asia, and low-intensity production in the humid and sub-humid tropics (FAO 1996). An estimated 70% of poultry and 58% of pigs are raised in high-intensity systems (Robinson *et al*. 2011) but only a minority of ruminants. In contrast, the production of low-intensity systems is relatively small,

but it involves many people: 600–700 million poor people keep livestock (IFAD 2004). In these systems, livestock are also a major source of power with more than 50% of arable land cultivated by draught animals (Ramaswamy 1994). In high-intensity systems, dense concentrations of industrial livestock production create vast quantities of manure and concentrated environmental problems. The situation in developing countries is compounded by ineffective environmental regulations and the location of agro-industry in or close to cities. In low-intensity systems, livestock faeces is an important commodity used for fertilizer, fuel, animal feed and building material. Excessive environmental loads may not be a problem but there are much greater human exposure risks from livestock faeces and hence a greater potential for zoonoses.

Table 3.1 Global total of various livestock species.

	2000	2012	% change
Cattle	1,314,813,626	1,428,636,207	8.7
Buffaloes	164,114,418	194,168,699	18.3
Sheep	1,059,759,106	1,078,948,201	1.8
Goats	751,440,392	921,431,865	22.6
Pigs	898,813,265	965,855,414	7.5
Horses	57,223,059	58,459,080	2.2
Asses	41,631,889	42,152,395	1.3
Chicken	14,401,862,000	19,458,571,000	35.1
Turkeys	457,993	449,442	−1.9
Duck	947,569,000	1,187,674,000	25.3

Data source: http://faostat.fao.org
Source: Food and Agriculture Organization of the United Nations 1996.

3.1.3 Pathogen loading by livestock and recreational waterborne zoonotic disease

There is growing concern among public health agencies from both developed and developing countries that zoonotic pathogens in livestock excreta pose an unacceptable waterborne public health risk (Cotruvo 2004). This concern is based on the large numbers of livestock kept (Table 3.1), the prevalence of water-borne zoonoses among livestock, outbreak investigations linking waterborne zoonotic illness in humans to livestock (e.g., Auld, McIver & Klaassen 2004), inferences drawn from case-control studies (Hunter & Thompson 2005), and direct human infectivity trials (DuPont *et al.* 1995, Okhuysen *et al.* 1999). Taken together, this growing body of evidence suggests

that under the right environmental, hydrological, host and other conditions, human ingestion of water contaminated with low levels of livestock excreta can result in illness from these pathogens. For waterborne transmission to occur, sufficient amounts of human-infective *Cryptosporidium parvum*, *G. duodenalis*, *E. coli* O157:H7, *Salmonella*, or *Campylobacter* need to be shed in the excreta of livestock, survive the numerous processes of the terrestrial or aquatic components of a catchment that attenuate and/or inactivate this pathogen load (e.g., filtration, UV inactivation, desiccation, dilution, senescence), contaminate recreational water and be ingested in infectious doses. As such, all else being equal, the amounts of these pathogens generated by livestock populations is related to the risk levels of waterborne transmission to humans. Therefore, an accurate calculation of the rate of pathogen production for the various livestock production systems will help assess livestock pathogen exposure risk and allow more effective targeting of load, transport, and exposure interventions on high risk livestock populations.

3.1.4 Environmental loading rate of zoonotic pathogens in livestock

The environmental loading rate per animal is a metric that allows comparison of the pathogen production rate between livestock species, different age classes or different animal production systems (Atwill *et al.* 2003, Dorner *et al.* 2004). It is defined as the total amount of a specific pathogen excreted per animal per day. Given that all five priority waterborne zoonotic pathogens are shed primarily in the faeces rather than the urine of infected animals, the environmental loading rate excludes urine production in its calculation. For pathogens such a *Leptospira*, the measurement of urine production would be critical. The environmental loading rate can be crudely estimated in various ways (Atwill *et al.* 2003, Dorner *et al.* 2004, Starkey *et al.* 2007, Ferguson *et al.* 2005), for example, daily faecal production per animal (f) × the point prevalence of faecal shedding (P) × mean intensity or concentration of pathogens excreted by infected animals (I_p), or fPI_p. Examples of faecal production rates are presented in Table 3.2. The amount an animal defecates per day is largely determined by dry matter intake (DMI) (Weiss 2004, Wilkerson *et al.* 1997), which, in turn, is influenced by the metabolic needs of the animal (e.g., body weight, lactation status), diet composition, and other factors. For example, an equation predicting wet excreta (faeces + urine) production per lactating dairy cow is: wet manure (kg/d) = 3.0 × DMI (kg/d) (Weiss 2004).

This approach can be used for confined and extensive livestock populations where individual faecal samples are collected from a representative sample of

Table 3.2 Examples of faecal production rates for various livestock species.

		Faecal production[1]		
	Index weight[2] (kg)	**kg animal^{-1} d^{-1}**	**% of body wt**	**Reference**
Dairy or beef calf: pre-weaned	50	1.65	3.3%	extrapolated from Wilkerson 1997
Dairy heifers: yearling	300	9.8	3.3%	ASAE Standards 2002[4]
Non-lactating cows	680	10.3	1.51%	Wilkerson. 1997
Lactating dairy cow	614	25.3		
average 14 kg/d	603	36.2	4.1%	Wilkerson 1997
milk average	NR	NR	6.0%	Wilkerson 1997
29 kg/d milk	NR	NR	7.1%	Johnson 2002
average 34 kg/d			8.6%	Johnson 2002
milk average				
51 kg/d milk				
Beef cattle	360	14.4	4.0%	ASAE Standards 2002
Feedlot steer: at entry	315	10.5	3.3%	Tucker & Watts 1993
Sheep	27	0.7	2.5%	ASAE Standards 2002
Pigs	61	2.7	4.5%	ASAE Standards 2002
Horses	450	18.5	4.1%	ASAE Standards 2002
Chickens	1.8	0.12[3]	6.4%[3]	ASAE Standards 2002
Layer	0.9	0.077[3]	8.5%[3]	ASAE Standards 2002
Broiler				
Turkeys	6.8	0.32[3]	4.7%[3]	ASAE Standards 2002
Ducks	1.4	0.15[3]	11%[3]	ASAE Standards 2002

[1]Excludes urine production, faecal mass as voided or wet weight.
[2]Weight (kg) used for estimating median or mean faecal production mass per animal per day.
[3]Includes all excreta.
[4]Erickson, Galen E.; Auvermann, B.; Eigenberg, R. A.; Greene, L. W.; Klopfenstein, Terry J.; and Koelsch, Richard K., "Proposed Beef Cattle Manure Excretion and Characteristics Standard for ASAE" (2003). *Conference Presentations and White Papers: Biological Systems Engineering*. Paper 2. http://digitalcommons.unl.edu/biosysengpres/2
NR = not reported.

animals. To illustrate an example of comparing two different host species using this metric, the environmental loading rate of *Cryptosporidium* spp. for a typical beef cow in the western USA ranged from 3,900 to 9,200 oocysts per animal per day (Atwill *et al.* 2003). In contrast, the oocyst production rate for the various genotypes of *Cryptosporidium* from California ground squirrels is about 58,000

oocysts per squirrel per day, a rate that is 6 to 15 times greater in oocyst production than adult beef cattle despite being less than one thousandth the size of a beef cow (Atwill *et al.* 2004). Probabilistic modeling and Monte Carlo estimation procedures for this parameter can generate uncertainty intervals for the oocyst loading rate (Dorner *et al.* 2004, Starkey *et al.* 2007). This rate can be further adjusted for animal numbers per acre or hectare (A), resulting in the calculated pathogen production rate for a specific animal stocking rate per unit area, fPI_pA. Pathogen production rates per acre or hectare can allow a more accurate match between the expected pathogen load at a site and the effectiveness or $\log_{10}$ reduction capacity of a load or transport intervention strategy (Tate *et al.* 2004). Lastly, the spatial pattern of faecal deposition by livestock predicts where the pathogen loads on a landscape will be deposited for extensive livestock production systems and draught animals. In general, this spatial pattern is not randomly distributed across the landscape, but often highly clustered or positively correlated with preferred foraging sites, loafing areas, staging areas for draught animals, drinking water, shade in warmer climates, and feed supplements (e.g., salt block, concentrates) (Tate *et al.* 2003, Lewis *et al.* 2005). It is likely that only a portion of the total spatial pathogen load is hydrologically connected, suggesting that the portion that has little risk of reaching water can be discounted or under-weighted in landowner- and catchment-scale modeling of waterborne pathogen transmission (Tate *et al.* 2003, Ferguson *et al.* 2005).

Accurate estimates of the environmental loading rate can also be generated by daily faecal sampling of representative groups of animals and calculating pathogen loading across the entire infectious cycle (Nydam *et al.* 2001), but the effort needed to generate this type of data precludes its widespread use, especially for extensive animal populations at low stocking densities. Alternatively, it may be cheaper to estimate the environmental loading rate as the mean intensity of a pathogen for pooled faecal matrices from all animals in a group (positive and negative animals) matched by age or weight class ($I_{p,n}$) × daily faecal production per animal for that age or weight class (f), $fI_{p,n}$. One concern about this approach is that pooling of positive and negative faecal samples will lower pathogen concentrations in the faecal matrix, and depending on assay sensitivity, generate excessive false negatives and under-estimates of pathogen loading.

Lastly, the advent of molecular fingerprinting tools has sharpened our understanding of host-adapted strains and virulence factors that are responsible for the majority of human illness, which has the effect of reducing both the prevalence of faecal shedding ($\downarrow P$) and the mean intensity of human-infective pathogens excreted among positive animals ($\downarrow I_p$), thereby reducing the calculated environmental loading rate for many of the priority pathogens. Examples where a subset of strains is responsible for much of the burden of human illness from a

pathogenic microbial species would be *E. coli* O157:H7 in North America, where more than 90% of sporadic and outbreak cases of haemorrhagic colitis and the haemolytic uremic syndrome (HUS) are associated this serotype (Gyles 2007, Karmali 2005, Karmali *et al.* 2009), specific multi-locus sequence typing (MLST) patterns for *Campy. jejuni* in the UK (Sheppard *et al.* 2009), assemblages A and B for *Giardia duodenalis* (Hunter & Thompson 2005), and the *Crypto. parvum* GP60 IIa subtype (Feltus *et al.* 2006).

The discovery of the host-specificity of certain pathogen subtypes has given rise to the suggestion that their genetic fingerprints may be useful in determining the sources of water contamination with animal wastes. However, to be successfully used in tracking host sources of faecal contamination, the agents have to be widespread in the host population, be shed in very large numbers, be relatively stable and be detectable using simple laboratory tests.

3.2 WATERBORNE ZOONOTIC PROTOZOA

Two protozoa, *Cryptosporidium parvum* and *Giardia duodenalis*, are classified as high priority waterborne zoonoses. *Crypto. parvum* and *G. duodenalis* are able to infect humans at very low doses (Okhuysen *et al.* 1999, Chappell *et al.* 2006), they can survive in cool water for extended periods of time (especially *Crypto. parvum*) and can resist many chemical disinfectants (especially *Crypto. parvum*; Korich *et al.* 1990, Erickson & Ortega 2006), and are unable to replicate in the environment. Once the faecal load of these protozoa has been deposited in the environment by animals, the (oo)cyst numbers begin to decline in both faeces and water due to a variety of natural processes, especially once temperatures exceed 40°C (Li *et al.* 2005, Gomez-Couso *et al.* 2009). The epidemiology and management of protozoal zoonoses hence differs in some respects from the bacterial waterborne zoonoses (*E. coli* O157:H7, *Salmonella enterica*, and *Campylobacter). Crypto. parvum* and *G. duodenalis* are found throughout the world, can coexist in the same host and are commonly transmitted using faecal-oral and waterborne transmission pathways. Although *Cryptosporidium hominis* is a common cause of human cryptosporidial infection in many locations around the world, this species is shed mostly by humans and only occasionally by other animals (Hunter & Thompson 2005).

3.2.1 *Cryptosporidium parvum*

Crypto. parvum is a protozoan or single-celled parasite that infects humans and a variety other mammalian species, including different species of livestock

(cattle, sheep, goats, and horses) and various other domestic and wild animals (Hunter & Thompson 2005, Xiao & Fayer 2008). As explained in Chapter 2, what was historically classified as *Crypto. parvum* in a variety of different livestock hosts has been shown more recently to be a group of different species or genotypes of *Cryptosporidium*, some of which appear to be relatively host-adapted and possibly of low infectious potential for humans. This has made accurate quantification of the environmental loading rate of DNA-confirmed *Cryptosporidium parvum* from the many livestock species found throughout the world problematic and subject to periodic revision. Prevalence and intensity data for presumptive *Cryptosporidium parvum* from the 1970s through the early 2000s probably overestimates the amount of oocyst shedding in domestic animals. Recent reviews or catchment-scale modeling of cryptosporidial loading by livestock struggle with this problem by tabulating oocyst loading data listed as *Cryptosporidium* (e.g., Dorner *et al.* 2004, Ferguson *et al.* 2009, Robertson 2009) or stratified by *Cryptosporidium* species (Starkey *et al.* 2007).

With these caveats in mind, high prevalence rates and high intensities of faecal shedding of presumptive *Cryptosporidium parvum* oocysts have been documented in young dairy calves from various regions of the world for several decades, with point prevalences of faecal shedding ranging from ~10 to ~80% in groups of one- to four-week old dairy calves and mean intensities ranging from 1×10^5 to 6×10^7 oocysts per gram among groups of infected animals (Goodgame *et al.* 1993, Xiao & Herd 1994, Atwill *et al.* 1998, Kuchzynska & Shelton 1999, Uga *et al.* 2000, Nydam *et al.* 2001, Moore *et al.* 2003; Applebee *et al.* 2005, Starkey *et al.* 2005, Santin *et al.* 2008). Figure 3.1 is an example of a group of young dairy calves shedding oocysts after oral inoculation of wild-type dairy calf *Cryptosporidium parvum*. Assuming that the body mass during the first 30 days of life ranges from 40 to 80 kg and mean daily faecal excretion is 3.3% of body mass (Table 3.2), the environmental loading rate for *Cryptosporidium parvum* oocysts for a group of young dairy calves could feasibly range from a few billion to hundreds of billions of oocysts per day depending on infection levels in the calves. Attempting to develop a single value for the environmental loading rate of *Cryptosporidium parvum* in this high risk population might convey a false sense of accuracy given the potential influence of animal husbandry practices, biosecurity of feed and water supplies, calving pen hygiene, and manure management practices on the prevalence of faecal shedding of oocysts in dairy calves (Maldonado *et al.* 1998, Sischo *et al.* 2000, Starkey *et al.* 2006, Silverlås *et al.* 2009). The duration of oocyst shedding has been associated with the number of ingested oocysts, suggesting that interventions that reduce the environmental load of *Cryptosporidium parvum* oocysts to which young dairy calves are exposed may lead to reduced

shedding durations (Moore *et al.* 2003). The prevalence of presumptive or DNA-confirmed *Cryptosporidium parvum* oocyst shedding is much less common in older dairy calves, yearlings, and especially adult dairy cows where less than 10% of mature cattle shed this protozoal species (Atwill *et al.* 1998, Sischo *et al.* 2000, Atwill & Pereira 2003, Santin *et al.* 2004, Starkey *et al.* 2005, Kvac *et al.* 2006, Coklin *et al.* 2007, Guerden *et al.* 2007, Santin *et al.* 2008).

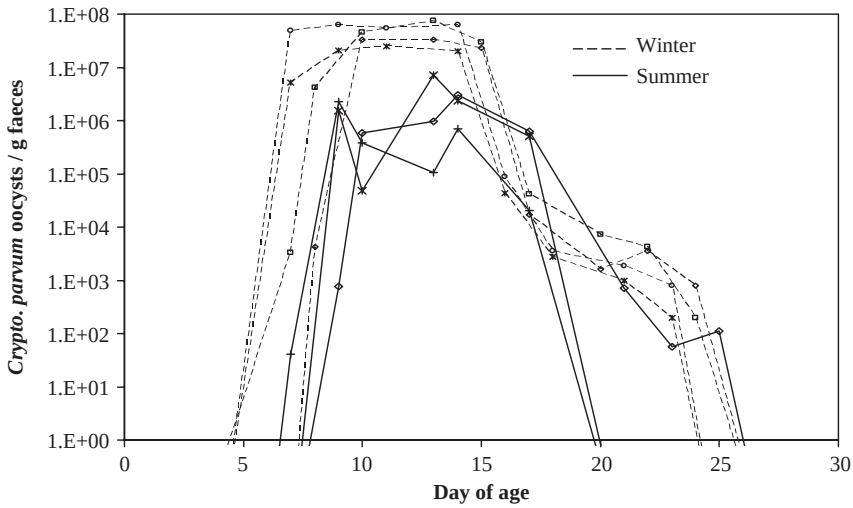


Figure 3.1 *Crypto. parvum* oocyst shedding among dairy calves in central California, USA, adapted from Moore *et al.* 2003 and unpublished data, 2007.

Beef calves and cows from cow-calf production systems, yearling beef replacement heifers, and feedlot steers all appear to be less frequently infected with *Cryptosporidium parvum* than young dairy calves. For example, fewer than 15% of preweaned range beef calves (two weeks to seven months) shed any species of *Cryptosporidium* (Atwill *et al.* 1999). A faecal survey of seven beef cow-calf herds in western North Dakota found no calves or cows infected with *Cryptosporidium parvum*; instead, 9.4, 6.6 and 1.4% of animals sampled were shedding *Cryptosporidium bovis*, *Crypto. ryanae or Crypto. andersoni*, respectively (Feltus *et al.* 2008). A survey of 22 feedlots in seven states (California, Washington, Colorado, Oklahoma, Texas, Nebraska, and South Dakota) found no (0/5274) fresh faecal pats with detectable *Cryptosporidium parvum* oocysts and 0.9% with *Cryptosporidium bovis* (Atwill *et al.* 2006). About

1% of the beef cows and 3% of their calves were positive for *Cryptosporidium* spp. in western Canada (Gow & Waldner 2006). About 0.5 to 1% of 367 pre-weaned beef calves and none out of 2381 post-weaned beef calves had detectable *Cryptosporidium parvum* oocysts in South Bohemia, Czech Republic. *Cryptosporidium parvum*, along with *Cryptosporidium bovis* and *Cryptosporidium suis* were detected in 12% of beef calves in Belgium; interestingly, the authors note that the production system for beef and dairy cattle in this part of Belgium are similar, functioning as a confined animal feeding operation rather than an extensive grazing beef herd (Geurden *et al.* 2007). The geometric mean intensity for all three species of *Cryptosporidium* was 780 oocysts/g faeces. *Cryptosporidium parvum* was detected in less than 10% of beef calves in Zambia (Geurden *et al.* 2006).

Cryptosporidium parvum in sheep and goat herds tends to occur more frequently in pre-weaned animals than in older animals (Robertson, 2009), but the reported prevalence rates of faecal shedding can vary widely from region to region. For example, 13% (63/477) of pre-weaned lambs had detectable oocysts compared to none (0/500) of post-weaned sheep in Australia, with infected lambs apparently shedding low oocyst numbers (Ryan *et al.* 2005, Yang *et al.* 2009). The prevalence of *Cryptosporidium* spp. was 13.1% in lambs and 9.5% in goat kids in Belgium, with almost none of the isolates in sheep yet all of the isolates in goats confirmed as *Cryptosporidium parvum* (Geurden *et al.* 2008). The mean intensity of oocyst shedding for all species of *Cryptosporidium* was ~6800 and ~232,000 oocysts per gram faeces for sheep and goats, respectively (not adjusted for % recovery) (Geurden *et al.* 2008). Assuming adult sheep produce 0.7 kg faeces per day, this would generate a mean daily environmental loading rate of 4×10^6 oocysts per sheep per day. In contrast, *Cryptosporidium parvum* was the only species identified among diarrheic lambs in Spain (Quilez *et al.* 2008). Sampling of lambs and adult sheep in the UK during human cryptosporidial follow-up investigations found 36% (94/261) of animals shedding *Cryptosporidium* spp., but only 9.2% were confirmed as *Cryptosporidium parvum* (Mueller-Doblies *et al.* 2008). Ortega-Mora (1999) measured a range of oocyst shedding intensities between 20 and 440 *Cryptosporidium* spp. oocysts/g faeces among adult sheep in Spain (*Crypto. parvum* not DNA-confirmed), with the average number of oocysts/g faeces being ~50 and ~90 on two different farms. Assuming adult sheep produce 0.7 kg faeces per day, this would generate a mean daily environmental loading rate between 35,000 and 63,000 oocysts. Lambs experimentally inoculated with a cervine-ovine isolate of *Cryptosporidium* spp. excreted an arithmetic mean of 4×10^9 oocysts/g faeces, of which half were determined to be non-viable and therefore reducing the estimated intensity by 50% (Bukhari &

Smith 1997). Lambs appear to shed fewer oocysts (*Crypto. parvum* not DNA-confirmed) if they ingest oocysts at two months of age compared to being infected at six days of age; mean peak oocyst shedding was 2.5×10^7 versus 2.2×10^9, respectively (Ortega-Mora & Wright 1994).

Faecal shedding of *Cryptosporidium parvum* among swine, chickens, geese and ducks is infrequent and typically not a significant environmental source of this species of protozoa (Hunter & Thompson 2005, Xiao & Fayer 2008). Similarly, most cross-sectional surveys conducted to date have found a moderately low prevalence of *Cryptosporidium* spp. in adult horses, with some reports indicating higher infections occurring in foals. For example, a survey on trail horses utilising public trails in Colorado, USA, found that 0.3% had detectable concentrations of *Cryptosporidium* spp. (Forde *et al.* 1997). Among the general equine population, two surveys detected *Cryptosporidium* in 27% (21/77) of normal foals and 29% (83/285) of diarrhoeic foals (Coleman *et al.* 1989, Browning *et al.* 1991). In a two-year survey by Coleman *et al.* (1989), 15% (8/55) of pasture-reared foals were found to be infected with *Cryptosporidium* the first year, but the subsequent year foals were negative. *Cryptosporidium* was detected in 15 to 31% of foals, 0 to 5% of weanlings and 0% of yearlings and mares (Xiao & Herd, 1994a). Cole *et al.* (1998) determined that 7% (5/70) of foals on breeding farms and 0.3% (1/366) of geldings, intact males and mares were infected with *Cryptosporidium*. Among horses used as packstock in the mountains of California, USA, none of 305 animals had detectable levels of *Cryptosporidium* (Atwill *et al.* 2000). About 3.5% of horses in Poland were found to shed low numbers of *Cryptosporidium parvum* oocysts (Majewska *et al.* 2004).

3.2.2 *Giardia duodenalis*

Giardia duodenalis like *Crypto. parvum* infects the intestinal tract of a wide range of hosts, including humans, dogs, cats, cattle, sheep, horses, rodents and a variety of other wild mammals. This species of *Giardia* is further divided into subgroups referred to as Assemblages A through G (see Chapter 2). Assemblage A infects humans and other primates, livestock, companion animals, rodents and other mammals; Assemblage B infects humans, other primates and dogs; assemblages C and D infect dogs; Assemblage E infects cattle and other hoofed livestock; Assemblage F infects cats; and Assemblage G infects rats (Hunter and Thompson 2005). Of particular concern for agricultural watersheds is Assemblage A because this type of *Giardia* is infective to humans and is also shed by livestock, dogs and cats, humans, and certain wildlife species.

Beef and dairy cattle have been shown to be commonly infected with *Giardia duodenalis* (Buret *et al.* 1990; Deshpande & Shastri 1981, O'Handley *et al.* 1999, Olson *et al.* 1997a, Olson *et al.*1997b, Paul *et al.* 2009, Quilez *et al.* 1996, Taminelli & Eckert 1989, Xiao *et al.* 1993, Wade *et al.* 2000). It would appear, however, that the majority of infections in cattle are of Assemblage E (non-zoonotic) and a smaller percentage of Assemblage A (human infective). Recent studies by Trout *et al.* (2004 through 2007) have shown that 13% to 15%, 3%, and 2% of calves, yearlings, and adult dairy cows, respectively, were shedding cysts of Assemblage A. The prevalence rate of faecal shedding of Assemblage E is much higher than that of Assemblage A, which may have led early investigators and public health officials to over-estimate the importance of livestock as a source of zoonotic *Giardia duodenalis*. Young cattle exhibit higher prevalence rates of faecal shedding compared to adult cattle. For example, previous studies have found that the prevalence rate of *Giardia duodenalis* shedding for beef and dairy calves ranges from 17 to 55% and for adult beef and dairy cattle from 0 to 17% (Fayer *et al.* 2000, Wade *et al.* 2000, Huetink *et al.* 2001, Appelbee *et al.* 2003, McAllister *et al.* 2005, Gow & Waldner 2006). A survey of 22 feedlots in seven states in the USA (California, Washington, Colorado, Oklahoma, Texas, Nebraska, and South Dakota) found 19% (1006/5260) of fresh faecal pats with detectable *Giardia duodenalis* cysts, with higher prevalence rates among recent arrivals and during winter time conditions (Hoar *et al.* 2009). The arithmetic mean intensity for the positive faecal pats was ~10,700 cysts/g faeces (Figure 3.2), or ~2030 cysts/g faeces for the entire sample of faecals (positives and negatives) from across middle and western US feedlots, with a small subset of samples exhibiting >500,000 cysts/g faeces (super-shedders). Assuming feedlot steers defecate between 10 and 40 kg, this would generate an environmental loading rate between 2×10^7 and 8×10^7 cysts per animal per day. About 34% (168/495) of Canadian beef calves had detectable *Giardia duodenalis* cysts, but almost all isolates were not human infective (Applebee *et al.* 2003). In an earlier study, faecal samples from cow-calf operations had a mean intensity of 5801 cysts/g faeces (Heitman *et al.* 2002). Approximately 25% of sheep and 36% of goats in Belgium were found positive for *Giardia duodenalis*, with a mean intensity of ~4600 and ~18,000 cysts/g faeces, respectively (not adjusted for % recovery) (Geurden *et al.* 2008). Assuming adult sheep produce 0.7 kg faeces per day, this would generate a mean daily environmental loading rate of 3.2×10^6 cysts per sheep per day. Interested readers are encouraged to read recent reviews to better understand the ongoing challenge of establishing a definitive role for livestock as a cause of human giardiasis (Thompson & Monis 2004, Hunter & Thompson 2005, Slifko *et al.* 2000).

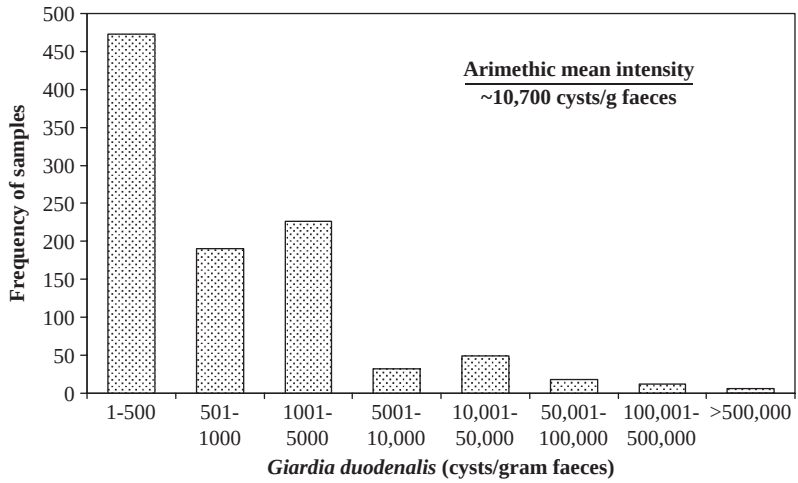


Figure 3.2 Frequency distribution of the intensity of *Giardia duodenalis* cyst in fresh faecal pats collected from feedlot pens from central and western USA, adapted from Hoar *et al.* 2009.

3.3 WATERBORNE ZOONOTIC BACTERIA

3.3.1 *E. coli* O157:H7

Although most *E. coli* strains are harmless and live in the intestines of healthy humans and animals, certain so called pathogroups of the organism consist of strains from specific serotypes that are associated with gastrointestinal and extra-intestinal infections (e.g. septicaemia, mastitis and urinary tract infections) in both humans and animals. Many of the members of these pathogroups share virulence attributes such as those necessary for gastrointestinal colonization. These factors include adhesins that allow them to attach to host-specific receptors in the intestine. In contrast to the other pathogroups, enterohaemorrhagic *E. coli* (EHEC) usually occur as part of the normal flora of the intestinal tract of ruminants and only cause disease when they are accidentally ingested by humans. EHEC produce one or more antigenic types of protein toxins, closely related to Shiga toxin, that cause cell death and apoptosis following receptor-mediated cell up-take (Karmali *et al.* 2009, Law 2000). *E. coli* O157:H7 and other EHEC (e.g., serotypes O26, O103, and O111) are associated with a spectrum of gastrointestinal illness in humans from simple diarrhoea to haemorrhagic colitis which is characterized by bloody diarrhoea. Occasionally, passage of toxins produced by the organism into the blood stream following the gastrointestinal form of the infection, result in a systemic illness called the

haemolytic uremic syndrome. This syndrome is characterized by haemolytic anaemia, kidney failure, coma and sometimes death (Law 2000, Rangel *et al.* 2005).

Karmali *et al.* (2004) have classified EHEC into different sero-pathotypes based on the frequency and severity of disease with which they are associated. *E. coli* O157:H7 is the sole member of seropathotype A because it is the most frequently associated with both sporadic cases and outbreaks of severe human illness. However, even within *E. coli* O157:H7 differences exist among genetic lineages in the frequency and severity of disease with which they are associated. Among the three genetic lineages recognized, lineage II is primarily bovine-associated and is infrequently associated with human disease whereas lineage I strains are the most frequently isolated group associated with human illness (Kim *et al.* 2001, Zhang *et al.* 2007). Lineage I/II contains members of a so called "hypervirulent clade" associated with higher levels of hospitalization and haemolytic uremic syndrome than other *E. coli* O157:H7 genetic groups (Manning *et al.* 2008).

E. coli O157:H7 can be passed from animal to human or human to human via several routes of transmission, such as ingesting contaminated food or water, direct contact with infected animals, direct contact with an animal's bedding or pens, and person-to-person direct contact transmission (reviewed in Rangel *et al.* 2005). It is a highly infectious organism for humans: ten to several hundred bacteria can cause clinical illness (Chart 2000, Tuttle *et al.* 1999, Willshaw *et al.* 1994). Young children and the elderly are the most susceptible to severe infections.

3.3.1.1 Risk factors for outbreaks of E. coli *O157:H7*

Consumption of contaminated food accounted for 52% of the 350 outbreaks and 61% of the 8,598 outbreak-associated human cases in the USA from 1982 to 2002, (Rangel *et al.* 2005). Contaminated hamburger and contaminated produce were responsible for 20% and 21% of these outbreak-associated human cases, respectively. Among produce-related foodborne illness, about half were due to cross-contamination in the kitchen, with the other half related to vegetable produce. Contamination of produce could originate from irrigation water, animal or human wastes in fields, and water used during harvesting, processing, or shipping (Rangel *et al.* 2005). Waterborne transmission accounted for about 18% of the outbreak-associated human cases, with the majority of these infections associated with contaminated drinking-water (15% of cases) and the rest associated with recreational exposure to contaminated water from lakes, ponds, and pools (3% of cases). Person-to-person transmission can occur if infected persons, especially food handlers, do not wash their hands. Direct contact with infected animals was a minor cause of outbreaks (Rangel *et al.* 2005).

3.3.1.2 Faecal shedding of E. coli *O157:H7 by livestock*

While studies have reported isolation of *E. coli* O157:H7 from a number of domestic and wild animal species, ruminants, in particular cattle, are considered the primary reservoir of this important human pathogen (Dunn *et al.* 2004; Gunn *et al.* 2007; Khaista *et al.* 2006; Hancock *et al.* 1997a, 1997b, reviewed in Renter & Sargeant, 2002, Renter *et al.* 2004). The importance of cattle as a reservoir is underlined by the high prevalence of the organism in the species. Serological evidence suggests that all beef cattle herds in the USA have been infected with the organism at one time or another (Laegreid *et al.* 1999). As well, many fattened cattle at slaughter can be shedding the organism in their faeces (van Donkersgoed *et al.* 1999, Elder *et al.* 2000). There is a strong epidemiological link between both sporadic cases and outbreaks of human infections and the consumption of undercooked beef and other foods, including vegetables, unpasteurized milk and fruit juices and water contaminated with ruminant faeces (Gyles 2007, Karmali *et al.* 2009). Recently, Walters *et al.* (2007) have also reported that the presence of the *Bacteroides* ruminant faecal marker in surface water is significantly higher in samples from which *E. coli* O157:H7 was isolated.

In cattle, calves become infected with the organism shortly after birth following a spike in peri-parturient shedding of the organism in their dam's faeces (Gannon *et al.* 2002). In the study, from 2 to 18% of dams and 23 to 26% of calves were shown to shed *E. coli* O157:H7 in their faeces in the period immediately following parturition. Many calves continue to shed the organism as they grow; there appears to be a secondary spike in shedding of the pathogen following weaning which is followed then by a decline through the fattening period (Smith *et al.* 1997). Elder *et al.* (2000) reported that 72% of lots of slaughter cattle and 28% of individual cattle in the Midwestern USA shed *E. coli* O157:H7 in their faeces during July and August. However, these likely represent high values as there is a seasonal peak in the frequency of shedding of the organism by young cattle during the summer months (van Donkersgoed *et al.* 1999). The lowest prevalence is found among adult cattle during the winter months.

When this bacterium infects cattle, it colonizes lymphoid-associated epithelial cells in the recto-anal junction using a type III secretion system which encodes an adhesin and a syringe-like apparatus which injects an adhesin receptor as well as a number of intracellular effector proteins into the cells. This changes the cells' morphology and physiology and allows *E. coli* O157:H7 to attach to them (Kenny 2002, Karmali 2004, Spears *et al.* 2006). These colonization proteins show promise as targets of vaccines to reduce faecal shedding of the organism by cattle.

The concentration of *E. coli* O157:H7 in faeces can vary widely, ranging from $< 10^2$ to over 10^6 bacteria or colony forming units per gram of faeces (CFU/g) (Sanderson *et al.* 1999, Shere *et al.* 2002, Fukushima & Seki 2004, reviewed in Renter & Sargeant 2002) and up to 10^6 CFU/g faeces for sheep (Ogden *et al.* 2005). Faecal shedding is often sporadic, fluctuating from 10^2 to 2×10^5 CFU/g faeces in as few as two days (Shere *et al.* 2002). The duration of faecal shedding is also highly variable, with some cattle and sheep shedding a few days to weeks with other cattle or calves shedding intermittently for up to 27 weeks following experimental infection (Cray & Moon 1995, Besser *et al.* 1997, Sanderson *et al.* 1999, Shere *et al.* 2002). The concentration of the pathogen in the faeces and the frequency of shedding among individuals in a herd is, however, skewed and does not follow a normal distribution. Instead, certain individuals within herds termed "super-shedders" appear to be the source of the majority of the organisms shed by groups of cattle (Chase-Topping *et al.* 2008). Super-shedders have been variously defined as cattle shedding either $>10^3$ or $>10^4$ CFU of *E. coli* O157:H7/g of faeces, depending on the study (Low *et al.* 2005, Naylor *et al.* 2003, Omisakin *et al.* 2003). The presence of super-shedders in herds is associated with a greater number of cattle shedding lower levels of the organism, suggesting that the super-shedders enhance transmission of the organism within the herd (Chase-Topping *et al.* 2007). Omisakin *et al.* (2003) reported that as many as 9% of *E. coli* O157-positive cattle at slaughter in Scotland were super-shedders. Chase-Topping *et al.* (2007) reported that phage type 21/28 was more frequently associated with supershedding than other *E. coli* O157 phage types, suggesting both the host and the pathogen may play a role in this phenomenon. In summary, super-shedders are thought to play a key role in spreading the organism to other cattle, other species of domestic and wild animals, contamination of beef, milk and field crops and water. Identification and treatment or removal of super-shedders from groups of cattle could significantly reduce the environmental load of this dangerous human pathogen. Super-shedders are also likely to play a role in the phenomenon of "clonal dominance" where a single genotype of *E. coli* O157:H7 predominates in a specific cattle herd. This characteristic of the organism has the potential to allow specific sources of food and environmental contamination and human infection to be traced to specific groups of animals based on the molecular fingerprint of the organism (Laing *et al.* 2009, Cooley *et al.* 2007).

E. coli O157:H7 has been shown to persist in water-trough sediment for at least four months (Hancock *et al.* 1997c) and inoculating calves with 10^6 CFU/L of water resulted in several weeks to over a month of faecal shedding of *E. coli* O157:H7 at concentrations of 10^2 to 10^6 CFU/g faeces (Shere *et al.* 2002). Livestock manure containing *E. coli* O157:H7 has been linked to or suspected as

the cause of a variety of food- and waterborne outbreaks of human illness over the past twenty years. 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). This underlines the need to carefully handle and dispose of stored manure and to encourage proper grazing management so as to reduce this route of transmission to humans (reviewed in Guan & Holley, 2003; Rangel *et al.* 2005). In addition to cattle and sheep, *E. coli* O157:H7 has been isolated from a wide variety of animals, including dogs, horses, white-tailed deer, elk, raccoon, various species of birds such as starlings, gulls, and geese, feral pig, flies, and others (reviewed in Renter & Sargeant 2002 and Pedersen & Clark 2007). However, it is not clear if all of these wildlife species are reservoirs of this pathogen or simply act as passive carriers of the organism derived from ruminant excreta. Therefore, we do not have a clear understanding of how wildlife populations in agricultural or rural watersheds participate in the environmental cycling of this pathogen (Pedersen & Clark 2007).

3.3.2 *Campylobacter*

Campylobacter spp. are the leading cause of human bacterial gastroenteritis in the developed world (Friedman *et al.* 2004). Campylobacteriosis is characterized by acute gastroenteritis and is occasionally associated with the Guillain-Barré Syndrome (a demyelinating polyneuropathy characterized by ascending paralysis) (Aspinall *et al.* 1994). There are several species of *Campylobacter* that have been associated with infections in humans; however, *Campy. jejuni* accounts for approximately 90% of human cases, with most of the remainder associated with *Campy. coli* (Friedman *et al.* 2004; Humphrey *et al.* 2007). As few as 500 to 800 organisms appear sufficient to cause clinical illness in humans (Robinson 1981, Black *et al.* 1988). Most cases of campylobacteriosis (ca. 80%) are thought to be foodborne in origin. The most important risk factor for sporadic campylobacteriosis is the consumption of undercooked poultry. Other risk factors include the consumption of raw milk (Teunis *et al.* 2005), exposure to pets and farm animals, especially when they have diarrhoea, and drinking untreated surface water (Friedman *et al.* 2004; Humphrey *et al.* 2007). Direct human-to-human transmission is uncommon (Tauxe 1992; Altekruse *et al.* 1994, Franco & Williams 1994, Adak *et al.* 1995), but direct animal-to-human transmission via contact with calves has been implicated in some cases (Smith, 1984; Friedman *et al.* 2004; Belongia *et al.* 2003). Foodborne cases are typically sporadic in nature and outbreaks associated with food sources are rare (Mead *et al.* 1999, Friedman *et al.* 2004). 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, there are two warm season peaks with *Campylobacter*, one in the late spring-early summer and another in the late summer-early fall (Stanley *et al.* 1998). Young children (0 to 4 years of age) living in close proximity to high densities of livestock are at a greater risk of *Campylobacter* infection than their urban-dwelling counterparts (Green *et al.* 2006), further suggesting that environmental exposure may play a role.

While waterborne outbreaks of campylobacteriosis are less common than those associated with the protozoal parasites, *Crypto. parvum* and *G. duodenalis*, and *E. coli* O157:H7, they do occur. Outbreaks of campylobacteriosis are typically associated with un-chlorinated 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 2300 cases of gastrointestinal illness was associated with infections by both *E. coli* O157:H7 and *Campy. 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 drinking-water-associated outbreaks have been reported in Norway, Finland and Sweden (Jakopanec *et al.* 2008, Schönberg-Norio *et al.* 2004). Finally, campylobacteriosis has also been associated with recreational use of water (Kärenlami *et al.* 2007, Schönberg-Norio *et al.* 2004).

3.3.2.1 Faecal shedding of Campylobacter *by livestock and wildlife*

Campylobacters are frequently shed in the faeces of livestock, poultry, wild mammals and wild birds (Stanley & Jones 2003, Skelly & Weinstein 2003, Humphrey *et al.* 2007). In addition, to domestic animals, *Campylobacter* spp. has been isolated from the faeces of a variety of wildlife species including but not limited to crows, common gulls, pigeons, puffins, ducks, Canada geese, Sandhill cranes, wild rabbits, rats, starlings, and sparrows (reviewed in Skelly & Weinstein 2003, Kwan *et al.* 2008). Companion animals such as dogs also can shed different species of *Campylobacter* (Hald *et al.* 2004) and are a risk factor for human infection. In a recent study in Scotland, it was reported that prevalence rates of *Campylobacter* ranged from 1.3% for dogs to 41.4% for poultry (Ogden *et al.* 2009). In this study, prevalence of *Campylobacter* in the faeces of cattle, sheep and pigs was very similar (ranging from 21.9% to 26.7%) as it was among ducks, geese, gulls, pigeons and wild birds of unknown origin (ranging from 23.7% to 27.8%). The average *Campylobacter* CFU/g of faeces

was also similar among the cattle, sheep, and pigs (ranging from 4×10^4 to 2×10^5) and ducks, geese, gulls, and pigeons (ranging from 6×10^4 to 2×10^5). Interestingly, poultry (the most commonly implicated source of the pathogen for humans) had the lowest average number of *Campylobacter* CFU/g of faeces of 1×10^2. With the exception of poultry, all positive individuals among animal groups shed 10^2 CFU/g or fewer *Campylobacter* in their faeces and a minority (<10%) shed from 10^6 to 10^8 CFU/g of faeces. Taken together, these data suggest that there are a large number of potential animal reservoirs of campylobacters that could transmit and cause enteric disease in humans.

Campylobacter spp. can be isolated from the majority of dairy cattle herds and sheep flocks (Oporto *et al.* 2007, Stanley *et al.* 1998) and from approximately 20 to 50% of individual dairy cattle in the UK and USA (Bae *et al.* 2005, Brown *et al.* 2004, Englen *et al.* 2007, Wesley *et al.* 2000). In cattle, a bimodal seasonal pattern has been noted in the prevalence of faecal shedding of *Campylobacter* with peaks in the spring and fall and the lowest levels shed in winter (Kwan *et al.* 2008). A summer peak in the faecal shedding of campylobacters has also been observed in sheep. Pasture gazing has been associated with the summer peak in faecal shedding of campylobacters in cattle but not in sheep (Grove-White *et al.* 2009). Calves appear to be rapidly colonized soon after birth, with peak shedding occurring a few months later (Nielsen 2002, Stanley & Jones 2003). The prevalence in dairy calves and the number of campylobacters shed per gram of faeces is significantly higher than in adults. In contrast to dairy cattle, a survey carried out in California found that only 5% of adult beef cattle shed *Campylobacter* in their faeces (Hoar *et al.* 1999; Hoar *et al.* 2001). In beef cattle feedlots, the prevalence of shedding of *Campylobacter* increases through the feeding period until just prior to slaughter when as many as 88% of animals are positive (Besser *et al.* 2005, van Donkersgoed *et al.* 2009).

3.3.2.2 Source attribution of Campylobacter *infections*

Recent advances in the molecular typing of campylobacters have allowed us to identify the most probable sources of human infections. Multi-locus sequence typing (MLST), which uses variations in the nucleotide sequences of essential *Campylobacter* genes, has allowed us to identify relatively stable clusters of related genotypes of the organism (Dingle *et al.* 2005). Using MLST, it has been shown that specific sequence types (ST) and clonal complexes (CCs) of the organism are much more commonly isolated from certain animal hosts than others. Further, certain of these animal STs and CCs are also much more common among human clinical isolates than others. Recently, Wilson *et al.* (2008) used a probabilistic attribution model to analyze ST data from a collection

of *Campy. jejuni* strains from Lancashire, UK, and concluded that 96.6% of human clinical infections could be attributed to farm livestock, 2.3% were from wild animals and only 1.1% from environmental sources. Among domestic animals, 56% of the clinical isolates could be attributed to chicken, 35% to cattle, 4.3% to sheep and only 0.8% to pigs. This and other MLST studies have shown that undercooked poultry is the most important source of *Campylobacter* associated with human disease and that cattle and sheep are an important secondary sources of this human pathogen (Sheppard *et al.* 2009). They also suggest that specific STs are host-adapted and may be useful in determining the host animal sources of campylobacters associated with disease in humans (Colles *et al.* 2008).

While multiple *Campy. jejuni* STs can occur in the same herd, spatial clustering of specific genotypes of *Campy. jejuni* is also observed within the same herd and for herds within one km of each other (Kwan *et al.* 2008). However, there is no evidence of geospatial clustering of bovine *Campylobacter* genotypes beyond this distance; rather, a number of relatively cattle-specific *Campy. jejuni* genotypes can be found in herds present in distant geographical regions (Rotariu *et al.* 2009). These data suggest that, 1) many genotypes can co-exist in the same herd, 2) there is a limited amount of inter-herd and cattle-independent (via vectors such as flies, wildlife, wind) transmission of the organism, and 3) certain genotypes appear to be relatively host-specific and are widespread in the cattle population. It is possible that the limited geospatial movement of certain *Campylobacter* genotypes is related to their high susceptibility to harsh environmental conditions such as desiccation and may also be related to host-specificity. Studies performed on *Campy. jejuni* isolates from surface water in New Zealand suggest that many of these water genotypes originating from wild birds and others belong to novel STs of unknown origin (Carter *et al.* 2009). While water-associated STs are not commonly associated with human clinical infections, Kärenlampi *et al.* (2007) reported that in Finland new and unassigned STs of *Campy. jejuni* were associated with illness following swimming in natural waters. Therefore, it is possible that *Campy. jejuni* from water are less frequently associated with human enteric disease simply as a result of less frequent exposure, rather than because they are less virulent than those from foods such as poultry. However, more research is required to better define the routes of transmission and relative virulence of different genotypes.

3.3.3 *Salmonella enterica*

More than 2500 serotypes of *Salmonella enterica* have been described and the organism can be isolated from a wide variety of host species, including humans, livestock, companion animals, reptiles, avian species, and mammalian wildlife.

3.3.3.1 Salmonella *serotypes associated with human infection*

Over 90% of human, avian, and other mammalian infections are attributed to serotypes from *Salm. enterica* subspecies *enterica*, with the majority of human infections in the European Union and the USA caused by a small number of serotypes within this subspecies, such as *Salm. enterica* subsp. *enterica* serotypes Typhimurium and Enteritidis (Olsen *et al.* 2001). It is estimated that in the USA 1.4 million human infections and 400 to 600 deaths occur each year from these various *Salm. enterica* subsp. *enterica* serotypes (Mead *et al.* 1999, Voetsch *et al.* 2004), with 95% of these infections due to a foodborne transmission (Mead *et al.* 1999). These infections can be fatal in the immunocompromised, in young children, and in the elderly. Most clinical illness appears to be sporadic, although outbreaks do occur. Foods often implicated in outbreaks include poultry and poultry products, meat and meat products, dairy products, egg products, seafood, and fresh produce. *Salm. enterica* subsp. *enterica* serotype Enteritidis is now one of the most common serotypes isolated from human disease cases and is associated with the eating of undercooked eggs (Mishu *et al.* 1994, Patrick *et al.* 2004). Interestingly, relative to the protozoan parasites, *Crypto. parvum* and *G. duodenalis*, and *E. coli* O157:H7, few outbreaks of *Salm. enterica* have been associated with exposure to recreational water or to drinking-water, despite its widespread occurrence in domestic and wild animals. Many of the serotypes of *Salm. enterica* shed by domestic animals and wildlife are infrequent causes of human illness, such as *Salm. enterica* serovar Dublin (CDC, 2005). Either illness associated with these rare serovars of *Salm. enterica* is seldom diagnosed, human exposure to these serovars is infrequent, or the infectious dose for humans is very high. It is also likely that there are host-adapted serovars of the organism that are rarely associated with disease because of inter-species barriers in transmission and survival. However, there are other *Salm. enterica* serovars which are widespread and are commonly isolated from multiple host species and are frequently associated with human infections, such as *Salm. enterica* serovar Typhimurium.

3.3.3.2 Shedding of Salmonella *by livestock*

Animals used for food production are common carriers of numerous serovars of *Salm. enterica*, with a wide range of prevalence in different livestock species. A survey of layer facilities in the USA found that 7.1% were positive for *Salmonella* Enteritidis (Garber 2003), with this bacterium frequently present in the litter (Davies & Breslin 2003). It is estimated that about 30% of dairy herds across the USA have cattle shedding *Salmonella* (NAHMS, 1996; NAHMS,

2002), with the herd-level animal prevalence ranging from 0 to 37% for lactating dairy cattle (Callaway *et al.* 2005). The prevalence of *Salmonella* in farm environmental samples in one study was shown to be 57.3%, 17.9% and 16.2% in swine farms, dairy farms, and poultry farms, respectively (Rodriguez *et al.* 2006). In New York dairies (440 dairy farms enrolled) *Salmonella* was isolated from 1.5% of milk filters (Hassan *et al.* 2000). In swine farms in the midwestern USA, the mean prevalence for *Salmonella* was 5% (Bahnson *et al.* 2006). The European Food Safety Agency reported that among EU member states in 2007 there was a mean *Salmonella* prevalence of 2.9% in laying hens (range 0–22.2%), 3.7% for broilers (range 0 to 25.3%), 10.6% for ducks (range 0 to 21.7%), 9.3% for geese (range 0 to 21.2%), and 7.8% for turkeys (range 0 to 14.8%) (EFSA, 2009). In the same report, *Salmonella* prevalence ranged from 0 to 19.3% in five EU member states reporting and from 1.6 to 7.7% for cattle in two member states reporting.

Serotypes of *Salm. enterica* in poultry farms in the USA include Typhimurium, Montevideo, Kentucky & Enteritidis (Liljebjelke *et al.* 2005) and eggs are a frequent source of *Salm. enterica* Enteritidis (Hogue *et al.* 1997).

3.3.4 Survival of zoonotic bacterial pathogens in the farm environment

E. coli O157 survives for at least 50 days in cattle faeces (Maule, 2000). Survival of the organism in bovine manure is dependent on the temperature with viable organisms found for 49 to 56 days at 22°C and 63 to 70 days at 5°C (Wang *et al.* 1996). The organism can survive up to 47 days in bovine manure heaps and up to 21 months in non-aerated bovine manure heaps (Kudva *et al.* 1998). Himathonkham *et al.* (1999) suggest that bovine manure be held for 105 days at 4°C or 45 days at 37°C to achieve a 5 log reduction in levels of *E. coli* O157 and *Salm. enterica* serovar Typhimurium.

It has also been shown that *E. coli* O157:H7 survives well in soil. An inoculum of 10^8 CFU/g of the organism was shown to decline between 1 to 2 logs after 130 days in soil cores of rooted grass. Jiang *et al.* (2002) reported detection of the pathogen for nearly 200 days in bovine manure amended soil and reported that survival was a function of the soil temperature, ratio of manure to soil and the presence of the indigenous microflora in the soils. Survival of *E. coli* O157:H7 in soil where bovine manure slurry has been applied is significantly longer than with surface application of solid bovine manure. However, for *Salm. enterica* serovar Typhimurium survival appears to be equivalent in manure and slurry-applied soils (Semenov *et al.* 2009). Campylobacters are particularly sensitive to factors in the external environment such as desiccation. Survival of *Campylobacter* in

bovine manure slurries has been shown to be 30 days compared with 90 days for *E. coli* O157:H7 and *Salm. enterica* Typhimurium (Nicholson *et al.* 2005). Similarly, Sinton *et al.* (2007) reported that *Campylobacter* was inactivated after 6.2 days in faecal pats compared with 38 days for *Salm. enterica*.

Lejeune *et al.* (2001a) reported isolation of *Salmonella* spp. from 2/235 (0.8%) and *E.coli* O157 from 6/473 (1.3%) water-troughs from cattle operations in the USA. Laboratory studies have shown that *E. coli* O157 can survive for 245 days in simulated water-trough sediments (Lejeune *et al.* 2001b). There is evidence that predation by protozoa reduces populations of "planktonic" enteric bacteria in surface waters. For example *E. coli* O157 populations decline in river water from 10^6 CFU/ml to undetectable levels after 27 days (Maule 2000). Wang and Doyle (1998) reported that *E. coli* O157 population declines are slowest in filtered lake water and at lower temperatures. *Campy. jejuni* populations decline more rapidly in surface water (7 to 15 days) than those of *E. coli*; however, the decline is also slower at lower temperatures (Korhonen & Martikainen 1991). Survival of *E. coli* O157 and other bacterial pathogens in sediments and on surfaces is thought to be prolonged as a result of biofilm formation.

3.4 METHODOLOGICAL CONCERNS REGARDING MONITORING PATHOGEN LOADS

There are several methodological concerns regarding sampling strategies and monitoring methods for characterizing livestock pathogen loads. Valid and precise quantitative estimates need to be generated for the daily faecal production per animal, the prevalence or incidence of pathogen shedding, and the intensity of pathogens excreted by infected animals. Calculating the loading rate facilitates a more accurate match between expected pathogen loads for a livestock production system and the efficacy of the intervention strategy for attenuating the pathogen load (Tate *et al.* 2004, Koelsch *et. al.* 2006). Current estimates for faecal production often rely on a single value to represent all adults or all juvenile livestock (Table 3.2), but using a percentage of body weight to estimate faecal production may allow a more accurate match to the group's weight and age composition (Atwill *et al.* 2004). One of the most important issues is that most of the animal infection literature is dominated by prevalence data which do not account for the up to 10^7 difference in shedding intensity between age classes, clinical status of animals, or different host species. For example, if 50% percent of animal group A sheds 10^2 *Salmonella* per day and 1% of animal group B sheds 10^6 *Salmonella* per day, on the basis of prevalence group A would be the priority host, but species B sheds 200-fold higher

amounts of *Salmonella* per day. Furthermore, probabilistic models for catchment-scale pathogen loading have been shown to be highly sensitive to estimates of shedding intensity (Dorner *et al.* 2004), underscoring their importance in load calculations.

Accuracy of estimates of pathogen prevalence in livestock populations depend on sample size, test sensitivity and specificity, prevalence and intra-herd correlation of infection status. The wide variation in diagnostic test sensitivity used to detect these pathogens can result in widely different prevalence estimates for the same infected population. For example, the addition of immunomagnetic bead separation prior to immunofluorescent microscopy (IMS-DFA) for detecting low levels of *Cryptosporidium parvum* oocysts in bovine faeces generates a 50% probability of detection (DT_{50}) at 1.0 oocyst/g faeces and 90% probability of detection (DT_{90}) at 3.2 oocysts/g faeces (Figure 3.3; Pereira *et al.* 1999). In contrast, immunofluorescent microscopy alone (DFA) generates a DT_{50} at 200 oocyst/g faeces and DT_{90} at 630 oocysts/g faeces. Using DFA alone to survey adult animals shedding low levels of oocysts will likely yield a downwardly-biased estimate of the shedding prevalence. For example, a prevalence rate of 7.1% compared to 0.6% (~12-fold difference) of *Cryptosporidium* spp. shedding was estimated using IMS-DFA compared to DFA in adult cattle (Atwill *et al.* 1998, 2003). However, using highly sensitive assays will detect more low shedders in the population, increasing the positive prevalence rate but potentially reducing the calculated mean intensity of pathogen shedding per gram of faeces (Atwill *et al.* 2003). Quantitative microbial assays that are used to estimate shedding intensity need to be adjusted for the percent recovery of the assay, which is well established in the protozoan literature but less commonly so in the bacterial literature. The use of the geometric mean compared to the arithmetic mean for pathogen intensity data underestimates the actual pathogen load deposited in the environment; the downward bias of the geometric mean becomes increasingly pronounced as the frequency distribution for intensity becomes more right-skewed (e.g., protozoan parasite eggs in juvenile animals, with a small percentage of individuals shedding very high levels).

Finally, the occurrence of many high priority waterborne zoonotic pathogens in livestock populations is highly seasonal, due to seasonal patterns of parturition or to unknown factors such as summer shedding of *E. coli* O157:H7 in beef cattle. *Cryptosporidium* spp. infection often increases after calving, lambing, foaling, and kidding occur. This suggests that accurate measures of prevalence and intensity need to sample across the year and include the various age classes of livestock, with clear tabulation of each age class matched to pathogen occurrence rather than generating a single estimate of prevalence rate and intensity for the entire livestock population (e.g., pooling data from neonates,

young stock, yearlings, and adults together). Such data will help match the seasonal environmental loading rate to seasonal intervention strategies and better alert water quality managers when highly infected populations are present on the watershed.

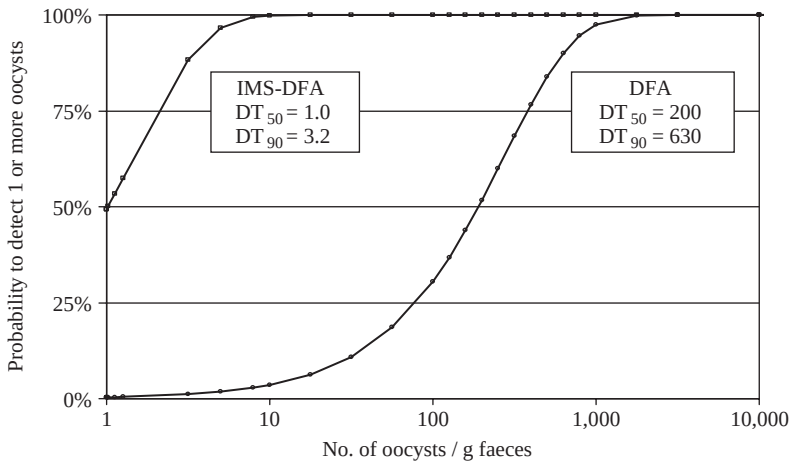


Figure 3.3 The probability of detecting *Crypto. parvum* oocysts in bovine faeces for two assays: immunomagnetic separation followed by immunofluorescent microscopy (IMS-DFA) and immunofluorescent microscopy alone (DFA). DT_{50} and DT_{90} are the oocyst concentrations detected with 50% and 90% probability, adapted from Pereira *et al.* 1999 and Atwill *et al.* 2003.

Certain age classes of animals tend to be more susceptible to infection than others and may even be infected by different subtypes of organisms than adults. It is known that manure from calves is likely to contain higher levels of zoonotic pathogens such as *Cryptosporidum parvum* and *E. coli* O157:H7 than manure from adults, and that on both a relative (concentration of organisms per unit of weight) and absolute basis (number of organisms excreted per animal) represent a greater risk as a source of these waterborne zoonotic pathogens than manure from adults. This "high risk" manure may need to be processed more thoroughly than wastes from adults to decrease pathogen numbers in the environment and the associated human health risks.

Certain human hosts, particularly those who are immunologically naïve or immuno-compromised (e.g. individuals with HIV infections, patients undergoing chemotherapy, and the elderly) are more susceptible to illness caused by waterborne pathogens. However, differences in the genetic susceptibility of individuals in the population also likely play a role in susceptibility to these infections. As in human hosts, there are also likely to be differences

in susceptibility among individual animals to colonization and infection by waterborne pathogens. It may be possible to use information concerning differential susceptibility to disease and/or colonization to target disease prevention programmes in humans and pathogen control programmes in animals.

Differences in the level and frequency of shedding and persistence of pathogen colonization among individuals in herds or groups of a specific age-class have also been reported. It has been stated that 20% of animals are responsible for 80% of the pathogen excretion in groups of animals. These animals have been referred to as super-shedders (although definitions vary). Targeted treatment or exclusion of these super-shedders in animal groups could substantially reduce risks associated with these waterborne zoonotic pathogens.

3.5 CONCLUSIONS

Animal husbandry plays a key role in the global economy. With the growth of the world population and the emergence of the economies of several large developing countries, an increase in the demand for animal protein is projected to continue. Livestock production systems use large quantities of limited resources such as fresh water and can cause environmental damage and loss of productivity of agricultural land if not properly managed. Animal husbandry generates significant amounts of wastes which can result in excessive nutrients and chemical pollution of waterways if improperly managed. In addition, these wastes are a source of zoonotic pathogens responsible for significant morbidity and mortality in human populations around the world.

Microbial pathogens associated with waterborne disease in humans are commonly excreted in the faeces or passed in the urine of most domestic and wild animal species. Most animal pathogens such as viruses and certain protozoa are host-restricted and are not associated with human disease (Carter *et al.* 2005, Fong *et al.* 2005). Among those microbial pathogens that have a broad host range that includes humans as a secondary or accidental host, only a small number have strong scientific evidence supporting their role as important water-borne zoonoses.

The pathogens reviewed in this chapter, *Cryptosporidium parvum*, *Giardia duodenalis*, *E. coli* O157:H7, *Salmonella*, and *Campylobacter*, have been reported in livestock populations worldwide and domestic livestock are believed to be important, and in some cases, the most important source of human infection. As such, understanding faecal shedding dynamics and the associated host, environmental, and agent factors driving these infection patterns is key to assessing risk to human health and developing control strategies.

Recent advances in the molecular characterization of micro-organisms has allowed us to identify subgroups of these pathogens that are limited in their distribution to one or a few closely-related host species and may not represent a risk to human health. The ability to better categorize genotypes of these agents according to human health risk, along with advances in ability to detect and measure pathogen loads will have a significant impact on the development of environmental policies designed to mitigate human health risks associated with these infections agents.

The majority of studies reviewed in this chapter were carried out in developed countries, yet it is likely that the burden of waterborne zoonoses is significantly higher in low- and middle income countries. Further research is needed to understand the importance of waterborne zoonoses and the contribution of domestic livestock to human health risks.

It is noteworthy that two of the five priority diseases are considered emerging diseases. Three quarters of new and emerging diseases have jumped species from animals to man and it is likely that, driven by climate change, livestock-intensification, environmental degradation, globalization and other mega-trends, the next few decades will see new waterborne zoonoses emerging.

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4

Zoonotic waterborne pathogens in livestock and their excreta – interventions

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4.1 INTRODUCTION

In Chapter 3, the prevalence of zoonotic pathogens in animal populations was discussed. In the present Chapter, methods used to reduce these zoonotic pathogens in animal populations and their waste products are presented. However, it must be borne in mind that farm-level interventions are rarely primarily aimed at reducing risks associated with waterborne disease in humans; most commonly the objective is to reduce foodborne zoonotic illness (caused by the same pathogens) or to reduce production losses in domestic animals. In the case of so-called "dual burden pathogens", that is micro-organisms with major

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deleterious impacts on both human and animal populations, the objectives are to both improve food safety and reduce production losses. A subsequent Chapter discusses some of the implications when effective control requires coordination of multiple actors and where costs are borne by one actor but benefits accrue to multiple actors, which may or may not include the actor investing in control.

4.2 RISK-BASED APPROACHES AND THE RATIONALE FOR ANIMAL-BASED INTERVENTIONS

Risk-based approaches provide a rationale for tackling waterborne zoonoses at the farm level. A cornerstone of risk analysis is the concept of a "risk pathway" that traces hazards from their source to the associated health outcome: paths commonly referred to as gate to plate, farm to fork, boat to throat and, when applied to water treatment, source to tap (Hamilton *et al.* 2006). This implies that controlling hazards at source will have benefits all the way down the risk pathway, and, indeed, it has been suggested that the farm is often the best place to control food and waterborne pathogens (Hafez 1999). Figure 4.1 sets out points at which pathogens can be controlled as they move from environment/other animals to infect farm animals, then establish infection in the gastrointestinal system (GIT) of livestock, and eventually are shed in excreta. These are considered in detail in the rest of the Chapter as four key control points to ensure animal excreta are free from waterborne zoonoses.

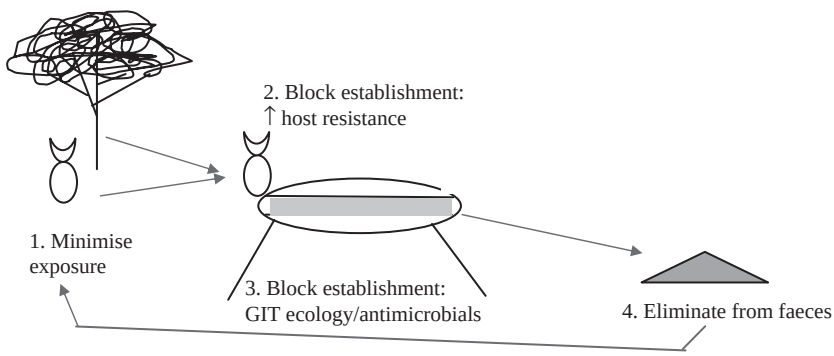


Figure 4.1 Points at which pathogen load can be reduced in farm animals and their excreta.

In keeping with the rest of the book, this chapter focuses on the five priority waterborne zoonoses identified in Chapter 2: *Salmonella enterica*, *Escherichia*

coli O157:H7, *Campylobacter* spp., *Cryptosporidium parvum* and *Giardia duodenalis* (a.k.a. *G. lamblia)*; other waterborne zoonoses (leptospirosis and schistosomiasis) are referred to as illustrative examples.

The concept of key control points or multiple barriers applies from farm through transport to human exposure: because absolute control is not possible at every level, multiple barriers are required for safety so if one barrier should fail then the presence of others ensures water and food quality are maintained, and health risks minimized.

Another insight from risk-based thinking is that processes are more manageable than products. This concept is at the heart of Hazard Analysis and Critical Control Points (HACCP), the genesis of which was when scientists from the food industry had to ensure safe meals for astronauts (Griffiths 1997). In setting up a HACCP system one attempts to identify all possible hazards and then identifies steps in the process where action is necessary to prevent negative health outcomes (critical control points). In the context of this chapter, the desired "product" is pathogen-free animal wastes.

Risks may have multiple sources and all sources must be synoptically considered; for example, halving the cases of salmonellosis in cattle will have little impact on public health if 99% of cases originate from other humans or from non-bovine animals. But risk is also incremental, and in this context even small interventions may reduce risk sufficiently to make them worth their cost. Justifying interventions is further discussed in a subsequent Chapter, but especially in low- and middle income countries the perfect is often the enemy of the good. High technology, high cost interventions remain all too often preferred over lower cost, more appropriate, somewhat less effective options that have far better prospects of sustainability.

Case Study 1: Successful National Programmes to Control *Salmonella* in Poultry – When Interventions at Farm Level Work

Salmonellosis in humans

Salmonella enterica serovar Typhi is a human-adapted pathogen associated with typoid fever and is a significant cause of waterborne disease in the developing world (Bhunia *et al.* 2009). It is thought that cases of human illness associated with this serovar have decreased in the developed world with the use of advanced systems to treat human sewage and chlorinate drinking-water (Leclerc *et al.* 2002). Non-typhoid *Salmonella* serovars are typically associated with gastroenteritis in humans. However, illness caused by these *Salmonella* serovars can also result in septicaemia and death. Non-typhoid salmonellae are commonly derived from animal sources and are

primarily transmitted to humans through contaminated foods but are also occasionally associated with waterborne disease (Callaway *et al.* 2007, Franklin *et al.* 2008, Schuster *et al.* 2005). While there are over 2500 serovars of *Salmonella*, only a handful of these are commonly associated with human disease. For example, in Europe more than 70% of human infections are associated with *Salmonella* serovars Enteriditis and Typhimurium (EFSA 2006).

Salmonella in poultry

Salmonella serovars Gallinarium and Pullorum were a common cause of production losses to the poultry industry in the first half of the 20th century (Cogan & Humphrey 2003). However, disease associated with these two serovars in poultry was successfully controlled through vaccination and led to their virtual eradication by the mid 1970s. It has been postulated that the decrease in these serovars in poultry provided a vacant niche which allowed *Salmonella* serovar Enteriditis to establish a foothold. In contrast to serovars Gallinarium and Pullorum, Enteriditis is not associated with disease in poultry but is very commonly associated with human disease. By 1997, foodborne illness associated with *Salmonella* serovar Enteriditis had risen from 10,000 to over 30,000 cases per year in the United Kingdom and accounted for about 70% of human *Salmonella* infections. It was shown that salmonellosis was associated with the consumption of poultry and that phage type 4 of *Salmonella* serovar Enteriditis-related disease was specifically associated with the consumption of shelled eggs. Similar Enteriditis-associated epidemics were also observed in other European countries and the USA at this time (Braden 2006, Patrick *et al.* 2004, Poirier *et al.* 2008, Wegener *et al.* 2003).

On farm control of Salmonella in poultry

Salmonella control programmes adopted in the European Union and in other developed countries around the world have certain elements in common but also may contain country-specific elements. Perhaps the most important of these is to have a *Salmonella* control plan with specific reduction targets. Many programmes emphasize the importance of strict biosecurity measures including the provision of physical barriers, limited access, "all in, all out" management systems and clean feed and water, in attempts to prevent horizontal transmission of these pathogens. While these measures may be suitable for keeping *Salmonella* out of clean flocks, they cannot be used to eliminate established infections. In order to do this one must start with clean seed stock. In both the broiler meat and table-egg industries significant efforts have been made towards a top-down approach to eliminate *Salmonella* from elite, grandparent and parent breeder flocks. Bacteriological and serological testing of samples is undertaken at multiple points in the production cycle of breeder flocks to determine their infection status. *Salmonella*-positive flocks are depopulated and barns

where they were housed are thoroughly cleaned and rested before repopulation. In the EU, governments compensate owners of depopulated flocks for their economic losses. While *Salmonella* eradication efforts began with the flocks at the top of the production pyramid, they have now worked their way down to the commercial level in many countries. This "test and slaughter" approach appears to have been very effective in the control of Enteriditis serovars in poultry and suggests that vertical (within egg or trans-ovarian) rather than horizontal (feed and/or environmental) transmission plays a key role in maintaining the infection in poultry. In Denmark, vaccines, competitive exclusion and antibiotics have not been part of the *Salmonella* control programme. They have reported a decline of *Salmonella*-infected broiler flocks from >70% in 1989 to <5% in 2001 (Wegener *et al.* 2003) and the number of infected table-egg layer flocks from 13.4% in 1998 to 0.4% in 2006 (Korsgaard *et al.* 2009). France has taken an approach similar to that of Denmark and it is estimated to have resulted in a decline of about 21% in the number of human infections with Enteriditis serovar and an 18% decline in the number of infections associated with Typhimurium serovar from 1992 to 2003 (Poirier *et al.* 2008).

In the USA, egg quality assurance programmes (EQAPs) were introduced by a number of states (Braden, 2006). While there is considerable variability among EQAPs, they also focus on strengthened biosecurity in poultry barns, bird and environmental testing for *Salmonella*, pasteurization of eggs from infected flocks, cold chain maintenance and consumer education. In states which have adopted EQAPs, there have been significant declines in the rate of human infections caused by the Enteriditis serovar (Mumma *et al.* 2004).

In the United Kingdom, the use of improved biosecurity procedures and use of a *Salmonella* serovar Enteriditis vaccine in laying hens may have contributed to the steep decline in human infections associated with this organism from 1998 to 2001 (Cogan & Humphrey 2003). These latter two measures also may have contributed to declines in *Salmonella*-positive flocks and levels of human infections in Belgium (Namata *et al.* 2009, Collard *et al.* 2009). In Finland, biosecurity, good hygiene and the use of competitive exclusion are thought to be responsible for the low flock prevalence of *Salmonella* (Maijala *et al.* 2004).

In the EU, there continues to be a great difference in the rates of human salmonellosis among member states (from 30 to >300 per 100,000) (ESFA 2006). The European Food Safety Authority has recently set *Salmonella* reduction targets for flocks of laying hens at <2% based on the success of these *Salmonella* control programmes in the member states mentioned above. It will also make vaccination of laying hens mandatory for member states with more than 10% of their flocks positive. In addition, it plans to introduce a trade ban on the sale of eggs from *Salmonella*-infected flocks. Collectively, these efforts reflect the progress that can be made in the control of zoonotic pathogens using targeted on-farm programmes.

4.3 CONTROL POINT 1: MINIMIZING EXPOSURE OF LIVESTOCK TO PATHOGENS

For livestock to become infected, and hence a hazard source, they must first be exposed to pathogens. Waterborne zoonoses can originate from infected livestock, other animals (including humans) or the environment; the latter is typical of free-living pathogens which cause accidental zoonoses. These sources of waterborne zoonoses can be present on or off the farm, and livestock may contract them directly (through animal to animal, or human to animal physical contact) or more commonly indirectly (through, e.g., vehicles (or fomites), vectors, air currents, dust particles, water, food or oral-faecal contact). In this section we consider how exposure of livestock to waterborne zoonoses can be reduced or eliminated.

4.3.1 Specific pathogen-free animals

Prevention is better than cure, and it is epidemiologically more sound to maintain animals free from pathogens than eliminate pathogens from infected animals. Therefore, the present Danish programme relies strictly on test and slaughter and not on vaccines and has placed emphasis on "cleaning up" the primary poultry breeder flocks and swine herds in *Salmonella* and *Campylobacter* control programmes.

Caesarean section-derived germ-free or gnotobiotic animals have been used in the swine industry to create disease-free animals. These animals are largely free from *Salmonella*, *Trichinella spiralis* and *Toxoplasma gondii* infections. Specific pathogen-free pig herds (free from *Y. enterocolitica* O:3/biovar 4) have also been established and maintained in Norway. According to serologic and cultural testing results, 15 of 16 of these herds tested have been free from *Y. enterocolitica* O:3/biovar 4 since 1996 (Nesbakken *et al.* 2007).

In technologically sophisticated livestock production systems such as those seen in high-income countries and many emerging economies, there is a demand for uniform, predictable food products, which in turn leads to demand for genetic homogeneity in animal populations. This is typically obtained by drawing on a very narrow range of high growth-potential genetic stock. This incidentally offers an opportunity for specific-pathogen-freedom cascading through generations to the animals producing for consumer markets. In poultry, for example, there is a production pyramid, with elite and grandparent breeder flocks at the top that provide the seed stock necessary for the lower levels in the production system that give rise to the production flocks that provide our poultry meat and eggs. Considerable effort is placed on maintaining these elite flocks free of enteric

pathogens. For poultry pathogens such as *Salm.* serovar Gallinarum, avian *Mycoplasma* and leukosis virus, specific pathogen-free flocks have been established on the elite and grandparent level. Efforts have also been made to prevent poultry from passing not only faeces-associated, but egg-associated pathogens such as *Salmonella* from moving down the production pyramid.

Of course, zoonotic pathogen control programmes for the creation of specific pathogen-free animals must be cost-effective and require diagnostic procedures able to detect all infected animals to be culled eg. serological procedures rather than microbial culture or detection methods have been found to be cost-effective in the Danish *Salmonella* control programme in swine (Wegener *et al.* 2003).

4.3.2 Biosecurity-raising livestock in a disease-free bubble

Pathogens enter livestock holdings by numerous routes: biosecurity management practices are designed to prevent the spread of disease by minimizing the movement of organisms and their living vectors (wild birds, rodents, flies, etc.) and inanimate vehicles (clothing, vehicles, feed, etc.) onto and within farm operations. Biosecurity systems are easiest to develop and implement for intensively farmed poultry flocks and swine herds, where controlling the environment is necessary for profitable production as well as zoonoses control. Specially designed barns can provide physical isolation of breeder and production animals (White *et al.* 1997). These systems can be designed to keep pathogens out by limiting entry of other animals of the same and of different species. Common practices include rodent, wild bird, and insect control procedures, and requiring human handlers to use a disinfectant boot bath, or even shower in and shower out of barns and farms. Other strategies include the maintenance of closed-herds or at least limiting the entry of new animals into the herd until they have been treated and/or undergone pathogen testing and/or a quarantine period has passed. In production facilities, animals of one age-class should be housed together and an "all in, all out" policy is recommended together with thorough cleaning and disinfection of the facility before it is restocked. Biosecurity systems such as these are reported to reduce the risk of poultry shedding *Campylobacter* in their faeces (Gibbens *et al.* 2001). It is also likely that manure from animals raised with high levels of biosecurity present a lower risk of food and water contamination. Organic systems often allow greater interaction with the external environment and it has been reported that Swedish organic free-range chickens have much higher levels of *Campylobacter* than chickens raised under conventional barn systems (Engvall 2001), providing supporting evidence of the effectiveness of reducing contact with the environment in reducing pathogen load.

Molecular fingerprinting of pathogens such as *C. jejuni* has allowed a more thorough examination of the movement of these important pathogens into and out of poultry barns and highlighted the importance of maintaining biosecurity (Bull *et al.* 2006). For example, these studies have underlined the importance of human and insect vectors and even wind in the transmission of these pathogens among flocks.

In animals such as cattle, sheep and goats, that are raised on pasture, and in feedlots and in free-range poultry flocks or swine herds, biosecurity is a significantly greater challenge. Transmission of waterborne pathogens such as *G. duodenalis*, *Crypto. parvum*, *Campylobacter*, *Salmonella*, *E. coli* O157:H7, and *Leptospira interrogans* among cattle in herds and between cattle and wildlife species is known to occur and is difficult to prevent in open pastures (Keene *et al.* 1997, Lomar *et al.* 2000, Thompson 2000, Daniels *et al.* 2003, Liebana *et al.* 2003, Wahlstrom *et al.* 2003). In cattle herds it is recommended to limit the introduction of new animals, to move dams and their calves away from birthing areas onto clean pastures after parturition, and to isolate dairy calves from other calves and the rest of the herd. This is particularly important for agents such as *Crypto. parvum* whose populations appear to be largely maintained by calf-to-calf transmission. It has also been demonstrated that reducing animal stocking densities reduces the exposure of susceptible animals to infected animals and their excreta. For example, reducing the stocking density in a high-cattle-use area was shown to be associated with a reduction in *G. duodenalis* cysts in rainfall-associated runoff.

Biosecurity is easy to recommend but can be hard to implement. The recent avian influenza pandemic in Southeast Asia has shown how difficult it is to maintain biosecurity in intensive production systems, while in smallholder systems in developing countries high levels of biosecurity are probably unattainable. Good hygienic practices may reduce the pressure of infection but converting the existing low input systems into biosecure systems might render them uneconomic and unworkable.

A more fundamental challenge in advocating of biosecurity messages is that, until recently, most recommended procedures have been based on commonsense rather than scientific evidence. Unfortunately, the most important sources of many zoonotic enteric pathogens in flocks and herds have not been quantified and are only starting to be investigated. Case-control and other risk-factor studies are needed to develop evidence-based biosecurity recommendations, where most emphasis is placed on steps shown to be critical in reducing the pathogen prevalence rate in animal populations. These are discussed in more detail later in this Chapter.

4.3.3 Reducing exposure from high risk animals

The law of the "vital few" and the "trivial many" applies to hosts as well as to zoonoses. For many diseases a minority of animals harbour a majority of pathogens; this may be the result of density-dependent and/or density-independent factors, and is especially important for parasitic infections. "Supershedding" and "superspreading" are more recently identified phenomena: super-shedders are individuals which shed exceptional numbers of pathogens (often temporarily) while super-spreaders are individuals with exceptional opportunities to infect other hosts (the phrase was first used in sexual disease transmission studies). As noted in Chapter 3, super-shedders are thought to be the most important source of the zoonotic pathogen *E. coli* O157:H7 and possibly other waterborne zoonoses (Chase-Topping *et al.* 2008) and are thought to be responsible for the perpetuation of herd infections.

Carriers are asymptomatically infected animals that shed constantly, intermittently, or when stressed and for periods varying from days to throughout their lives: the carrier state is important in salmonellosis and leptospirosis. Carriers are difficult to identify without laboratory testing procedures such as the serological tests that are used to identify pigs asymptomatically carrying *Salmonella,* as part of the European SALINPORK programme (Wegener *et al.* 2003).

Understanding epidemiological phenomena that result in high-risk animals offers the possibility of new intervention modalities based on identification and removal, treatment, or isolation for the period of shedding (where temporary). The carrier state presents a particular challenge in that control procedures such as vaccination and antimicrobials may control the level of pathogens excreted but not eliminate the carrier state and, indeed, simply mask hidden infections. However, for some pathogens (e.g. leptospirosis) antibiotics are effective in eliminating the carrier state.

4.3.4 Reducing exposure from wildlife

Although briefly considered in the section on biosecurity, wildlife transmission deserves a place to itself.

Many waterborne zoonoses have wildlife reservoir hosts and in many cases these have key roles in disease epidemiology. And, while in wild animal populations, parasite and host may live in an ecological balance with little or no obvious effect on the natural host reservoir population, health effects are likely to be observed when human hosts are accidentally exposed to these zoonotic agents. Eradication programmes have been, and continue to be, successfully

used to control infectious diseases in domestic animals and may be applicable to animal pests such as rodents and mosquitoes, but this approach is not acceptable for wildlife species thought to play an important ecological role or that are considered endangered. In this situation, control in wildlife is more difficult, which in turn fosters persistence of disease in livestock populations e.g. it is thought that attempts to eradicate bovine tuberculosis have been frustrated by the inability to control *Mycobacterium bovis* infections in badger (*Meles meles*) populations in the United Kingdom (Delahay *et al.* 2003) and possums in New Zealand. Another example is leptospirosis which mainly occurs in humid tropical climates due to contamination of water with urine from infected wild or feral animal species. Control of leptospirosis in these species is challenging, given the variety of *Leptospira interrogans* serovars that are encountered in the wild animal reservoir and the cost of vaccination and eradication programmes.

There are some non-lethal approaches to control zoonoses in wildlife, especially use of vaccines. One of the few successful uses has been in the control of fox rabies in northern Europe and Switzerland (Wandeler *et al.* 1988). In the vaccination programme, food baits impregnated with a modified-live rabies virus were distributed in the countryside. Aguilar-Setien *et al.* (2002) reported on the potential use of a vaccinia-rabies glycoprotein recombinant virus aerosol for control of rabies in vampire bats (*Desmodus rotundus*). Attempts have been made to vaccinate raccoons, skunks, coyotes, mongooses, and bats (Creekmore *et al.* 1994, Aguilar-Setien *et al.* 2002, Hanlon *et al.* 2002, Linhart *et al.* 2002) with either aerosol or bait vaccines against rabies virus. Vaccines have also been developed against brucellosis in feral swine and wild ruminants (Davis & Elzer 2002) and the plague bacillus, *Yersinia pestis*, in rodents. Aerosol or bait vaccines may be useful for the control of certain waterborne zoonotic pathogens. Also, contraceptive vaccines have been developed to bring about control of wildlife populations that may carry zoonotic diseases (Barber 2000, Mate *et al.* 2003, Smith & Cheeseman 2002).

4.3.5 Reducing exposure from litter and bedding

Litter and bedding can provide an ideal environment for pathogen survival and growth. Poultry litter is commonly treated with agents such as aluminium sulfate and sodium bisulfate to limit the survival of bacterial pathogens. These litter treatments significantly reduced *Campylobacter* colonization in the caeca of chickens, but had no effect on *Salmonella* colonization (Line 2001). If poultry litter has high moisture content, very high bacterial cell numbers may be found. To prevent wet litter, moisture absorbing bedding material (softwood shavings) is used and heating and ventilation systems monitored to allow control of

humidity (Doyle & Erikson 2006). Studies on *E. coli* O157:H7 have shown that it persists longer in sawdust used for cattle bedding than in sand (LeJeune & Kauffman 2005).

4.3.6 Reducing exposure from feed

Given that most enteric pathogens are transmitted by the faecal-oral route, clean feed is essential in their control. It has been shown that many of the *Salmonella* serovars shed by poultry probably originated from meat and fish products in poultry feeds, introduced to increase protein content (Crump *et al.* 2002). From 5 to 20% of livestock feeds have also been reported to have *Salmonella* contamination (McMullan 2000), although the upper estimates may be exceptional, heating during processing of poultry and swine "complete" feeds is efficacious in the killing of most microbial pathogens (Cox *et al.* 1986, Daniels *et al.* 2003). Gamma-irradiation also appears to be effective in reducing pathogen loads in animal feeds (Leeson & Marcotte, 1993). A maximum dose of 15 to 35 kGy is sufficient to produce *Salmonella*-free feed under commercial conditions. As well as processing to reduce pathogens, feeds must be stored in a manner that will prevent recontamination. Contamination of commercial feed is probably a greater problem in developing countries where quality assurance of feed is often lacking, and animals are usually fed directly from the ground. Moreover, cultural practices, such as the feeding of household waste to animals will increase the likelihood of zoonotic pathogen presence in feed. In some pig-keeping communities, farmers defecate directly into pig-pens as a way of providing additional feed: this practice facilitates the transmission of "reverse zoonoses", that is, diseases readily transmissible from man to animals. For some diseases, such as cysticercosis, the animals in turn can be an important source of infection for humans.

4.3.7 Reducing exposure from water

LeJeune & Wetzel (2007) noted that there is a strong association between the presence of pathogens such as *E. coli* O157:H7 in drinking-water and the presence of pathogens in cattle faeces. Water for poultry also frequently contains *Salmonella*. However, it is not always clear if the water contamination is the cause or simply the consequence of gastrointestinal colonization of the animals by these enteric pathogens. Bacteria in drinking-water systems are thought to escape antibacterial agents present in the water by adhering to the surfaces of tanks and tubes and forming resistant structures termed "biofilms" (Tuschewitzki *et al.* 1983, Zimmer & Slawson 2002, Kalmokoff *et al.* 2006); therefore, thorough cleaning and sanitation of animal water troughs is

recommended. The use of acidified water has been reported to reduce carriage of *Salmonella* in the crop and caecal carriage of *Campylobacter* (Chaveerach *et al.* 2004, Russell 2002). Chemical and physical treatments to reduce pathogens in the water supply include chlorination, ozonation, frequent cleaning, raising water troughs, use of nipple drinkers, and screens that reduce organic solids in the troughs. Many chemical treatments have been evaluated for their usefulness in the control of pathogens in water sources for livestock and poultry. In cattle operations, treatments such as acidification of water or addition of chemicals (chlorine, ozone, sodium chlorate) have had limited success because of the difficulty of eliminating rumen content or manure from water troughs (Callaway *et al.* 2002). Other chemical treatments can be effective even in the presence of manure or rumen content in the laboratory, but so far strategies have not been able to demonstrate a significant affect on herd prevalence rates of zoonotic enteric pathogens, and extensive investment in clean water delivery may not be warranted (LeJeune & Wetzel 2007). In developing countries animals are often given water considered unfit for human consumption, this practice obviously increases the likelihood of exposure to pathogens.

4.4 CONTROL POINT 2: INCREASING HOST IMMUNITY/RESISTANCE

Exposure to waterborne zoonotic agents, even in high doses, does not necessarily result in infection. The innate or genetic ability to resist infection varies with species, breed and individual. In addition, resistance can be increased by management practices or conferred by active or passive immunization.

4.4.1 Disease resistant livestock

Research has been conducted on the breeding of pathogen-resistant animals (Adams & Templeton 1998). However, disease resistance is usually complex, involving many genetic loci with incremental rather than absolute levels of resistance, making it difficult to breed for resistance. However, new approaches, such as population-based, whole genome-wide association studies may be more successful than conventional breeding programmes in identifying and developing disease-resistant livestock (Calenge *et al.* 2010; Morris 2007; Huang *et al.* 2011).

4.4.2 Vaccination

Vaccination creates immunological resistance to pathogens and has been a mainstay of disease control for over a century. However, because many

food- and waterborne pathogenic bacteria do not cause illness nor illicit a strong immunological response in the host animal, development of vaccines against these pathogens has been difficult. In the context of the five agents identified as frequently associated with waterborne zoonotic disease, good success has been achieved in vaccines against *Salmonella* responsible for disease in swine and dairy cattle (House *et al.* 2001) and to reduce *Salmonella* colonization in poultry (Zhang-Barber *et al.* 1999). In the United Kingdom, many breeder and layer flocks are now vaccinated against *Salmonella* serovar Enteritidis, which has led to a reduced prevalence of this serovar in poultry (Barrow 2007). Live *Salmonella* vaccines generally confer better protection than killed vaccines, because the former stimulate both cell-mediated and humoral immunity (Imersaal *et al.* 2005). Vaccines have also been developed for use in cattle to reduce faecal shedding of *E. coli* O157:H7 (Judge *et al.* 2004, Moxley *et al.* 2009, Sargeant *et al.* 2007).

While vaccination has long been used to prevent animal diseases of economic importance, many of the zoonotic waterborne pathogens seem to have limited or no effect on the health of animals; swine and poultry frequently excrete *Salmonella* and *Campylobacter,* and young calves excrete *E. coli* O157 in their faeces, without apparent ill effect. The gold standard for vaccines has traditionally been prevention of disease and economic loss associated with animal disease. However, in the case of zoonotic pathogens, vaccines should not only prevent faecal shedding but also ideally eliminate the carrier state. Until recently, veterinary vaccines have been very crude and have consisted only of killed or modified-live pathogenic organisms. This lack of sophistication is related to the required low cost of production of these vaccines and difficulties with regulatory approval of recombinant vaccines. There is also concern about loss in quality of meat and poultry products as a result of vaccination via intramuscular routes, and more and more emphasis is being placed on adjuvants and routes of vaccination that stimulate mucosal immunity and prevent colonization and the carrier state.

It has been reported that infection of calves with zoonotic pathogens such as *E. coli* O157:H7 and *Crypto. parvum* occurs within hours and days of birth (Harp *et al.* 1996, Gannon *et al.* 2002). Therefore, it may be more logical to mount a specific immune response in the dams through vaccination prior to birth so that protection against colonization and infection can be provided passively through colostrum and milk. This approach was initially developed for protection of lambs and calves against K99-bearing enterotoxigenic *E. coli* (Sojka *et al.* 1978, Nagy 1980). Perryman *et al.* (1999) reported that the severity of *Cryptosporidium parvum* infection and oocyst shedding can be reduced by feeding calves immune colostrum obtained from dams vaccinated with a

recombinant *Crypto. parvum* surface antigen, but other trials evaluating vaccination or colostral immunity did not consistently reduce shedding oocysts or reduce the severity of *Crypto. parvum* infection (Harp *et al.* 1989, Thompson *et al.* 2008).

Properly formulated vaccines have the potential to be valuable tools in controlling leptospirosis in domestic animal reservoirs. The degree of protection provided by *Leptospira* vaccines is serovar-specific and has led to the use of multivalent vaccines with antigens from several serovars. However, some of the multivalent vaccines on the market have not been shown to provide long-lasting protection against clinical disease or urinary shedding in cattle (Bolin & Alt 2001). Other vaccines with *Leptospira interrogans* serovar harjo alone or in combination with serovar pomona have been shown to decrease urinary shedding in cattle and are reported to decrease the incidence of leptospirosis in humans in contact with the cattle (Mackintosh *et al.* 1980, Bolin & Alt 2001).

Passive immunity is conferred to an animal by the uptake of specific antibodies derived from another animal. As mentioned above, drinking the colostrum of vaccinated dams is known to protect calves from diarrhoea-causing *E. coli* strains. Similarly, oral feeding of chicks with egg yolk derived from hens vaccinated against specific *Salmonella* serovars can provide passive immunity and reduce the likelihood of colonization by this pathogen (Cox & Pavic 2009, Rahimi *et al.* 2007). Antibody producing genes have also been cloned in to plants and "plantibodies" could potentially enjoy both prophylactic and therapeutic use someday in the control of animal infections (Judge *et al.* 2004).

Case Study 2: Vaccinating Water Buffaloes to Control Schistosoma Japonica

Human schistosomiasis, or bilharzia, is a chronic parasitic disease that affects around 200 million people in 76 countries and is second only to malaria in public health importance. There are three major types of schistosomiasis, of which only one, *Schistosoma japonicum*/*S. Mekongi*/*S. malyayensis* complex, is an important zoonosis. This causes intestinal schistosomiasis and is found in Southeast Asia; around 40 reservoir hosts have been identified. Schistosomiasis is a water-based infection, contracted when free-swimming infective larvae (cercariae), released from the snail intermediate host, penetrate the skin. Infections can result in serious illness characterized by hepatosplenomegaly and cerebral symptoms, and the disease is considered a major obstacle to economic development.

Not all zoonoses are best tackled at farm level and before targeting pathogen load reduction in the livestock host, it is necessary to fully understand the role of livestock in the transmission cycle. The presence of pathogens in animals and in humans tells

us little about disease epidemiology, but in the case of *Schistosoma japonicum*, there is strong evidence that cattle and water buffaloes play an important role in the transmission cycle. Historically, schistosomiasis was eradicated from Japan without medical interventions, with the switch from bovines to horses as draught animals considered to have played a role in this. Field studies in China and elsewhere indicate an important role for cattle and water buffaloes which shed hundreds of thousands of eggs per day in their faeces. Water buffaloes spend most of their time in the water and thus are constantly exposed to infected snails. The molecular biology of *S. japonicum* distinguishes between two main clusters: those from humans and bovines versus those from domestic animals, again supporting the importance of bovines in human transmission. Mathematical models, parameterised by data collected from the field suggest that water buffalo account for approximately 80% of transmission in China and in their absence infection cannot be maintained. Finally, intervention studies showed that if bovines are not treated for schistosomiasis along with humans, infection rates remain high.

This assembly of independent evidence from different disciplines provides strong evidence that *Schistosoma japonicum* is a good candidate for livestock-based interventions to reduce risk to humans and this is now a component of most control programmes. As for other complex diseases, current control of schistosomiasis takes an integrated approach with interventions targeting human behaviour; disease ecology; management of bovines; and treatment of humans, bovines and some other animals. Praziquantal is an effective treatment for schistosomiasis and relatively inexpensive. Although used in ongoing control initiatives, the need for repeated treatments and the possibility of development of resistance are impediments to widespread use. More recently there has been some success in the development of subunit vaccines: field trials in China show these are as effective as chemotherapy in reducing the infection rate in water buffaloes and could have an important role in disease control.

A lesson from this case study is that while not all zoonoses are best or even usefully tackled at the farm level, when domestic livestock plays an essential role in transmission, then including livestock in integrated disease control programmes also is essential.

Case Study 3: Vaccinating Cattle for *E. Coli* O157:H7

Escherichia coli serotype O157:H7 was first recognized as a human pathogen in 1982, following a ground beef-associated outbreak of haemorrhagic colitis in Michigan (Riley *et al.* 1983) and was subsequently associated with the haemolytic uremic syndrome (HUS) in children (Karmali *et al.* 1985). *E. coli* O157 produces Shigatoxins (a.k.a. Verotoxins) that enter the blood stream following damage to the large intestine and kill endothelial cells lining the small vessels in the intestine, kidney and brain (Karmali *et al.* 2009). HUS is characterized by kidney failure and haemolytic anemia and has a mortality of up to 15% in young children (Gould *et al.* 2009).

E. coli O157:H7 has been associated with large outbreaks of human infection and sporadic infections in almost all regions of the world (Cooley *et al.* 2007, Bell *et al.* 1994, Yukioka & Kurita 1997, Effler *et al.* 2001, Machino *et al.* 1999, Dundas *et al.* 2001). In the USA, Canada and the United Kingdom regional differences in rates of *E. coli* O157:H7 infection have been noted (Innocent *et al.* 2005, Gould *et al.* 2009, Waters *et al.* 2004, Michel *et al.* 1999) ranging from <1 to >10 cases per 100,000 population per year. *E. coli* O157:H7 infections have been associated with a variety of food products including ground beef, unpasteurized dairy products, raw field vegetables, radish sprouts and fruit juices (Erickson & Doyle 2007). In addition, outbreaks of human infections have been associated with recreational and drinking-water. In Walkerton, Ontario, Canada contamination of well water contributing to the town water supply and a failure in the chlorination system resulted in over 2300 illnesses, 28 cases of HUS and 7 deaths (Auld *et al.* 2004). In Swaziland an estimated 42,000 individuals suffered from *E. coli* O157 infections as a result of contaminated river water (Effler *et al.* 2001).

Cattle and E. coli *O157:H7 infections in humans*

The primary source of the pathogen in most of these food- and environment-associated disease cases is thought to have been ruminant faeces. Cattle and other ruminants are thought to be natural hosts of *E. coli* O157:H7; however, they are not associated with clinical disease in this animal reservoir. The linkage between *E. coli* O157:H7 infection in cattle and humans is based on a number of lines of evidence: (1) Eating of undercooked ground beef and unpasteurized milk are risk factors for acquiring *E. coli* O157:H7 infections, (2) *E. coli* O157 infections occur more frequently in regions with high cattle density (Michel *et al.* 1999), (3) Argentina has the highest rate of HUS and the highest beef consumption per capita, and cultural practices such as feeding raw beef juice ("jugo de carne") to infants have been associated with HUS (Rivas *et al.* 2008), and (4) isolates of the pathogen from outbreaks of human illness have been shown to share the same molecular fingerprint as epidemiologically linked cattle isolates (Louie *et al.* 1999).

Control of E. coli *O157:H7 in cattle*

A significant amount of attention has been focused on control of *E. coli* O157:H7 in slaughter houses, beef processing plants, restaurants and at home by both governments and industry in a number of developed economies (Gannon 1999). This arose from clear evidence that *E. coli* O157:H7 from ground beef was associated with human disease, threats of litigation and trade restrictions, loss of consumer confidence and significant costs to industry associated with the recall of millions of tons of contaminated ground beef. Measures adopted to control the pathogen included the

implementation of HACCP systems, carcass pasteurization procedures, trimming and chemical treatments of carcasses, cold chain maintenance, irradiation of ground beef, implementation of the five log reduction rule for cooking, and other bactericidal food processes and consumer education.

In addition, studies on "pre harvest" or on-farm control of *E. coli* O157:H7 were also initiated. Studies on the epidemiology and ecology of this organism in cattle revealed it to be very widespread in the cattle population (Karmali *et al.* 2009). *E. coli* O157:H7 could be isolated on most farms from calves shortly after birth and again at weaning and throughout the growing and finishing period. In North America, peak faecal shedding of this pathogen occurs during the summer months. Within groups of cattle the distribution of colonization, and of the duration and number of organisms shed by cattle is not normal; instead, specific individuals in a group known as "super-shedders" are responsible for most of the faecal output of this pathogen (Chase-Topping *et al.* 2008) and represent the largest source for contamination of the environment and of carcasses at slaughter.

E. coli O157:H7 possesses a genomic island termed the locus of enterocyte effacement (LEE) which encodes a type three secretion system (TIIISS) (Dean & Kenny 2009). The system consists of a syringe-like structure which injects a series of proteins directly into the host cell and allows the bacteria to attach to the intestinal cell surface. The LEE-encoded protein intimin and other TIIISS proteins have been used as vaccine components in attempts to disrupt or prevent intestinal colonization by this pathogen. However, studies carried in the United Kingdom reported that vaccination of young calves with recombinant intimin and EspA do not prevent colonization and subsequent faecal shedding of *E. coli* O157:H7 (Dziva *et al.* 2007, van Diemen *et al.* 2007). In contrast, a vaccine prepared from the culture supernatant of *E. coli* O157:H7 strains (and shown to contain several key LEE proteins) was shown to reduce faecal shedding of the organism in groups of challenged and naturally infected feedlot cattle in Canada (Potter *et al.* 2004). However, when the latter vaccine was tested in a large study involving cattle from nine commercial feedlots no significant difference in shedding of the organism was found between vaccinates and non-vaccinates (Van Donkersgoed *et al.* 2005). Subsequent studies with this vaccine in USA feedlot cattle have shown that its efficacy increases with the number of doses given (Peterson *et al.* 2007, Moxely *et al.* 2009). Other bovine *E. coli* O157 vaccines have also been tested or are undergoing development, including a plant-expressed intimin vaccine and others based on the O157 lipopolysaccharide, H7 flagellar antigen and a siderophore receptor/porin (SRP) preparation from the organism (Conlon *et al.* 2000, MacNeilly *et al.* 2008, Fox *et al.* 2009, Thompson *et al.* 2009). The SRP vaccine like the TIIISS vaccine also appears to significantly reduce faecal shedding of *E. coli* O157:H7 in feedlot cattle. In addition to different vaccine formulations, alternative routes of delivery such as intranasal vaccination are also being explored (Babiuk *et al.* 2008). It has been estimated that there are over 74,000 cases of *E. coli* O157:H7 infection per

year in the USA and that each case on average costs $6,276 (2007 US$) (Withee *et al.* 2009). If there is a linear relationship between the number of head of cattle colonized with the organism and human disease incidence, maximum vaccine (or other treatment) costs could range from $2.29 to $9.14 per dose and yield an economic benefit. However, the cost is dependent on the vaccine efficacy and the coverage required in order to achieve herd immunity. The market for such a vaccine is large, given that about 32 million cattle are slaughtered each year in the USA.

In addition to vaccines, many other measures have been evaluated to determine if they can reduce the shedding of *E. coli* O157:H7 by cattle. These include the use of prebiotics, probiotics, chlorate, specific antimicrobials, and bacteriophage therapy (Lejeune & Wetzel 2007, Sargeant *et al.* 2007). It is likely that the most efficacious of these treatments will eventually be used in tandem to reduce health risks associated with this important human pathogen.

4.4.3 Management practices to increase resistance to disease

Natural exposure to infection can lead to acquired resistance and is recommended to aid in the control of some diseases. However, it appears that for the more important waterborne pathogens (e.g. *E. coli* O157:H7 and *Salmonella*) natural exposure does not confer protection to the host (Gyles 1998).

Although it is often assumed that stress may lead to increased carriage or shedding of food-borne pathogens, little is actually known about the importance or mechanism of stress in influencing the shedding of water- and foodborne pathogens (Rostagno 2009).

4.5 CONTROL POINT 3: MANIPULATION OF THE MICROBIAL ECOLOGY OF THE HOST'S GASTROINTESTINAL TRACT

Colonization of animals by pathogenic micro-organisms is a natural process, but the set of micro-organisms that colonize one animal species or one age-class of animal is by no means universal. The basis for this variability in host susceptibility is thought to be related to the specific environmental conditions that exist in the gastrointestinal tracts of different animals. The gastrointestinal tract is populated by competing micro-organisms which produce toxic (antimicrobial) substances, send out hormone signals that limit growth or stimulate the host's immune system, and physically exclude others from protected or otherwise favourable spaces. In addition, viruses and bacterivorous uni-cellular and multi-cellular eukaryotes prey on other microbes, including

pathogens. Diet-dependent, digestive processes also create physical and chemical stress for the organisms. Therefore it is not surprising that certain zoonotic pathogen control efforts have focused on using these obstacles to tip the ecological balance in the gastrointestinal tract in favour of the beneficial microflora and against the survival of pathogenic species.

4.5.1 Prebiotics, probiotics and competitive exclusion

Decades ago it was discovered that feeding the caecal contents of adult birds to young chicks makes them resistant to colonization with *Salmonella* (Rantala & Nurmi 1973), and, in contrast to the use of antimicrobials in feeds, inhibitory effects on the shedding of *Salmonella* persisted long after the treatment was stopped. This method of combating *Salmonella* in poultry, called "competitive exclusion," became widely used in Finland in their *Salmonella* control programme. Hirn *et al.* (1992) reported that less than 5% of the Finnish poultry flocks were *Salmonella*-positive and that 70–80% of salmonellosis cases in humans in Finland were acquired abroad. Stern *et al.* (2001) in the USA also used anaerobic cultures derived from mucosal scraping of the intestines of adult chickens in competitive exclusion experiments and demonstrated that their cultures reduce *Salmonella* in the caeca of chickens as well as faecal shedding of *Campylobacter*. However, attempts to control *Campylobacter* in poultry using competitive exclusion have been less consistent.

The biological basis of competitive exclusion is poorly understood; however, it may be related to enhancing the immune function of the host, nutrient competition among bacteria, or the elaboration of toxic substances such as volatile fatty acids, antimicrobial peptides, or bacteriocins (Joerger 2003). Studies on bacterial populations also suggest that certain bacterial hormones allow "communication" among different bacterial species and may play a role in controlling population levels of specific bacterial species (a process called quorum sensing) for example a recent report suggests that a common anaerobe in the human intestine, *Bacteroides thetaiotaomicron*, can repress expression of Shiga toxin by *E. coli* O157:H7 (de Sablet *et al.* 2009).

Cultures of micro-organisms that limit pathogenic bacterial populations in the gut have been termed probiotics. Other micro-organisms, such as lactobacilli and *Bifidobacterium*, and complex carbohydrates that promote the growth of populations of these members of the normal gut flora have been extensively studied as agents to control faecal shedding of enteric bacterial pathogens such as *Salmonella* in poultry (Stavric 1992, Gusils *et al.* 1999, Fernandez *et al.* 2002). The strategy of using probiotic bacteria is also being explored for control of other bacterial pathogens in other animal species. Tkalcic *et al.*

(2003) reported that a mixture of probiotic *E. coli* strains isolated from adult cattle reduced faecal shedding of *E. coli* O157:H7 and enterohaemorrhagic *E. coli* serotype O111:NM from young cattle within 8–30 days and 6–12 days following treatment, respectively. Sargeant *et al.* (2007) reported that, based on a systematic review of several subsequent studies in the literature, feeding probiotic bacteria was the only one of the many pre-harvest treatments which they investigated that was efficacious in reducing the faecal shedding of *E. coli* O157:H7 by cattle.

Coupling the use of competitive exclusion and prebiotics, in a process known as synbiotics, could yield a synergistic effect in the reduction of foodborne pathogenic bacterial populations in animals for consumption.

Oligosaccharides and other organic compounds which enhance growth of the normal gastrointestinal flora are termed prebiotics: they promote the growth of probiotic species. Probiotic bacteria, such as lactic acid bacteria, are thought to create an antagonistic environment for pathogens through a number of possible mechanisms that parallel those thought to play a role in competitive exclusion. These include production of organic acids, competition for colonization sites, competition for nutrients and enhancement of the host immune system (Doyle & Erickson 2006). It has been shown that as populations of *Lactobacillu*s and *Bifidobacterium* spp increase there is a reduction in *Salmonella* prevalence in birds (Fernandez *et al.* 2002, Xu *et al.* 2003). Prebiotics in feeds such as β-glucans (Lowry *et al.* 2005) and fructo-oligosaccharides appear to reduce *Salmonella* colonization in chickens (Donalson *et al.* 2007, Babu & Raybourne 2008).

Bacteriocins are bactericidal toxins produced by certain bacteria which eliminate other competing bacterial species including subtypes within their own species. Bacteriocin-producing bacteria are being studied for the control of *E. coli* O157:H7 faecal shedding in cattle (Duncan *et al.* 1999, Schamberger & Diez-Gonzalez 2002, Zhao *et al.* 2003) and faecal shedding of *Salmonella* in poultry (Wooley *et al.* 1999).

4.5.2 Bacteriophages

Bacteriophages are viruses that form an important part of the normal "microflora" of the gastrointestinal tract of animals. Bacteriophages can be either lytic or temperate. Lytic bacteriophages enter the bacterial host, take over cell processes such as DNA and protein synthesis, and then burst the cell to release new phage progeny. In contrast, temperate bacteriophages exist in a latent state integrated into the genomes of bacteria. Stressors on the bacterial cell cause the latent bacteriophage DNA to be excised from the bacterial genome and trigger the lytic cycle. Lytic bacteriophages were first investigated for their role in

controlling *E. coli*-associated diarrhoeal diseases in calves, lambs, and piglets (Smith & Huggins 1983, Smith *et al.* 1987). While the results of these studies using mixtures of bacteriophages were promising, the work was not pursued further until recently. Kudva *et al.* (1999) isolated bacteriophages that lyse *E. coli* O157 strains. However, the use of bacteriophages to control *E. coli* O157: H7 in cattle has met with mixed success (Sheng *et al.* 2006, Rozema *et al.* 2009).

4.5.3 Antimicrobials

Antimicrobials have been used at low levels for growth promotion in animal production since the 1950s. They act primarily to increase feed efficiency; the mechanism of action is not known but is mediated largely through the effects of antimicrobials on gut bacteria and inhibition of pathogenic bacteria is believed to be one of multiple pathways. A number of studies have shown the benefits of incorporating antimicrobials into the diets of farm animals in reducing the levels of enteric bacterial pathogens associated with foodborne and waterborne diseases (Rantala & Nurmi 1973, Goodnough & Johnson 1991, Johnson 1992). Antimicrobials such as penicillin and dihydrostreptomycin have also proven useful in controlling leptospirosis infections and eliminating the carrier state in domestic animals (Alt *et al.* 2001). As mentioned above, transmission of enteropathogens from the hen to the egg in elite, grandparent and parent breeder flocks has been shown to be critical in decreasing levels of these zoonotic pathogens in commercial flocks. Several antibiotics, including gentamycin, have been effective at reducing bacterial cell numbers in poultry semen without negatively influencing fertility. Antimicrobial dips to sanitize egg surfaces have also been proved efficacious in reducing *Salmonella*-positive eggs (Berrang *et al.* 2000, Cox *et al.* 2002). Treatment of cattle with neomycin sulphate has been shown to decrease faecal shedding of *E. coli* O157:H7 (Elder *et al.* 2002, Woerner *et al.* 2006). However, approval of this antimicrobial for routine use in cattle is unlikely because it and other closely related antibiotics are used in the treatment of human infections.

Despite their potential use in the control of zoonotic pathogens in animals, much concern has been expressed about the overuse of antibiotics in animal feeds for the purposes of growth promotion. Concern is greatest where there is the possibility of cross-resistance developing between antibiotics used in animal feeds and those used in human clinical medicine. The development in *Salmonella* of resistance to fluoroquinolones and extended spectrum β-lactamases as well as the emergence of multi-antibiotic resistance are thought to be a direct consequence of antibiotic supplementation of animal feeds (Threlfall 2002). These antibiotic resistance determinants are encoded by naturally occurring mobile genetic elements that

can quickly be transferred both within and between bacterial species. This has caused concerns about the long-term effectiveness of these measures and, more importantly, about the transfer of these genetic elements into a broader range of human pathogens. While there are attempts to separate classes of antibiotics used in animals for growth promotion from those used in human therapeutics, the trend is to greater restriction on the use of antibiotics for growth promotion in farm animals. Between 1997 and 1999, the European Union implemented bans on five different antimicrobials: avoparcin, spiramycin, tylosin, bacitracin, and virginiamycin, which are used for growth promotion in animals (Frei *et al.* 2001, Emborg *et al.* 2003, Evans & Wegener 2003). It was initially feared that these regulatory developments might increase levels of enteric pathogens in animal excreta. However, Evans & Wegener (2003) have reported decreases in *Salmonella* levels in poultry and pigs and equivalent levels of *Campylobacter* in poultry in Denmark three years after the ban on the use of these antimicrobials in feeds.

Crypto. parvum is naturally resistant to most anti-coccidial drugs (Coombs & Muller 2002). Joachim *et al.* (2003) reported that halofuginone decreases but does not eliminate faecal shedding of *Cryptosporidium* oocysts by calves and suggested that the drug, used together with good sanitation and disinfection procedures, may limit infection by this parasite in calves. Likewise, prophylactic feeding of decoquinate in experimentally challenged dairy calves did not reduce oocyst shedding nor clinical severity (Moore *et al.* 2003). Few, if any chemotherapeutic drugs have been adopted for treatment of this parasite in livestock populations due to cost or lack of efficacy (Thompson *et al.* 2008).

Chlorate compounds have selective toxicity for enteric pathogens such as *E. coli* and *Salmonella*. In these bacteria the intracellular molybdenum-containing enzyme nitrate reductase changes chlorate to the toxic chlorite form. Interestingly, this chemical has little or no effect on most other members of the gastrointestinal microbial flora because they either lack this enzyme or possess a second enzyme that changes chlorite to the non-toxic chloride form. Sodium chlorate, given by mouth to cattle, sheep and pigs has been shown to reduce *Salmonella* serovar Typhimurium and *E. coli* O157:H7 in the intestine (Loneragan & Brashears 2005). Similarly, chlorate administered to broilers prior to slaughter has been shown to reduce *Salmonella* contamination in the crop and the caecum (Byrd *et al.* 2003, Byrd *et al.* 2008). Unfortunately, resistance to chlorate appears to develop quickly in *Salmonella* and is likely to develop in *E. coli* O157:H7 (Oliver *et al.* 2009).

Short-chain fatty acids such as acetate, butyrate and formate have also been shown to reduce *Salmonella* populations in the crops and caeca of broiler chickens (Immerseel *et al.* 2005). However, the use of combinations of agents

such as organic acids, sodium nitrate and chlorate may be more effective than any single agent alone (Byrd *et al.* 2001, 2003; Jung *et al.* 2003).

Zinc oxide in diets of weanling pigs helps maintain the stability of the intestinal microflora, making the gut less susceptible to colonisation by pathogens, either by competing for the same niche or by suppressing pathogen growth (Katouli *et al.* 1999). Zinc and copper are both widely used supplements; more recently there has been interest in essential oils and spice extracts as growth promoting alternatives to antibiotics (Pasteiner 2006).

4.6 CONTROL POINT 4: TREATMENT OF ANIMAL WASTES TO REDUCE ZOONOTIC PATHOGENS

As we noted in Chapter 3, certain zoonotic pathogens can survive for months and perhaps even years in moist excreta in the environment. It is also important to remember that excreta from young animals may contain high levels of certain zoonotic pathogens and should be an important focus of on-farm waste treatment efforts. For example, given the high levels of *Crypto. parvum* oocysts in young calf faeces, careful management of these excreta should substantially reduce off-site discharges of this pathogen (Miller *et al.* 2008). Reducing the levels of these pathogens in animal excreta is one of the most important steps in the control of waterborne zoonoses that arise from livestock. Composting and anaerobic digestion at high temperatures are very effective procedures in reducing the levels of zoonotic pathogens in livestock excreta. Depending on the excreta treatment system, the equipment and land devoted to process this material can be expensive and are not commonly used, but even simple methods such as drying in the sun or passive stacking of manure solids can reduce levels of pathogens.

Woodchip corrals are outside enclosures, bedded with large woodchips for over-wintering cattle and sheep. As the dung and urine is washed through the woodchips it appears to be digested by microbes so that reasonably clean water enters the soil.

In developing countries alternative systems such as biogas and vermiculture have been promoted with some success. In the small systems that predominate in developing countries, manure is a valued resource used for fertiliser, fuel, and building material. While this means that waste build-up is rarely a problem it also results in high levels of human exposure to manure. The problem can be compounded by culture. For example, many Hindu dairy farmers regard cow manure as sacred and innocuous, reducing their motivation to protect themselves from contact with it.

Case Study 4: Manure Management in Developing Countries

In intensive production systems of developed countries animal waste is more likely to be considered a problem than a product. In contrast, developing countries typically suffer from a scarcity of organic fertilizer and manure is regarded as a valuable output: for example agro-pastoralists in West Africa rank manure higher than milk in terms of benefits provided by cattle (Grace *et al.* 2009). The demand for manure in developing countries opens the door to incentive-based methods for manure management that, as an externality or side-effect, reduce pathogen loads in manure and/or divert manure to other uses and so stop pathogen-laden faeces entering water.

Using animal waste to produce biogas is an example of such an intervention. This is a relatively simple process in which gas is produced by anaerobic fermentation in sealed vessels and then under low pressure supplied to cooking stoves: "free" fuel is of course a major incentive for farm households for whom energy for cooking is an important part of the household budget. An additional benefit is the digestant which is odourless and highly valued as a fertiliser. Moreover, biogas is more efficient than cattle dung fuel and contributes less to greenhouse gases, and cooking with biogas results in much less exposure of women and children to particulate matter which is a major cause of disease. At the same time, anaerobic digestion will reduce most pathogens by more than 99% and thus can be considered an effective strategy for rendering animal waste safe; biogas, therefore, offers the basis for a win-win solution providing both economic benefits and mitigation of zoonotic hazards.

Of course, things are not always so simple and this case study summarises the historical development of biogas use in India to draw lessons for the introduction of manure management technology. Biogas (called "gobar gas" in India, as nearly all comes from cows) was first used in 1897; however, it was not widely promoted until the energy crisis of the 1970s. Early models were technically efficient but had a number of disadvantages including: high cost of parts which had to be brought from outside the village, use of milled steel gas holders which required annual painting to remain rust free, and dependence on a high level of masonry skills for building and repair. As with many transferred technologies, the challenge proved not to be installing plants but rather maintaining them, and as much as 80% of these first generation units fell into disuse.

Realization of these problems led to the development of simpler and more appropriate models and the increasing involvement of non-governmental organizations in delivery. Units currently used are much simpler and can be constructed with materials available in the locality; using the waste of four head of cattle a farmer can get enough cooking gas to meet the needs of a household of six to eight people. The biogas installation programme is subsidized by the Government of India and targets have been established for each state; at present around 200,000 per year are being constructed and most are found to be working on follow-up visits. Yet, despite these impressive results, biogas still has a very minor role in supplying the

energy needs of rural India and, despite 40 years of promotion, doubt remains with some concluding that the lifetime social and economic benefits of the heavily subsidized Indian family-scale biogas plants do not equal the costs of construction and maintenance.

This case-study underlines the importance of socio-cultural factor in influencing uptake. In India, there is a long tradition of use of cow manure: dried cow dung is an important fuel in rural areas and dung mixed with other substances "gobar lipa" is used for coating the floor, walls and yard, of village homes; this undoubtedly increased the acceptability of the innovation. The importance of the positive attitude towards cattle dung is highlighted by the great reluctance to use methane produced from human faeces: even connecting the toilet to household biogas plants is resisted by almost all people.

Other lessons from this case study are the need for simple, easily maintained technology, the benefits of continued adaptation and re-invention of innovations, the importance of support from both government and non-governmental organizations in disseminating new technologies, and, finally, the challenges of mass uptake of even heavily-promoted, socially acceptable, environmentally sound, subsidized, manure-management innovations that deliver major health and financial benefits to the adopters.

4.7 CROSS-CUTTING ISSUES

Most of this Chapter has been organised around the concept of key control points where intervention can reduce the burden of waterborne zoonoses in domestic livestock. This final section focuses on some important cross-cutting issues: control challenges in developing countries, identification of new control interventions, and emerging trends that may influence future control at the farm level.

4.7.1 Developing country issues in controlling animal pathogens at the farm level

The studies reported in this Chapter on control of zoonotic pathogens were mostly carried out in the developed world, reflecting the preponderance of research. However, there is no reason to expect that either pathogens of concern, disease epidemiology or optimum control strategies at farm level will be the same in the developing world. Firstly, in developed countries the so-called "classical zoonoses" have been controlled and their potential as waterborne pathogens is rarely considered. However, in developing countries many of these are uncontrolled and the extent, if any, of waterborne transmission unascertained. For example, *Taenia solium,* is a zoonotic tapeworm transmitted among humans

and between humans and pigs is eliminated from most developed countries but is a major problem in many developing countries. Tapeworm eggs can survive in soil and water and one of the consistent risk factors is drinking surface water (Cao *et al.* 1997). However, the extent of waterborne transmission is unclear.

Another crucial difference is that in developing countries most of the population drinks untreated water (including surface water) and even when treated water is available, breakdowns in treatment are common. *Crypto. parvum* is a high profile waterborne zoonosis partly because it is not eliminated by typical water treatments used in developed countries: in developing countries, all waterborne zoonoses, rather than just a few, risk escaping treatment.

Low- and middle-income countries also differ in their proportion of susceptible hosts: infants and children are a higher proportion of the population and a large proportion of the global HIV-AIDS burden is borne by these countries: this would be expected to increase the relative importance of zoonoses associated with immuno-suppression such as listeriosis. Different socio-cultural practices also influence the presence and prevalence of pathogens. For example, in Ethiopia meat is commonly eaten raw resulting in high levels of *Cysticercus bovis* infection. At the same time, not all characteristics of developing country farms are risk enhancing. The small number of animals kept and lack of vertical integration decrease opportunities for microbial spread and because value chains are short and comprise numerous actors, failure is both less likely and of less impact when it occurs. Moreover, many indigenous farming and food-handling practices are risk mitigating (Grace *et al.* 2008).

Finally, most poor countries are tropical countries where the disease ecosystem is very different from the temperate and wealthy countries beyond the tropics of Capricorn and Cancer. In most tropical countries, there is no distinct cold season where freezing temperatures can kill off bacteria in soil and water. The hot, humid conditions that prevail are more conducive to growth and survival of many pathogens and vectors and some water-associated zoonoses (e.g. schistosomiasis) are only found in tropical areas.

Low- and middle income countries not only have different priorities in terms of waterborne zoonoses, they also have different challenges in managing them. Control of waterborne zoonoses at farm level in developing countries is constrained by structure, lack of awareness, inadequate services, and incentive failures. Small farmers are numerous, poor, and lack infrastructure; compared to the average farmer, they are more likely to be female, illiterate, non-participants in farmer associations, and remote from veterinary services (Grace *et al.* 2008). These structural factors will obviously make control on farms difficult. Compounding this, public veterinary services in developing countries are often under-resourced and dysfunctional while private veterinary services have yet to

emerge as a major service provider. In low-income countries, there is a median number of just 139 private veterinarians per country, woefully inadequate given the number of farms and animals. In these circumstances conventional disease management through command and control mechanisms are unlikely to work, and new models must be developed based on stakeholder engagement and enlightened self-interest.

4.7.2 Epidemiological studies to identify putative risk factors

A starting point for developing new intervention strategies is the identification of host, management and environmental factors associated with an elevated risk of animal infection, otherwise known as risk factors. Risk factors are correlational and not necessarily causal, because correlation does not imply causation. For example, as mentioned previously, farms where livestock are infected with a specific pathogen may have the same pathogen present in livestock drinking-water. However, this does not imply that treating the drinking-water will reduce the prevalence in livestock: it could be that the water is contaminated by livestock but that this is not important in maintaining the agent. Nonetheless, risk factor studies can be powerful tools for generating hypotheses that can be tested more rigorously using follow-up study designs such as randomized clinical trials. Examples of this epidemiological approach would include cross-sectional or longitudinal studies on management risk factors for infection of *Cryptosporidium* spp. in beef calves in the USA (Atwill *et al.* 1999), US and Mexican dairy calves (Maldonado *et al.* 1998, Garber *et al.* 1994), and Swedish dairy cattle (Silverlås *et al.* 2009), *G. duodenalis* infection in U.S. feedlot steers (Hoar *et al.* 2009), *E. coli* O157:H7 infection in Canadian cattle (Callaway *et al.* 2003, Dargatz *et al.* 1997, LeJeune & Wetzel 2007), *Salmonella* infection in European swine (Fosse *et al.* 2009), and *Campylobacter* colonization in broilers in the United Kingdom (Ellis-Iversen *et al.* 2009). Common recommendations from many of these studies are improved biosecurity of housing facilities, clean water supplies, improved manure management practices, physical separation of non-infected and infected groups, and calving pen hygiene, along with segregation of different age classes of animals (e.g. pre-weaned from post-weaned).

4.7.3 Emerging trends in the control of zoonotic pathogens at the farm level

All diseases are dynamic and waterborne zoonoses are no exception. The last century has seen unprecedented change in livestock farming systems: never

before have such large populations been kept in such small spaces requiring such complex management. While some believe that the apex of intensification has been achieved and the future will see a shift to small-scale organic local production, it may be more realistic to hypothesize that growing populations and demand for animal protein can only lead to further intensification and industrialisation (Steinfeld *et al.* 2006). Technological and methodological advances have undoubted potential to revolutionise control of waterborne zoonoses at farm level. Molecular epidemiology is increasing our understanding of transmission of pathogens and providing unexpected insights which can lead to new control strategies. Genomic studies offer the opportunity to scan for potential vaccine antigens, and engineer new vaccines. Genetic modification, if acceptable to the public, could be used to introduce disease resistance to livestock. Risk-based approaches and disease modelling bring new ways of understanding and managing harm associated with pathogens. The related concepts of "One World, One Health, One Medicine" and "EcoHealth" which view animal, human and ecosystem health as inextricably linked, may likewise herald a radical rethink of the prevention and control of zoonoses (Zinstag *et al.* 2007). Innovation will be undoubtedly needed in the next decades as the effects of climate change are increasingly felt. This is likely to affect pathogen loads at farm level through multiple pathways, as yet poorly understood, but including: pathogen survival in the environment, livestock nutrition, livestock susceptibility, breeds of livestock kept, and farming systems (Thornton *et al.* 2008)

4.8 CONCLUSIONS

The idea of risk pathways and the concept of "source to tap" offer at once a rationale for pushing pathogen control down the value chain and a method for identifying the points where control can be applied. In this Chapter we identify four sequential key control points: minimising exposure of livestock to pathogens; increasing livestock resistance to infection; minimising establishment of pathogens by altering gut ecology and using antimicrobial agents; and eliminating pathogens from livestock manure. However, while there are many interesting strategies for reducing pathogen loads in animals there are far fewer examples of strategies which are targeted, effective, affordable and widely adopted. The greatest success has been seen in industrial poultry production where biosecurity measures, pathogen-free birds, vaccination, and competitive exclusion have been widely applied and have resulted in decreases in the loads of potentially waterborne pathogens, especially *Salmonella*. The ability to highly control the environment and exclude wildlife and alternative hosts undoubtedly contributes to this success. In the case of ruminants, there are no widely used

strategies targeted at specific-pathogen reductions; however, there are many promising research avenues being currently pursued. General biosecurity and good hygienic practices would be expected to reduce pathogen loads; however, there is limited information on the effectiveness of individual or packages of measures against waterborne pathogens and even less on their cost-effectiveness. In developing countries, the pathogen load is probably much higher in animals; at the same time the awareness of the problem and investments in mitigation are much lower. The dichotomy between zoonoses control in the developed and developing world is widely acknowledged to be a disaster waiting to happen and recent years have seen expanding and converging programmes on zoonoses control at national, regional and global levels. This chapter has reviewed the current status of waterborne zoonoses control at the farm level: future scanning would suggest that technology advances, demographic and societal change, and climate change may have major repercussions on farm-based strategies used to control zoonotic pathogens.

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5

Transport of microbial pollution in catchment systems

Christobel Ferguson and David Kay

5.1 POTENTIAL OF MICROBIAL MODELS TO DESCRIBE CATCHMENT COMPLEXITY

Humans, wildlife and domestic livestock are all potential sources of fecal indicator organisms (FIOs) and/or pathogens in surface waters. To assess the microbial risks to surface water quality in drinking-water catchments it is necessary to quantify both the contributions from each of these sources and the processes that govern their fate and transport to water bodies and waterways. Catchment characteristics vary widely over space and time. Such a quantification and attribution therefore requires an understanding of the natural, riparian, farm and urban processes that can occur in drinking-water catchments. An initial approach is to develop a conceptual model that assists in the identification of the relevant natural and anthropogenic processes, as presented in Figure 5.1. These include environmental inactivation, soil retention, host prevalence, buffer strip entrapment, manure treatment, wetland

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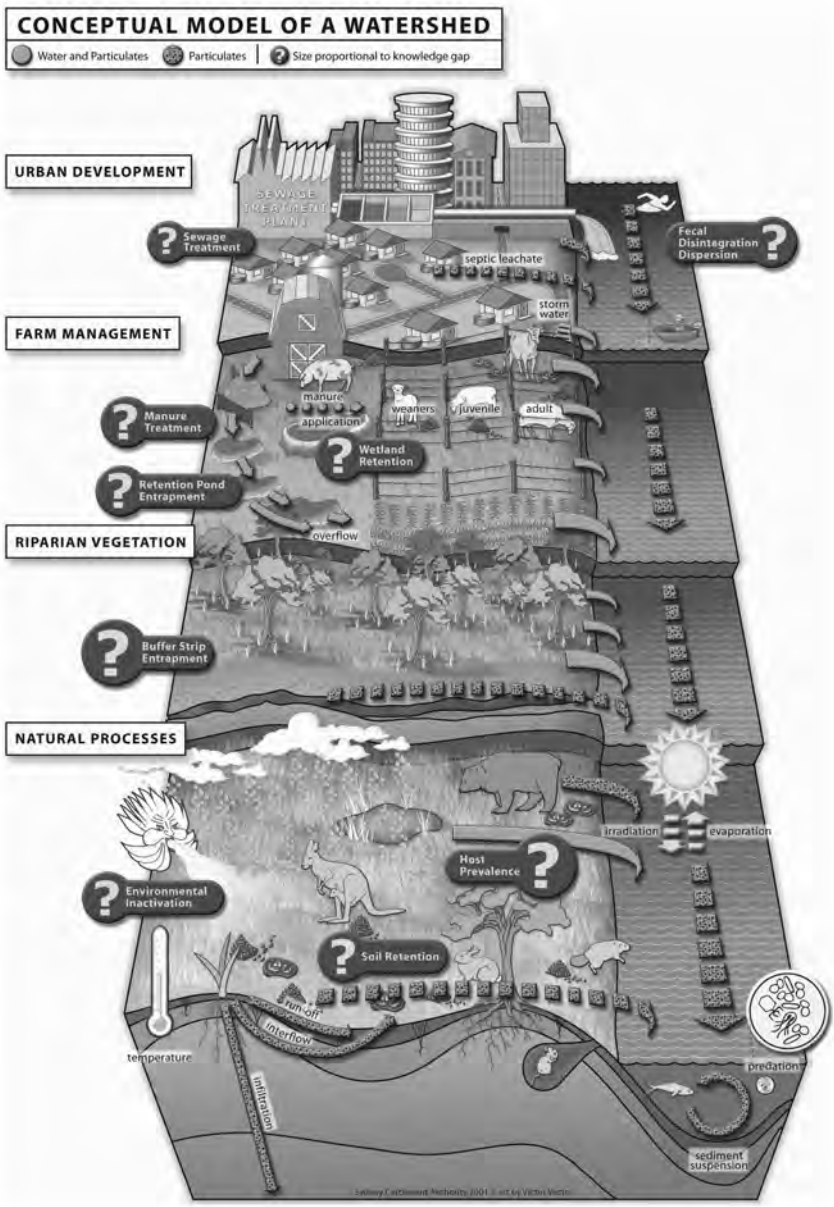


Figure 5.1 Conceptual model of pathogen sources, fate and transport in a drinking-water catchment (*with permission from JAWWA*).

and retention pond entrapment, faecal disintegration and dispersion, and sewage treatment (Ferguson 2003a).

It is, however, a daunting task to quantify all of these processes in detail for every catchment. Hence, an efficient approach is to identify the most significant biophysical processes, quantify them and then use models to apply this knowledge more broadly. Although hydrologic modelling of catchments to describe the movement of water, nutrients and sediment is a well established practice, the application of modelling techniques to quantify and predict the fate and transport of microorganisms (Tian *et al.* 2002, Steets & Holden 2003, Kay *et al.* 2005, Haydon & Deletic 2006), particularly pathogens, is a relatively recent and rapidly evolving field of research (Walker & Stedinger 1999, Medema & Schijven 2001; Dorner 2006; Ferguson 2007). The advantage of using mathematical models is that they have both predictive and hypothesis-testing functions which enable knowledge to be applied generically in simulations to any sufficiently attributed catchment. Modelling of microbial risks to drinking-water catchments will identify hotspots that can be prioritised for mitigation and facilitate scenario testing of mitigation techniques for a variety of climatic conditions.

The majority of catchment modeling platforms can be classified as either simplified or complex hydrological models. Simple models such as IHACRES (Jakeman *et al.* 1990) generally require a small number of parameters, resulting in modest data requirements, and are easy to calibrate and test. While these models give a modest set of outputs, the confidence in the estimated values can be high provided sufficient data are available for testing or, even better, validation. The more complex hydrology-based models generally simulate and predict the volume and movement of contaminants, often incorporating a Geographical Information System (GIS) framework. However, the output of models is only as good as the combination of the calibration input and the validity of the assumptions used. Hence, the collation of quantitative data on microbial sources and processes is essential to elucidate the mechanisms of pathogen transport to surface waters. A number of isolated and problem-driven investigations have been reported with a developed nations' bias and principal focus on FIOs rather than pathogens, and these studies have informed the modelling of microbial fluxes to date.

5.2 EMPIRICAL DATA ON ANIMAL MICROBIAL SOURCES

Although humans, wildlife and domestic livestock are known sources of faecal pollution of drinking-water catchments, relatively few studies have quantified or

analysed the factors contributing to variation in microbial risks associated with these sources. Even when the source matrix is perceived to be the same (for example, cattle faeces) the actual risk will vary dependent on specific pathogen prevalence, shedding concentrations, geographic, animal diet, seasonal and temporal distributions, availability of hosts and treatment and mitigation measures. Quantification of microbial sources (particularly prevalence and concentration) and the extent of their variability is necessary for constructing predictive models of microbial loads within catchments. It is also essential for the cost-effective prioritisation of catchment management actions. The following information summarises an analysis of the existing data on prevalence and concentrations of *Escherichia coli*, *Cryptosporidium* oocysts and *Giardia* cysts as indices of microbial risk arising from wildlife and livestock in drinking-water catchments.

A global review of the literature published up to 2005 was conducted to determine the extent of variation in reported concentrations of prevalence and pathogen shedding intensities in animal faeces. Data from each study were pooled and analysed by animal type and where possible by geographic location for Europe/United Kingdom, Australasia and North America. The majority of studies examined prevalence of pathogens in animal faecal matrices with fewer studies reporting pathogen concentrations excreted by animals. Although domestic livestock such as cattle and sheep have been frequently tested for the prevalence of *Cryptosporidium* ($n = 23$ and $n = 12$, respectively), fewer studies have tested samples from wildlife ($n = 1$ for some species). Also, different studies had widely different numbers of samples analysed. For example, estimates of *Cryptosporidium* oocyst prevalence in adult cattle were derived from between 19 and 8064 individual samples depending on the study, and hence data need to be interpreted with caution.

Ranked by mean prevalence, *Cryptosporidium* oocysts in animal faeces scored highest in juvenile sheep faeces, adult sheep faeces, juvenile cattle and pig faeces, respectively (see Table 5.1). The mean prevalence of *Cryptosporidium* in adult cattle faecal material was less than expected (ranking 11th), below that of kangaroos and goats. The prevalence of *Cryptosporidium* oocysts in animal faeces is presented graphically in Figure 5.2.

Table 5.1 Mean prevalence rates of *Cryptosporidium* oocysts in animal faeces.

Ranking	Animal	Age	n*	Mean prevalence	Median prevalence
1	Sheep	juvenile	5	0.524	0.710
2	Sheep	adult	7	0.346	0.270
3	Cattle	juvenile	23	0.283	0.210

(*Continued*)

Table 5.1 (*Continued*)

Ranking	Animal	Age	n*	Mean prevalence	Median prevalence
4	Pigs	juvenile	5	0.261	0.219
5	Hedgehogs	adult	1	0.250	0.250
6	Pigs	adult	7	0.221	0.054
7	Rodents	adult	9	0.209	0.220
8	Poultry	adult	3	0.200	0.270
9	Kangaroos	adult	2	0.194	0.194
10	Goats	adult	3	0.187	0.190
11	Cattle	adult	22	0.152	0.082
12	Badgers	adult	1	0.150	0.150
13	Geese	adult	2	0.117	0.117
14	Horses	juvenile	1	0.100	0.100
15	Horses	adult	3	0.100	0.089
16	Deer	juvenile	1	0.088	0.088
17	Racoons	adult	2	0.085	0.085
18	Rabbits	adult	2	0.068	0.068
19	Cats	adult	3	0.065	0.083
20	Deer	adult	5	0.060	0.060

*Number of published studies (**not** the number of samples analysed).

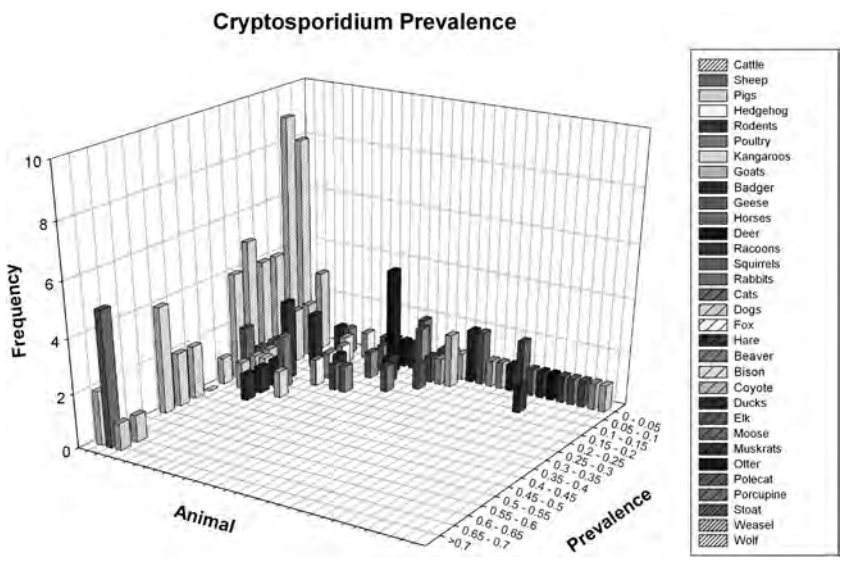


Figure 5.2 Mean prevalence rates of *Cryptosporidium* oocysts by animal species.

The mean concentration of *Cryptosporidium* oocysts in animal faeces is shown in Table 5.2. The highest mean concentration of *Cryptosporidium* oocysts excreted per gram of animal faeces was found for juvenile cattle (mean = 38115, $n = 4$), squirrels (mean = 27000, $n = 2$), rodents (mean = 25250, $n = 4$) and juvenile sheep faeces (mean = 9135, $n = 2$), respectively. The most numerous studies on *Cryptosporidium* oocyst prevalence in animal faeces have been carried out on domestic livestock animals, cattle and sheep. These studies were pooled for adult and juvenile animals to enable comparison of geographical regions (Figures 5.2 and 5.3).

Table 5.2 Concentration of *Cryptosporidium* oocysts in animal faeces.

Ranking	Animal	Age	n*	Mean concentration/g	Median concentration/g
1	Cattle	juvenile	4	38115	22500
2	Squirrels	adult	2	27000	27000
3	Rodents	adult	4	25250	27000
4	Sheep	juvenile	2	9135	9135
5	Cattle	adult	12	3830	46.25
6	Hedgehogs	adult	1	3000	3000
7	Badgers	adult	1	3000	3000
8	Bison	adult	1	2369	2369
9	Poultry	adult	1	2100	2100
10	Rabbits	adult	2	2100	2100
11	Deer	adult	3	2004	3000
12	Elk	adult	2	1871	1871
13	Fox	adult	2	1500	1500
14	Horses	adult	2	1033.5	1033.5
15	Sheep	adult	5	780.4	88
16	Beavers	adult	1	509	509
17	Pigs	juvenile	1	472	472
18	Kangaroos	adult	1	204	204
19	Pigs	adult	3	24.1	14.3
20	Geese	adult	1	0	0

*Number of published studies (**not** the number of samples analysed).

The majority of studies of *Cryptosporidium* oocyst prevalence rates in cattle have been carried out in either Europe or North America with fewer studies in Australasia (Figure 5.3). Sporadic high rates of prevalence (>70%) were

reported in both Europe and Australasia; however, the highest prevalence reported in North America was only in the range of 50–55%. Most studies of cattle faeces in North America report prevalence rates in the range of 5–10% ($n = 7$) while studies in Europe and Australasia show a broader distribution of prevalence (Figure 5.3). Figure 5.4 shows that it is common for prevalence of *Cryptosporidium* oocysts in sheep faeces to range between 5–30% in European and North American studies. However all three regions had at least one study that reported prevalence of >70% in sheep faeces. The higher rates of prevalence of *Cryptosporidium* in cattle faeces were predominantly associated with juvenile compared to adult animals (Figure 5.5). The majority of studies showed that the mean prevalence rate in adult cattle was frequently in the range of 0–10%.

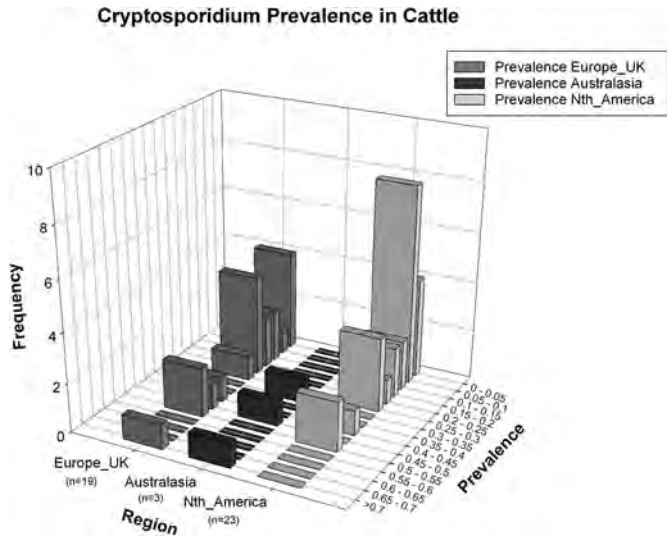


Figure 5.3 Mean prevalence rates of *Cryptosporidium* oocysts in cattle faeces by region.

The mean prevalence of *Giardia* cysts in animal faeces was highest in cat, muskrat, juvenile sheep and juvenile cattle faeces, respectively (see Table 5.3). The mean prevalence of *Giardia* cysts in animal faeces is also shown graphically in Figure 5.6. Although there were fewer studies investigating *Giardia* compared to *Cryptosporidium* prevalence, it is interesting to note the low prevalence of *Giardia* cysts in both adult and juvenile animals (Table 5.3, Figures 5.6 and 5.7).

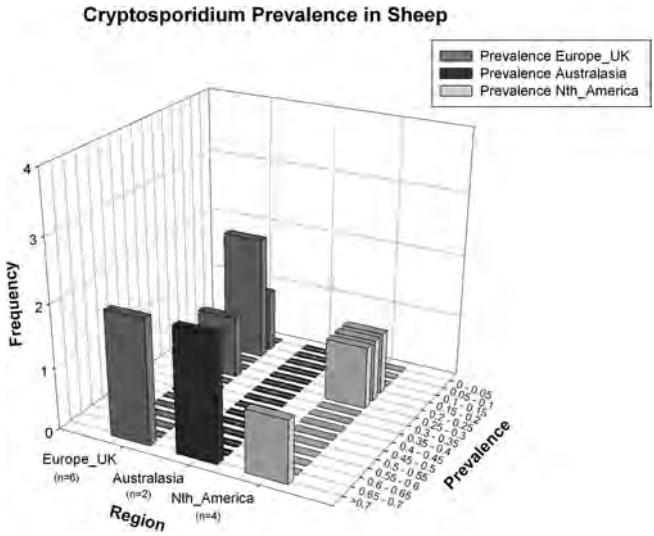


Figure 5.4 Mean prevalence rates of *Cryptosporidium* oocysts in sheep faeces by region.

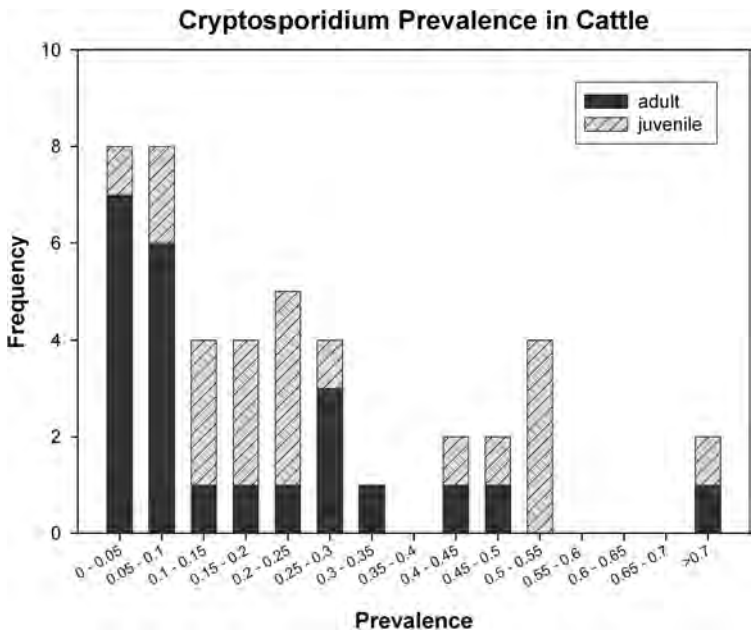


Figure 5.5 Comparison of adult and juvenile mean prevalence of *Cryptosporidium* oocysts in cattle faeces.

Table 5.3 Mean prevalence of *Giardia* cysts in animal faeces.

Ranking	Animal	Age	n*	Mean prevalence	Median prevalence
1	Cats	adult	1	0.8	0.8
2	Muskrat	adult	2	0.632	0.6315
3	Sheep	juvenile	1	0.57	0.57
4	Cattle	juvenile	3	0.467	0.31
5	Beavers	adult	2	0.269	0.269
6	Ducks	adult	1	0.24	0.24
7	Sheep	adult	2	0.149	0.149
8	Bison	adult	1	0.146	0.146
9	Cattle	adult	8	0.132	0.105
10	Horses	adult	2	0.125	0.125
11	Dogs	adult	2	0.102	0.102
12	Pigs	adult	6	0.09	0.078
13	Elk	adult	2	0.079	0.079
14	Coyotes	adult	1	0.05	0.05
15	Pigs	juvenile	3	0.041	0.033
16	Deer	juvenile	1	0.029	0.029
17	Deer	adult	2	0.006	0.006
18	Moose	adult	1	0.006	0.006
19	Fox	adult	1	0	0
20	Geese	adult	1	0	0

*Number of published studies (**not** the number of samples analysed).

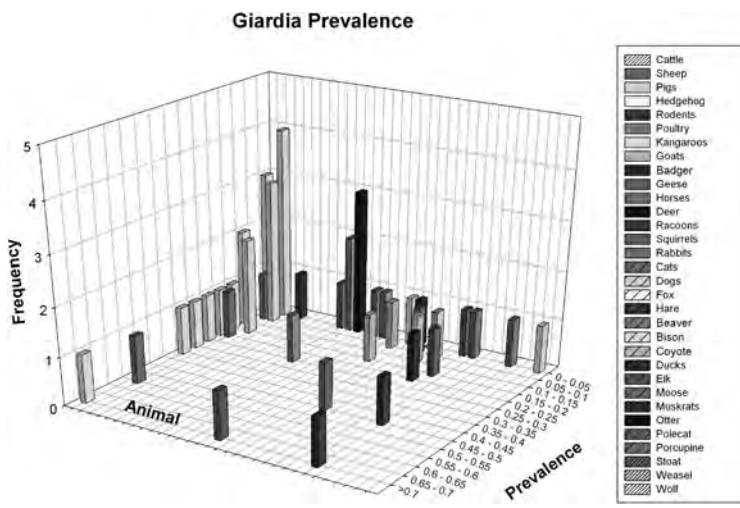


Figure 5.6 Mean prevalence of *Giardia* cysts by animal species.

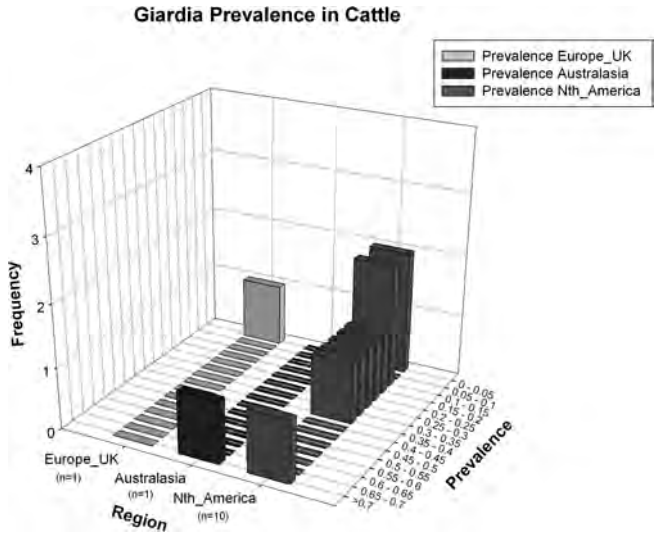


Figure 5.7 Mean prevalence of *Giardia* cysts in cattle faeces by region.

The mean concentration of *Giardia* cysts in animal faeces is shown in Table 5.4. The highest mean concentration of *Giardia* cysts excreted per gram of animal faeces was for muskrats (mean = 9574, $n = 1$), bison (mean = 2649, $n = 1$), beavers (mean = 1654, $n = 1$) and coyotes (mean = 1577, $n = 1$), respectively.

The most numerous studies on *Giardia* cyst prevalence in animal faeces have been carried out on domestic livestock animals: cattle and pigs. The studies on cattle faeces were pooled for adult and juvenile animals to facilitate comparison of geographical regions (Figure 5.6). Unfortunately the majority of studies were carried out in North America ($n = 10$) where *Giardia* prevalence was frequently in the range of 0% to 40%, with only one study reporting prevalence greater than 70%. Two studies were performed outside North America: one in Europe recorded a prevalence rate of 3.6% in adult cattle and one in Australasia recorded a prevalence rate of 89% in juvenile cattle. The paucity of data makes comparison of regions difficult for prevalence rates of *Giardia* even in domestic livestock animals.

The lack of quantitative data on pathogen prevalence and concentrations in animal faeces makes it difficult to gauge the relative risks of various animal species that can inhabit drinking-water catchments. Farm management practices can reduce the impact of animal faecal source loadings if they are directed at factors that control the level of microbial risk. The lack of quantitative data

severely limits the effectiveness of control measures and prevents prioritisation of source water protection activities in catchments. Without such data it is difficult to determine which factors are most important in mitigating catchment risks since control of animal population density, volume and location of excreta, and animal behaviour can only be effective if measures target the right animals.

Table 5.4 Concentration of *Giardia* cysts in animal faeces.

Ranking	Animal	Age	n*	Mean concentration/g	Median concentration/g
1	Muskrat	adult	1	9574	9574
2	Bison	adult	1	2649	2649
3	Beavers	adult	1	1654	1654
4	Coyotes	adult	1	1577	1577
5	Cattle	juvenile	2	1519	1519
6	Deer	adult	1	1168	1168
7	Cattle	adult	6	1013	34.2
8	Elk	adult	2	832.5	832.5
9	Ducks	adult	1	444	444
10	Moose	adult	1	168	168
11	Pigs	adult	2	42	42
12	Sheep	adult	1	20	20
13	Fox	adult	1	0	0
14	Geese	adult	1	0	0
15	Hare	adult	1	0	0
16	Horses	adult	1	0	0
17	Porcupine	adult	1	0	0
18	Rabbits	adult	1	0	0
19	Rodents	adult	1	0	0
20	Squirrels	adult	1	0	0

*Number of published studies (**not** the number of samples analysed).

The number and size of domestic livestock studies performed were probably sufficient for use in initial modelling efforts. However, the *Cryptosporidium* prevalence data suggested there was some geographic variability that needs to be considered when estimating risk at a specific location. Although the risks from wildlife species are thought to be lower than from domestic livestock, there were few studies and sample numbers were often low. Hence, relying on published data sets alone to estimate risk would not be recommended. It is recommended that to estimate the risk posed by wildlife at a specific location, local studies of pathogen prevalence and concentrations are performed to

provide sufficient information for a reliable assessment of the level of risk posed by these animal sources.

5.3 EMPIRICAL DATA ON HUMAN MICROBIAL SOURCES

A recent paper on FIO concentrations in sewage effluents provides empirical data on the contribution of human wastewater as a source of faecal contamination in catchments. Kay *et al.* (2008a) note the lack of published empirical data on FIO concentrations in sewage-related discharges to natural waters and the significant information gap for the research and management communities. They reported FIO data (presumptive total coliforms (TC), faecal coliforms (FC) and enterococci (EN)) for 1933 samples taken from a range of different types of sewage-related effluent in the United Kingdom and on the island of Jersey at the locations specified in Figure 5.8. These data cover discharges/effluents associated with the following "levels" of treatment:

- untreated sewage – including various storm sewage overflows as well as crude sewage discharges;
- "primary" treatment – that is "physical" treatment such as settlement of solids as might be achieved in a primary settlement tank at a municipal wastewater treatment plant or a domestic septic tank;
- "secondary" treatment – that is involving biological processes such as would be produced in an activated sludge plant or trickling filter system; and,
- "tertiary" treatment – that is final "cleaning" of effluent from secondary treatment, in some cases designed specifically to remove FIOs, notably UV disinfection, but often to reduce levels of nutrients (e.g. in reedbed/grass plot systems) in addition to FIOs.

Given the worldwide utilisation of the treatment technologies investigated, these data provide indicative geometric means (GMs) and ranges for FIO concentrations likely to be encountered in other comparable geographical regions. It should be noted, however, that sewerage systems in the United Kingdom predominantly take both sewage and runoff from built-up areas (i.e. "combined" systems), which leads to high variations in flows. Thus, the reported data may be less applicable to systems where foul sewage and surface drainage are collected separately. Some specific technologies are not covered, notably chemical dosing and combined chemical/UV systems, both of which are not commonly employed in the United Kingdom; and lagoons/constructed wetlands, which were not encountered in the areas studied.



Figure 5.8 Location of sewage and stream sampling to generate the data on FIO transport at the catchment scale in the United Kingdom.

Interestingly, this study reported flow effects on FIO concentrations in sewage effluents and the results are split into dry weather conditions and response to rainfall events. Table 5.5 provides details on the sample numbers for each type of treatment and Table 5.6 provides results of the FIO concentrations in the different treatment systems by flow condition.

Table 5.5 Types of sewage treatment/effluent reported in Kay *et al.* (2008).

Level of treatment [a]	Specific effluent types[a]
Untreated sewage (69)	Crude sewage discharges (16)
	Storm sewage overflows[b] (53)
Primary treatment (12)	Primary settled sewage effluent (7)
	Stored settled sewage effluent (2)
	Settled septic tank effluent (3)
Secondary treatment (67)	Trickling filter effluent (38)
	Activated sludge effluent[c] (17)
	Oxidation ditch effluent (3)
	Trickling/sand filter effluent (1)
	Rotating biological contactor effluent (8)
Tertiary treatment (14)	Reedbed/grass plot effluent (6)
	Ultraviolet-disinfected effluent (8)

[a] Figures in brackets indicate number of different treatment plants sampled (numbers of valid enumerations (n) are shown in Table 5.6).
[b] Specific effluent types comprise treatment plant inlet overflows, stormwater retention tank overflows and combined sewer overflows (CSOs); high-flow data only.
[c] Activated sludge effluent includes deep-shaft activated sludge effluent at one site.

5.4 MICROBIAL TRANSPORT

Both Clean Water Act TMDL investigations and EU Water Framework Directive (WFD) Programmes of Measures require information at the drainage basin scale. There has been work at this scale on the impacts of land use on stream FIO concentration and transport.

Bales *et al.* (1995) examined phage (a viral tracer) and FIO transport in an aquifer at Cape Cod, USA and reported phage attenuation higher than that for the bacteria. The phages were easily remobilised, however, by enhanced flow which suggests continued viability whilst adsorbed to the sandy aquifer matrix. Sinton *et al.* (2005) reported vertical microbial tracer transport from areas of slurry spreading to a 16.8 m deep aquifer in New Zealand. The breakthrough curves suggested travel speeds of between 15.7 and 39.2 $m.hr^{-1}$. The tracers

Table 5.6 Summary of faecal indicator organism concentrations (cfu 100 mL^{-1}) for different treatment levels and individual types of sewage-related effluents under different flow conditions: geometric means (GMs), 95% confidence intervals (CIs)[a]; and results of *t*-tests comparing base- and high-flow GMs for each group and type [b]; and (in footnote) results of *t*-tests comparing GMs for the two untreated discharge types and the two tertiary-treated effluent types (the results of other comparisons for the groups and other effluent types are present in Tables 5.7 and 5.8).

Indicator organism Treatment levels and specific types	Base-flow conditions				High-flow conditions			
	n[c]	Geometric mean	Lower 95% CI	Upper 95% CI	n[c]	Geometric mean	Lower 95% CI	Upper 95% CI
TOTAL COLIFORMS								
Untreated	**253**	**3.9×10^7 *(+)**	**3.2×10^7**	**4.6×10^7**	**279**	**8.2×10^6 *(–)**	**7.0×10^6**	**9.6×10^6**
Crude sewage discharges[d]	253	3.9×10^7 *(+)	3.2×10^7	4.6×10^7	79	1.2×10^7 *(–)	8.2×10^6	1.6×10^7
Storm sewage overflows[d]					200	7.2×10^6	5.9×10^6	8.4×10^6
Primary	**130**	**3.0×10^7 *(+)**	**2.3×10^7**	**3.9×10^7**	**14**	**1.2×10^7 *(–)**	**4.0×10^6**	**3.7×10^7**
Primary settled sewage	61	3.8×10^7	3.0×10^7	4.7×10^7	8	2.2×10^7	–	–
Stored settled sewage	26	2.4×10^7	1.2×10^7	5.1×10^7	1	1.1×10^6	–	–
Settled septic tank	43	2.5×10^7	1.3×10^7	4.2×10^7	5	7.5×10^6	–	–
Secondary	**853**	**1.1×10^6**	**9.5×10^5**	**1.2×10^6**	**183**	**1.3×10^6**	**1.0×10^6**	**1.7×10^6**
Trickling filter	472	1.4×10^6	1.2×10^6	1.7×10^6	76	1.4×10^6	1.0×10^6	1.9×10^6
Activated sludge	256	7.8×10^5 *(–)	6.2×10^5	1.0×10^6	92	1.4×10^6 *(+)	8.6×10^5	2.1×10^6
Oxidation ditch	35	8.1×10^5	4.6×10^5	1.4×10^6	5	3.1×10^6	–	–
Trickling/sand filter	10	6.4×10^5	2.8×10^5	1.4×10^6	8	2.7×10^5	–	–
Rotating biological contactor	80	6.8×10^5	4.6×10^5	1.0×10^6	2	4.0×10^6	–	–
Tertiary	**182**	**5.5×10^3**	**3.4×10^3**	**9.0×10^3**	**8**	**3.8×10^3**	**–**	**–**
Reedbed/grass plot[e]	73	3.7×10^4	1.5×10^4	8.1×10^4	2	2.3×10^4	–	–
Ultraviolet disinfection[e]	109	1.5×10^3	9.9×10^2	2.6×10^3	6	2.1×10^3	–	–

(*Continued*)

Table 5.6 (*Continued*)

Indicator organism Treatment levels and specific types	Base-flow conditions				High-flow conditions			
	n[c]	Geometric mean	Lower 95% CI	Upper 95% CI	n[c]	Geometric mean	Lower 95% CI	Upper 95% CI
FAECAL COLIFORMS								
Untreated	**252**	**1.7×10^7 *(+)**	**1.4×10^7**	**2.0×10^7**	**282**	**2.8×10^6 *(–)**	**2.3×10^6**	**3.2×10^6**
Crude sewage discharges[d]	252	1.7×10^7 *(+)	1.4×10^7	2.0×10^7	79	3.5×10^6 *(–)	2.6×10^6	4.7×10^6
Storm sewage overflows[d]					203	2.5×10^6	2.0×10^6	2.9×10^6
Primary	**127**	**1.0×10^7 *(+)**	**8.4×10^6**	**1.3×10^7**	**14**	**4.6×10^6 *(–)**	**2.1×10^6**	**1.0×10^7**
Primary settled sewage	60	1.8×10^7	1.4×10^7	2.1×10^7	8	5.7×10^6	–	–
Stored settled sewage	25	5.6×10^6	3.2×10^6	9.7×10^6	1	8.0×10^5	–	–
Settled septic tank	42	7.2×10^6	4.4×10^6	1.1×10^7	5	4.8×10^6	–	–
Secondary	**864**	**3.3×10^5 *(–)**	**2.9×10^5**	**$3.7 \times x10^5$**	**184**	**5.0×10^5 *(+)**	**3.7×10^5**	**6.8×10^5**
Trickling filter	477	4.3×10^5	3.6×10^5	5.0×10^5	76	5.5×10^5	3.8×10^5	8.0×10^5
Activated sludge	261	2.8×10^5 *(–)	2.2×10^5	3.5×10^5	93	5.1×10^5 *(+)	3.1×10^5	8.5×10^5
Oxidation ditch	35	2.0×10^5	1.1×10^5	3.7×10^5	5	5.6×10^5	–	–
Trickling/sand filter	11	2.1×10^5	9.0×10^4	6.0×10^5	8	1.3×10^5	–	–
Rotating biological contactor	80	1.6×10^5	1.1×10^5	2.3×10^5	2	6.7×10^5	–	–
Tertiary	**179**	**1.3×10^3**	**7.5×10^2**	**2.2×10^3**	**8**	**9.1×10^2**	**–**	**–**
Reedbed/grass plot[e]	71	1.3×10^4	5.4×10^3	3.4×10^4	2	1.5×10^4	–	–
Ultraviolet disinfection[e]	108	2.8×10^2	1.7×10^2	4.4×10^2	6	3.6×10^2	–	–
ENTEROCOCCI								
Untreated	**254**	**1.9×10^6 *(+)**	**1.6×10^6**	**2.3×10^6**	**280**	**4.9×10^5 *(–)**	**4.2×10^5**	**5.6×10^5**
Crude sewage discharges[d]	254	1.9×10^6 *(+)	1.6×10^6	2.3×10^6	79	8.9×10^5 *(–)	6.7×10^5	1.2×10^6
Storm sewage overflows[d]					201	3.8×10^5	3.2×10^5	4.5×10^5
Primary	**128**	**1.3×10^6**	**1.1×10^6**	**1.7×10^6**	**14**	**9.8×10^5**	**4.4×10^5**	**2.2×10^6**

Primary settled sewage	61	2.4×10^6	2.1×10^6	2.7×10^6	8	1.9×10^6	–	–
Stored settled sewage	26	6.2×10^5	3.2×10^5	1.1×10^6	1	2.9×10^5	–	–
Settled septic tank	41	9.3×10^5	5.3×10^5	1.6×10^6	5	4.3×10^5	–	–
Secondary	**871**	**2.8×10^4 *(–)**	**2.5×10^4**	**3.2×10^4**	**182**	**4.7×10^4 *(+)**	**3.6×10^4**	**6.1×10^4**
Trickling filter	483	4.1×10^4	3.5×10^4	4.7×10^4	76	5.7×10^4	4.2×10^4	8.3×10^4
Activated sludge	262	2.1×10^4 *(–)	1.8×10^4	2.7×10^4	91	4.1×10^4 *(+)	2.7×10^4	6.0×10^4
Oxidation ditch	35	2.0×10^4	1.0×10^4	4.0×10^4	5	1.2×10^5	–	–
Trickling/sand filter	11	2.1×10^4	1.0×10^4	5.3×10^4	8	1.1×10^4	–	–
Rotating biological contactor	80	9.6×10^3	6.7×10^3	1.4×10^4	2	3.7×10^5	–	–
Tertiary	**177**	**3.0×10^2**	**1.8×10^2**	**5.0×10^2**	**8**	**2.1×10^2**	**–**	**–**
Reedbed/grass plot[e]	73	1.9×10^3	7.1×10^2	4.3×10^3	2	2.3×10^3	–	–
Ultraviolet disinfection[e]	104	8.3×10^1	4.6×10^1	1.1×10^2	6	9.7×10^1	–	–

[a] CIs only reported where $n \geq 10$

[b] t-tests comparing low- and high-flow GM concentrations only undertaken where $n \geq 10$ for both sets of samples; only statistically significant ($p < 0.05$) differences between base- and high-flow GM concentrations are reported: indicated by *, with the higher GM being identified as *(+) and the lower value by *(–)

[c] n indicates number of valid enumerations, which in some cases may be less than the actual number of samples.

[d] t-tests comparing the GM concentrations between the two untreated discharge types show high-flow GM concentrations to be significantly higher in crude sewage discharges than storm sewage overflows for TC ($p < 0.05$) and EN ($p < 0.001$).

[e] t-tests comparing the GM concentrations between the two tertiary-treatment effluent types show GM TC, FC and EN concentrations to be significantly higher ($p < 0.001$) in reedbed/grass plot effluents than effluents from UV disinfection for base-flow conditions (there are too few high-flow samples for these tertiary effluents for meaningful comparisons to be made for high-flow GM concentrations).

Table 5.7 Reduction in faecal indicator organism (FIO) concentrations in specific primary, secondary and tertiary treatment effluents compared with the geometric mean concentrations for the level of treatment which provides the input to these treatment plants (i.e. untreated sewage and primary- and secondary-treated effluent, respectively), based on the geometric mean concentrations reported in Table 5.6[a].

Treatment type	FIO[b]	Reduction (cfu 100 mL^{-1}):		Reduction (%):	
		Base flow	High flow	Base flow	High flow
Primary effluents compared with untreated sewage[c]					
Primary settled sewage	TC	8.5×10^5		2.21	
	FC	0		0	
	EN	0		0	
Stored settled sewage	TC	1.5×10^7		38.56	
	FC	1.1×10^7		66.13	
	EN	1.3×10^6		67.66	
Settled septic tank	TC	1.4×10^7		35.55	
	FC	9.4×10^6		56.68	
	EN	9.9×10^5		51.69	
Secondary effluents compared with primary-treated effluent					
Trickling filter	TC	2.9×10^7	1.1×10^7	95.22	88.47
	FC	1.0×10^7	4.1×10^6	95.88	88.08
	EN	1.3×10^6	9.3×10^5	96.98	94.17
Activated sludge	TC	2.9×10^7	1.1×10^7	97.39	88.69
	FC	1.0×10^7	4.1×10^6	97.34	88.95
	EN	1.3×10^6	9.4×10^5	98.43	95.88
Oxidation ditch	TC	2.9×10^7	–	97.30	–
	FC	1.0×10^7	–	98.03	–
	EN	1.3×10^6	–	98.51	–
Trickling/sand filter	TC	2.9×10^7	–	97.88	–
	FC	1.0×10^7	–	97.97	–
	EN	1.3×10^6	–	98.41	–
Rotating biological contactor	TC	2.9×10^7	–	97.73	–
	FC	1.0×10^7	–	98.46	–
	EN	1.3×10^6	–	99.29	–
Tertiary effluents compared with secondary-treated effluent					
Reedbed/ grass plot	TC	1.0×10^6	–	96.59	–
	FC	3.2×10^5	–	95.94	–
	EN	2.6×10^4	–	93.24	–
UV disinfection	TC	1.1×10^6	–	99.86	–
	FC	3.3×10^5	–	99.92	–
	EN	2.8×10^4	–	99.71	–

[a] Reductions only calculated where $n \geq 10$ (– indicates $n < 10$).

[b] FIOs: TC = total coliforms, FC = faecal coliforms, EN = enterococci.

[c] No meaningful figures can be calculated for high-flow conditions (see text).

were attenuated rapidly which was attributed to early exclusion from macropore flow but significant groundwater contamination was considered possible from animal waste applied to land.

Byappanahalli *et al.* (2003) suggested that significant environmental reservoirs of faecal coliform organisms in stream bed sediments in the mid-west of the USA existed, indicating protracted survival and potential re-growth outside the alimentary canal within freshwater stream environments. Jamieson *et al.* (2003) noted the probable existence of a stream bed store of FIOs in a 1000 ha watershed in Ontario and noted a general pattern of of Canadian recreational water quality criteria being exceeded in catchment streams draining the livestock farming and residential area studied.

McDonald *et al.* (1982), Wilkinson *et al.* (1995, 2006), Wilkinson (1995), Wilkinson (2006) and Muirhead *et al.* (2004) have used artificial releases of water to assess the in-channel sedimentary contribution to stream water concentrations. Bai and Lung (2005) adopted a similar approach but then applied a model based on Environmental Fluid Dynamics Code (EFDC) to model sedimentary and FIO transport. They suggest the approach facilitates quantification of the channel sedimentary store contribution and the catchment derived inputs at specific points, although the published model validation data appear much better for the sediment than for the FIO parameters measured. Collins and Rutherford (2004) developed a catchment simulation model to predict coliform concentration in New Zealand streams draining livestock grazing areas. The model used daily livestock data and simulated surface and sub-surface FIO fluxes as well as direct deposition at locations where livestock could access the stream channel. They note the uncertainty regarding a number of these processes and the model sensitivity to the distance between the location of faecal inputs and the catchment. A scenario analysis suggested that riparian buffer areas could be effective both through livestock exclusion and microbial attenuation.

Land use impacts on catchment-scale FIO budgets have been reported by Fraser *et al.* (1998) who used a GIS-based sediment delivery model (SEDMOD) calibrated for 12 sub-watersheds of the Hudson River, New York. The sediment delivery model together with a GIS layer describing livestock density explained 50% of the variance in "average" faecal coliform output. Crowther *et al.* (2002, 2003) reported a multiple regression approach relating percentage land use in a series of sub-catchments with both high- and low flow geometric mean faecal indicator concentrations observed at sub-catchment outlets during the summer bathing season. The models produced higher explained variance (commonly 60–70%) when predicting high-flow concentration which is the key period of maximum delivery (Wilkinson *et al.* 1995, 2006, Wilkinson 2001). Storm flow water quality, discharged to coastal waters was examined by Lewis *et al.* (2005)

in a study of on-farm remedial measures (BMPs) implemented in California. Similarly, Haydon and Deletic (2006) modelled land use impacts on surface and sub-surface FIO flux using wash-off and loss equations linked to a hydrological model (SimHyd). The model required water quality data for each catchment to calculate the FIO coefficients to predict microbial flux for a range of rural, peri-urban and urban catchments within Australia.

Tong and Chen (2002) report an application of the USEPA BASINS model calibrated against 11 water quality parameters including faecal coliforms in Ohio. FIO monitoring data will commonly have runs of low flow and low concentration interspersed with occasional high values caused by agricultural diffuse pollution and intermittent discharges from sewers. The authors report (Page 379) that: "*In the data set for [… … …] and faecal coliform, there are a few outliers (in the form of extremely high values). These outliers as well as missing data and "zero" values were deleted from the data set*". Information on the proportion of data deleted is not given, but modelling the episodic nature of FIO flux would need to address the high values which are the periods of peak contamination and health risk.

In one of the rare longitudinal (before and after) catchment-scale studies designed to quantify the effect of a BMP (in this case cattle exclusion from catchment streams) on FIO flux from a 56.7 ha drainage basin, Line (2003) reported data derived from a 7.5 years sampling period which suggested 65.9% and 57.0% reductions in faecal coliform and enterococci export, respectively. It is also reported that the provision of an alternate water supply without fencing was not effective in producing FIO reduction (see also Shreeram & Mostaghimi 2002). In a two year longitudinal investigation in the United Kingdom of FIO export through a period of de-stocking due to an outbreak of foot-and-mouth disease Chalmers *et al.* (2005) and Sanders *et al.* (2005) report a surprisingly slow improvement in water quality following the most drastic BMP of >95% stock removal from the 254.6 ha Caldew catchment in Cumbria. A longitudinal study at Brighouse Bay in Scotland examined the effects of BMPs on water quality in catchment streams and at an adjacent bathing water beach. The principal BMP was stream bank fencing to create a RBS with associated provision of drinking troughs. Containment of dirty water from farms was also implemented. The stream water quality data suggested extreme seasonality with the summer period having markedly higher FIO concentrations in catchment streams. However, comparison with an unmodified adjacent control catchment suggested a 66% reduction in *E. coli* summer high-flow export coefficient (in $\text{cfu} \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$) with a parallel 81% reduction in intestinal enterococci export. Detailed monitoring through a rainfall event in the post-remediation period suggested that even this improvement would be insufficient to guarantee bathing

water compliance with Directive 160/76/EEC (Kay *et al.* 2007b, Dickson *et al.* 2005). The separate effects of RBS and steading dirty water control have been addressed in a longitudinal study of 60 monitored catchments in Scotland by Kay *et al.* (2005d). Here, significant improvements were recorded in FIO flux when compared to "control" catchments but a relatively high intensity of "measures" was required (i.e. >30% of stream bank length protected by RBSs).

The regional (i.e. multi-catchment) scale sources of agricultural diffuse pollution on Ayrshire bathing waters has been assessed by Aitken *et al.* (2001). Following from this work, The impacts of faecal indicator fluxes from this catchment was examined using three modelling strategies, the first a soil transport model, the second a regression model and the third a more distributed catchment model (PAMIMO). The regression model gave the best prediction of bathing water quality and the authors concluded that preventing surface runoff would prove most protective of bathing water quality (Vinten *et al.* 2004b).

Bacterial source tracking has been employed by Hyer and Moyer (2004) to inform TMDL studies in the USA. Pond *et al.* (2004), Domingo *et al.* (2007), Wuertz and Field (2007) and USEPA (2005) provide excellent overviews of the potential for the source tracking methods currently available to contribute to FIO flux source apportionment. These methods use either species and or sub-species of organisms thought to be associated with faecal matter from humans or defined animal groups, or chemical markers indicative of human sewage. There is currently no single and definitive approach with which to identify exact proportions of human and animal derived FIOs, but this area is developing rapidly and may provide operationally useful data in the medium term (Stapleton *et al.* 2009).

A consortium commissioned by the regulators and government in Scotland to produce a screening tool as part of the preparation for WFD implementation reviewed a series of FIO modelling approaches (Anon. 2006). The screening tool is loosely based on research carried out at the Scottish Agricultural College (SAC) (Vinten *et al.* 2002, Ogden *et al.* 2001, McGechan & Vinten 2003, McGechan & Vinten 2003, Vinten *et al.* 2004a). It provides what is termed a "smart dynamic export coefficient" approach. It is driven by land use data at a one km^2 resolution and provides export estimates for each one km^2 grid based on:

(i) steading and other farm losses;
(ii) soil applied organic wastes;
(iii) livestock numbers or soil burden;
(iv) channel contribution related to ditch and small stream density in each cell;
(v) a flow-dependent mobility factor incorporating sub-surface and overland components; and

(vi) the probability density function of overland- and through-flow in each one km^2 cell.

The Screening Tool authors note the lack of empirical ground-truth data on riverine faecal indicator concentrations which would be needed to assess the predictive accuracy of this approach. However, they produce annual FIO (i.e. *E. coli*) runoff loadings for each one km^2 grid cell covering the whole of Scotland and Northern Ireland, suggesting annual ranges in FIO export of between $<1 \times 10^{13}$ cfu $km^{-2}.pa^{-1}$ to $>1 \times 10^{14}$ cfu $km^{-2}.pa^{-1}$ (see Anon. 2006: pages 128 and 129).

The only environmental FIO ground-truth data available to the Screening Tool authors were the bathing beach compliance data collected as required by Directive 76/160/EEC, the EU Bathing Water Directive which required FIO measurement at over 500 coastal bathing waters around the coast of the United Kingdom (Anon. 1976). They sought to test the modelling approach by parametric correlation analysis between the number of samples (i.e. of the 20 samples collected each bathing season at each compliance point in the United Kingdom) achieving the Directive 76/160/EEC Guide value for faecal coliform (i.e. 100 cfu $\cdot$ 100 mL^{-1}) and the annual mean (i.e. arithmetic mean) value of the *E. coli* export from all one km^2 grids which fell within the hydrological contributing catchment thought to affect specific coastal bathing water compliance locations. The FIO export models for 60 correlating pairs gave poorly explained variance of the number of compliant samples with r^2 values ranging between 0.01 and 0.33 (see pages 176 to 180 of Anon. 2006). This is, perhaps, unsurprising given: (i) the unpredictable and inherently dissimilar, near-shore dilution and transport effects which would be found at different coastal locations linking riverine inputs to the bathing water compliance point where water quality is measured; (ii) the seasonal mismatch between the "summer" compliance data (dependent) and the "annual" loading (predictor) variables which were used in the correlation analysis; (iii) the probable right skew in the predictor variable and (iv) the effect of using the mean loading for each one km^2 grid to characterise catchment derived flux to the bathing water (i.e. at the catchment outlet which may have a greater proportion of high FIO-export land use than, for example, afforested headwater areas).

Efforts at the EU scale are evident to develop integrated modelling strategies able to address the needs of WFD implementation (Moore & Tindall 2005). However, operationally useful, that is fully white box, deterministic and process-based faecal indicator models able to predict the effects of individual remedial "programmes of measures" (POMs) or "best management practices" (BMPs) on catchment scale FIO fluxes do not exist at the present time.

Predictive black-box modelling of faecal indicator flux, using satellite and GIS-derived data in the 1500 km^2 Ribble catchment in Lancashire, UK, has been

reported in Kay *et al.* (2005d), and Crowther *et al.* (2002, 2001, 2003) have reported similar investigations on other smaller catchments in the United Kingdom draining to recreational waters. The objective of this approach has been to predict high-flow geometric mean FIO concentrations at sub-catchment outlets. This information has been used by the British regulators to target remediation efforts to "hot spot" sub-catchments which exhibit high positive residuals identified through model comparison with empirical data acquisition. These catchment scale studies followed early process-based modelling studies (Jenkins 1984, Jenkins *et al.* 1983, Kay & McDonald 1980). Neural network black-box models have been applied to identify non-point pollution sources by Brion and Lingireddy (1999) and Collins (2004) reported that a simple statistical model using flow and solar irradiance was able to explain 87% of the variance in *E. coli* during five rainfall events monitored in a pastoral wetland in New Zealand.

In an excellent review paper Jamieson *et al.* (2004a) state:

> *Liquid and solid wastes generated from both animal and domestic sources can significantly impair drinking, irrigation and recreational water sources in rural areas. The assessment and management of non-point sources of microbial pollution, in particular, is an issue of great interest. A representative watershed scale water quality model would be an invaluable tool in addressing microbial pollution issues. [… … … ….] A complete watershed scale microbial water quality model includes subroutines which (i) characterize the production and distribution of waste and associated microorganisms, (ii) simulate the transport of microorganisms from the land surface to receiving streams, and (iii) route microorganisms through stream networks. Current watershed scale models only account for microbial transport to surface waters through overland flow and ignore subsurface transport. The movement of microorganisms on the soil surface is predicted using simple empirical equations or by assuming that microorganism transport is only associated with sediment erosion. However, several studies have indicated that the assumption that microorganism transport is directly linked with sediment transport may not be valid. The simulation of microorganism survival and transport in receiving streams is complicated by sediment/microorganism interactions. More research is needed to be able to quantitatively assess and model microbial processes in alluvial streams.*

The same team have conducted a series of studies in Ontario to clarify these areas covering the fate and transport of FIOs in catchment systems (Jamieson *et al.* 2004a,b, Jamieson *et al.* 2005a,b).

Diffuse source transport of FIOs from farming activities were presented in Kay *et al.* (2008b) (see Table 5.8). This report was based on 205 stream monitoring sites

for which high- and low-flow FIO data were available for the summer bathing season and 11 sites for which parallel winter data were available. Satellite-derived land use data were used to characterise the catchment land use upstream of these monitoring sites. These data have been used to derive relationships between land use and water quality during both high- and low-flow conditions and to quantify the effects of seasonality on FIO transport. This is important with respect to non-seasonal water uses such as abstraction for drinking-water, particularly private supplies, and where receiving waters are used for shellfish harvesting. Table 5.8 presents the observed transport rates of FIOs from these catchments expressed as faecal indicator concentration observed at catchment outlets with different land uses in the UK summer and comparative winter data for 11 catchments. Table 5.9 expresses these data as export coefficients which are the more common requirement of catchment transport models.

The information in Tables 5.8 and 5.9 represent an attempt to provide the modelling community with a peer-reviewed accessible data resource to drive catchment microbial transport models. They are, of course, regionally specific, highly biased to the developed nations and narrowly restricted to FIOs, that is not having pathogen transport data and certainly not having specific information describing zoonotic pathogen transport. The current status of transport data for the FIO microbial parameters could compare with that available for the nutrient parameters some 25 years ago in North America (see e.g. PLUARG, 1983).

5.5 CONCLUSIONS

(1) Nutrient transport, as quantified by the export coefficient approach within a GIS, is exemplified for nitrogen by Mattikalli and Richards (1996). In many respects, this parallels the drivers of microbial transport outlined in Kay *et al.* (2005, 2008b) in the United Kingdom. Other literature sources of export coefficient for the FIOs are not, at present, available. However, it should be noted that FIO transport is highly episodic in nature and highly seasonal. Thus, coefficients for high and low flow and for winter and summer are likely to be needed in addition to coefficients for different land use types. For large-scale catchment appraisal, this approach is likely to provide sufficient detail for the policy community wishing to prioritise "measures" designed to reduce FIO concentrations and where the key questions relate to the balance between point and diffuse inputs and the likely impact of specific interventions e.g. enhanced attenuation and storage to prevent sewerage system spills

Table 5.8 Geometric mean (GM) and 95% confidence intervals (CIs) of the GM faecal indicator organism (FIO) concentrations (cfu 100 ml^{-1}) under base- and high-flow conditions at the 205 sampling points and for various subsets, and results of paired, *t*-tests to establish whether there are significant elevations at high flow compared with base flow.

FIO		Base flow			High flow		
Sub-catchment land use	**n**	**Geometric mean**	**Lower 95% CI**	**Upper 95% CI**	**Geometric mean[a]**	**Lower 95% CI**	**Upper 95% CI**
TOTAL COLIFORMS							
All sub-catchments	205	5.8×10^3	4.5×10^3	7.4×10^3	7.3×10^4**	5.9×10^4	9.1×10^4
Degree of urbanisation[b]							
Urban	20	3.0×10^4	1.4×10^4	6.4×10^4	3.2×10^5**	1.7×10^5	5.9×10^5
Semi-urban	60	1.6×10^4	1.1×10^4	2.2×10^4	1.4×10^5**	1.0×10^5	2.0×10^5
Rural	125	2.8×10^3	2.1×10^3	3.7×10^3	4.2×10^4**	3.2×10^4	5.4×10^4
Rural sub-catchments with different dominant land uses							
≥ 75% Improved pasture	15	6.6×10^3	3.7×10^3	1.2×10^4	1.3×10^5**	1.0×10^5	1.7×10^5
≥ 75% Rough grazing	13	1.0×10^3	4.8×10^2	2.1×10^3	1.8×10^4**	1.1×10^4	3.1×10^4
≥ 75% Woodland	6	5.8×10^2	2.2×10^2	1.5×10^3	6.3×10^3*	4.0×10^3	9.9×10^3
FAECAL COLIFORMS							
All sub-catchments	205	1.8×10^3	1.4×10^3	2.3×10^3	2.8×10^4**	2.2×10^4	3.4×10^4
Degree of urbanisation[b]							
Urban	20	9.7×10^3	4.6×10^3	2.0×10^4	1.0×10^5**	5.3×10^4	2.0×10^5
Semi-urban	60	4.4×10^3	3.2×10^3	6.1×10^3	4.5×10^4**	3.2×10^4	6.3×10^4
Rural	125	8.7×10^2	6.3×10^2	1.2×10^3	1.8×10^4**	1.3×10^4	2.3×10^4
Rural sub-catchments with different dominant land uses							
≥ 75% Improved pasture	15	1.9×10^3	1.1×10^3	3.2×10^3	5.7×10^4**	4.1×10^4	7.9×10^4
≥ 75% Rough grazing	13	3.6×10^2	1.6×10^2	7.8×10^2	8.6×10^3**	5.0×10^3	1.5×10^4
≥ 75% Woodland	6	3.7×10	1.2×10	1.2×10^2	1.5×10^3**	6.3×10^2	3.4×10^3

(*Continued*)

Table 5.8 (*Continued*)

FIO		Base flow			High flow		
Sub-catchment land use	**n**	**Geometric mean**	**Lower 95% CI**	**Upper 95% CI**	**Geometric mean[a]**	**Lower 95% CI**	**Upper 95% CI**
ENTEROCOCCI							
All sub-catchments	205	2.7×10^2	2.2×10^2	3.3×10^2	5.5×10^3**	4.4×10^3	6.8×10^3
Degree of urbanisation [b]							
Urban	20	1.4×10^3	9.1×10^2	2.1×10^3	2.1×10^4**	1.3×10^4	3.3×10^4
Semi-urban	60	5.5×10^2	4.1×10^2	7.3×10^2	1.0×10^4**	7.6×10^3	1.4×10^4
Rural	125	1.5×10^2	1.1×10^2	1.9×10^2	3.3×10^3**	2.4×10^3	4.3×10^3
Rural sub-catchments with different dominant land uses							
≥ 75% Improved pasture	15	2.2×10^2	1.4×10^2	3.5×10^2	1.0×10^4**	7.9×10^3	1.4×10^4
≥ 75% Rough grazing	13	4.7×10	1.7×10	1.3×10^2	1.2×10^3**	5.8×10^2	2.7×10^3
≥ 75% Woodland	6	1.6×10	7.4	3.5×10	1.7×10^2**	5.5×10	5.2×10^2

[a]Significant elevations in concentrations at high flow are indicated: ** $p < 0.001$, * $p < 0.05$.

[b]Degree of urbanisation, categorised according to percentage built-up land: "Urban" (≥10.0%), "Semi-urban" (2.5–9.9%) and "Rural" (<2.5%)

Table 5.9 Summary of geometric mean faecal indicator organism (FIO) export coefficients ($\log_{10}$ cfu km^{-2} hr^{-1}) under base- and high-flow conditions at the 205 sampling points and for various subsets, and results of paired, 1-tailed *t*-tests to establish whether there are significant elevations at high flow compared with base flow.

FIO		Base flow			High flow		
Subcatchment land use	**n**	**Geometric mean**	**Lower 95% CI**	**Upper 95% CI**	**Geometric mean[a]**	**Lower 95% CI**	**Upper 95% CI**
TOTAL COLIFORMS							
All subcatchments	205	1.8×10^9	1.4×10^9	2.4×10^9	9.5×10^{10}**	7.2×10^{10}	1.2×10^{11}
Degree of urbanisation[b]							
Urban	20	8.5×10^9	3.3×10^9	2.2×10^{10}	4.1×10^{11}**	1.6×10^{11}	1.1×10^{12}
Semi-urban	60	4.2×10^9	2.6×10^9	6.7×10^9	1.5×10^{11}**	8.3×10^{10}	2.7×10^{11}
Rural	125	9.3×10^8	6.9×10^8	1.3×10^9	6.1×10^{10}**	4.6×10^{10}	8.0×10^{10}
Rural subcatchments with different dominant land uses							
≥ 75% Improved pasture	15	2.9×10^9	1.4×10^9	6.0×10^9	2.8×10^{11}**	1.6×10^{11}	4.9×10^{11}
≥ 75% Rough grazing	13	7.1×10^8	3.5×10^8	1.4×10^9	5.3×10^{10}**	2.6×10^{10}	1.1×10^{11}
≥ 75% Woodland	6	3.1×10^8	5.7×10^7	1.6×10^9	1.4×10^{10}**	6.0×10^9	3.4×10^{10}
FAECAL COLIFORMS							
All subcatchments	205	5.5×10^8	4.1×10^8	7.2×10^8	3.6×10^{10}**	2.7×10^{10}	4.8×10^{10}
Degree of urbanisation[b]							
Urban	20	2.8×10^9	1.1×10^9	7.2×10^9	1.3×10^{11}**	4.8×10^{10}	3.6×10^{11}
Semi-urban	60	1.2×10^9	7.4×10^8	1.9×10^9	4.6×10^{10}**	2.5×10^{10}	8.6×10^{10}
Rural	125	2.9×10^8	2.1×10^8	4.0×10^8	2.6×10^{10}**	1.9×10^{10}	3.5×10^{10}
Rural subcatchments with different dominant land uses							
≥ 75% Improved pasture	15	8.3×10^8	4.3×10^8	1.6×10^9	1.2×10^{11}**	6.5×10^{10}	2.2×10^{11}

(*Continued*)

Table 5.9 (*Continued*)

FIO		Base flow			High flow		
Subcatchment land use	**n**	**Geometric mean**	**Lower 95% CI**	**Upper 95% CI**	**Geometric mean[a]**	**Lower 95% CI**	**Upper 95% CI**
≥ 75% Rough grazing	13	2.5×10^8	1.1×10^8	5.7×10^8	2.5×10^{10}**	1.1×10^{10}	5.5×10^{10}
≥ 75% Woodland	6	2.0×10^7	4.7×10^6	8.2×10^7	3.3×10^9**	1.3×10^9	8.8×10^9
ENTEROCOCCI							
All subcatchments	205	8.3×10^7	6.6×10^7	1.1×10^8	7.1×10^9**	5.5×10^9	9.3×10^9
Degree of urbanisation[b]							
Urban	20	4.0×10^8	2.1×10^8	7.6×10^8	2.7×10^{10}**	1.1×10^{10}	6.2×10^{10}
Semi-urban	60	1.5×10^8	9.8×10^7	2.2×10^8	1.1×10^{10}**	6.1×10^9	1.9×10^{10}
Rural	125	4.9×10^7	3.7×10^7	6.5×10^7	4.7×10^9**	3.5×10^9	6.3×10^9
Rural subcatchments with different dominant land uses							
≥ 75% Improved pasture	15	9.6×10^7	5.2×10^7	1.8×10^8	2.2×10^{10}**	1.3×10^{10}	3.8×10^{10}
≥ 75% Rough grazing	13	3.3×10^7	1.2×10^7	9.0×10^7	3.6×10^9**	1.3×10^9	9.7×10^9
≥ 75% Woodland	6	8.5×10^6	3.8×10^6	1.9×10^7	3.8×10^8**	1.3×10^8	1.1×10^9

[a] Significant elevations in export coefficients at high flow are indicated: ** $p < 0.001$

[b] Degree of urbanisation, categorised according to percentage built-up land: "Urban" (≥ 10.0%), "Semi-urban" (2.5–9.9%) and "Rural" (<2.5%)

measured against best management practices to reduce diffuse source contributions.

(2) As the scale reduces, the policy questions focus on the impacts of specific on-farm interventions such as stock exclusion from stream banks, filter strip width, installation of wetlands and farm dirty water containment and disposal which are addressed in Chapter 6 of this book. It is at this scale that the uncertainties and contradictions in much of the empirical data in this area become most problematic. This problem is present for all catchment modelling studies and scaling issues have been extensively discussed for the nutrient parameters, see for example Brazier *et al.* (2005). The "flow connectivity simulation" as exemplified by Heathwaite *et al.* (2005) for phosphorus is likely to be an extremely useful approach and just as applicable to FIO transport modelling as to the nutrient parameters. Where high connectivity has been observed linking farmyard impervious surfaces in Scotland with adjacent streams, single steading areas have been shown to increase stream high flow FIO loadings by over 300% (Kay *et al.* 2003) in a catchment used predominantly for livestock rearing where 7 km^2 of the catchment was above the monitoring point. These, sub one km^2 effects can be the most important factors in determining sub-catchment transport of FIOs but would not be addressed where input data to GIS driven models using export coefficient remains at the one km^2 resolution.

(3) The further development of existing process-based modelling approaches developed in New Zealand (Collins, 2004), Canada (Jamieson *et al.*, 2004a,b, 2005a,b), Australia (Ferguson *et al.* 2003a,b, 2005), the United Kingdom (Vinten *et al.* 2002, 2004a,b) and the USA (Bai & Lung 2005) is essential if model outputs are to inform the design of farm-scale (<1 km^2) BMPs. There are, however, many areas where basic information on microbial kinetics in different catchment compartments is inadequate. There is very little information on, for example, the survival of faecal indicators and pathogens in freshwaters and their associated sediments (Evison 1988, Duran *et al.* 2002, Zerfas 1970) when compared to the extensive literature on marine systems (Fujioka *et al.* 1981, Fujioka and Yoneyama 2002, Kay *et al.* 2005, Sinton *et al.* 1999, 2002, 2005, Flint 1987). Indeed, the assumption that microbial disappearance outside the alimentary canal is characterised by simple first order kinetics (Chick 1910) is at variance with reported empirical data suggesting continued viability for protracted periods in faeces and sediments which might imply environmental re-growth which is a process rarely considered in modelling studies. The transport kinetics of FIO through porous media

and wetland systems has also been intensively researched using jar scale experiments (Fontes *et al.* 1991), but field validation data are very sparse (Foppen 2006) preventing policy guidance on some potentially useful remediation strategies such as unlined wetlands (Harrington *et al.* 2005).

(4) The key model output required by the policy community is the "episodic" transport of FIOs during storm events (which is the key risk period for bathing water and shellfish non-compliance) but very few studies have measured FIO dynamics through events to facilitate model calibration and validation (McDonald & Kay 1981). More commonly data have been collected through regular monitoring over periods of a year or more (Tong & Chen 2002) and researchers have sought to disentangle complex relationships by retrospective correlation analysis (Kim *et al.* 2005). This will not uncover the most significant processes where the original data are biased to low flow conditions because of the "regular" sampling regime and perhaps 95% of the transport flux occurs in relatively short-term high-flow events which are "missed" by the sampling regime employed. This "episodic" transport is the condition found in livestock farming areas where the zoonotic pathogen loadings originate. Thus, studies seeking to inform TMDL estimates or the design of Programmes of Measures should characterise both high- and low-flow water quality which is logistically difficult but essential. Monitoring data are generally not suitable for microbial transport model calibration.

The reasonable assumption has often been made that FIOs associate with sediment and sediment models can be employed to predict FIO flux, but very few studies have sought to understand how, or indeed whether, FIOs partition with different sedimentary fractions and how particle size and mineralogy influence attachment, transport and survival in freshwater systems. The limited estuarine marine work in this area may not be applicable to freshwater systems (Kay *et al.* 2005). A study by Characklis *et al.* (2005) indicates that different microorganisms will have different partition coefficients and that these may also vary with the composition of the water matrix, for example between low-flow and high-flow events. Hence, extrapolation between FIO and multiple pathogen species will likely be inaccurate and the relationships will vary with composition of the water matrix.

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6

Effectiveness of best management practices for attenuating the transport of livestock-derived pathogens within catchments

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6.1 INTRODUCTION

Waterborne pathogens (and associated faecal indicator organisms (FIOs)) derived from human and animal faeces are a significant water quality concern in many parts of the world. In the United States of America (USA) "pathogens" (actually FIOs: coliforms and enterococci) are the most frequent cause of "impairment" (i.e. non-compliance) in waters covered by the US Clean Water Act (see: Figure 6.1) (USEPA 2009). The design and implementation of measures to

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attenuate the transport of pathogens and FIOs from catchment source areas to points of water resource use are now legal requirements in Europe and North America. In the European Union (EU), for example, the Water Framework Directive (WFD; (CEC 2000)) requires Member States to design a "*programme of measures*" (POM) to ensure compliance with the microbiological standards set out in daughter directives, such as those relating to bathing waters (the World Health Organisation (WHO) health-based standards recently agreed by the EU Parliament; (WHO 2003, Kay, Bartram *et al.* 2004, CEC 2006)); and to near-shore and estuarine shellfish-harvesting waters, which are identified as a "protected area". POMs usually need to address both human and livestock sources within catchments. The former are mostly point-source discharges of treated or untreated sewage effluent and, as such, are relatively easy to identify, monitor and regulate. Livestock sources, by contrast, are mostly "diffuse" (e.g. inputs of fresh faeces or application of stored manures to land), though specific point sources can often be identified (e.g. farm steadings and feedlots used by cattle, manure heaps). They are generally more variable in character, depending on local conditions, and, therefore, more difficult to investigate and characterise than sewerage sources. The effectiveness of potential remediation strategies is more difficult to assess. Indeed, in many cases there is a lack of empirical data.

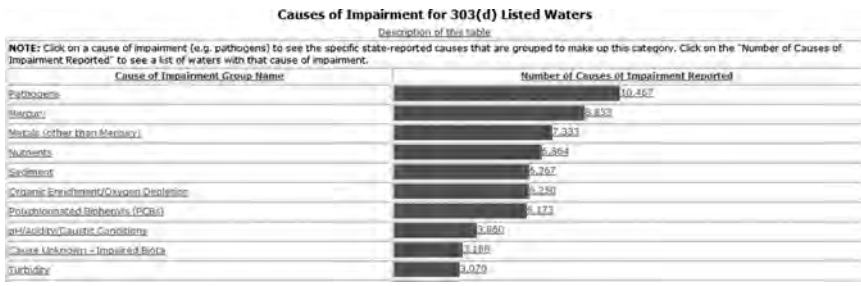


Figure 6.1 Reasons for impairment in all USA waters covered by the Clean Water Act on 6th September 2009. The actual microbial parameters implied by the word "Pathogens" are actually the coliforms and enterococci (USEPA 2009).

In many countries, legislation has been introduced governing certain livestock-related farming operations that pose a high risk to the environment. In England and Wales, for example, the *Control of Pollution (Silage, Slurry and Agricultural Fuel Oil) Regulations* (Statutory Instrument (SI) [England & Wales] 1991) place strict requirements on farmers concerning the storage and handling of manure, especially slurry. In the USA, technology standards are set for the processing of animal feedlot runoff (Federal Register (US) 2003). In

addition, a wide range of best management practices (BMPs) have been advocated for reducing pollution from agriculture (e.g. Cuttle 2007, Scottish Environment Protection Agency (SEPA) 2009), many of which are embodied within national Codes of Good Agricultural Practice (CoGAPs) – for example, Scottish Executive 2005, Department of Agriculture and Rural Development (DARD) [N. Ireland] 2008, Department for Environment Food and Rural Affairs (Defra) 2009. Some measures are designed to reduce pathogen numbers at the source (see: Chapter 5). However, the majority, as identified in Table 6.1, seek to attenuate pathogen transport within catchments by either reducing their transfer to, or survival within, watercourses.

Table 6.1 Best management practices (BMPs) for attenuating the transport of livestock-derived pathogens/FIOs within catchments.

BMPs to attenuate pathogen transfer to watercourses
Containment of farm steading/feedlot sources
Reducing amounts of slurry/contaminated water
Runoff interception/containment
On-farm treatment of contaminated water
Ponds* [Table 6.2/A]
Vegetative treatment areas (VTAs) for feedlot runoff* [Table 6.2/B]
Constructed farm wetlands (CFWs)* [Table 6.2/C]
Control of livestock on farmland
Stream bank fencing and bridging
Minimising livestock congregation areas and soil poaching
Exclusion from areas and at times of high pollution risk
Woodchip corrals* [Table 6.2/D]
Control of manure application to land
Location and timing of application
Incorporation of manures in soil
Vegetated buffer strips (VBSs), inc riparian buffer strips (RBSs)* [Table 6.2/E]
BMPs to attenuate pathogen concentrations within watercourses
Grassed waterways (or 'swales'), inc interception of track runoff
In-stream ponds* [Table 6.2/F]

*BMPs for which summary datasets are presented in Table 6.2. [letter indicates section]

While there is quite an extensive literature on the mitigation of nutrients, pesticides, BOD and suspended sediments, less is known about the effectiveness of agricultural BMPs in addressing the microbial parameters, and a lot of these microbial data are relatively inaccessible to those agencies responsible for the

implementation of POMs. For example, the Scottish Environment Protection Agency (SEPA 2009) identifies more than 30 specific BMPs for reducing FIO concentrations in Scotland, but attenuation efficiency data (for *E. coli*) are presented only for six: one is expressed quantitatively (74% reduction, based on a single study), whereas the others are simply rated as "high" or "medium", with no source being cited. Similarly, Cuttle (2007) adopts an "expert judgment" approach (rather than citing actual empirical data) in assessing the BMP effectiveness in England. To meet the requirement for empirical evidence to inform the design and implementation of POMs, the Centre for Research into Environment and Health (CREH) is compiling a database on the effectiveness of BMPs in attenuating the transport of livestock-derived pathogens within catchments. This chapter presents a summary of the current database; assesses the relative effectiveness of those BMPs for which attenuation data are available; examines the evidence relating to the impact of catchment-scale implementation of BMPs on water quality; and, identifies the major gaps in the evidence base that need to be targeted in future empirical studies.

6.2 CATCHMENT DYNAMICS OF LIVESTOCK-DERIVED PATHOGENS

6.2.1 Hydrological pathways

Apart from direct voiding of faeces to streams or the possibility of manure entering during application to the land, microbial contamination of watercourses depends on the transport of organisms, either independently or attached to particles of soil or faeces, via hydrological pathways (Tyrrel & Quinton 2003). These range from slow, diffuse soil seepage, through more rapid bypass flow through soils and artificial soil drainage, both of which have the capacity to carry significant microbial loads (Ross & Donnison 2003), to surface runoff from the land, including artificial surfaces (e.g. roofs, yards, tracks and roads) and the conveyance of contaminated water to watercourses via artificial drains. Rainfall is significant in two ways: it enhances catchment connectivity (Heathwaite, Quinn *et al.* 2005) by activating potential pollutant flow paths that do not operate under dry conditions (surface runoff from ground surfaces, upstream extension of stream network into source areas and sites of ephemeral flow, etc.); and it increases the volumes of water flow and hence the capacity for pollutant transport throughout the hydrological system. Detailed reviews of these various transmission pathways and the microbial delivery to receiving waters from pastures are presented by Hickey, French *et al.* (2002), Collins (2005), Oliver (2005), Collins (2007) and Tyrrel & Quinton (2003), and are discussed further in Chapter 5.

6.2.2 Processes of microbial attenuation

Once excreted from the digestive tracts of animals, the numbers of pathogens and associated FIOs tend to diminish as a result of natural die-off through a combination of aging, in an environment which is generally unfavourable for microbial re-growth; exposure to adverse environmental conditions (UV-light, desiccation, fluctuating temperatures); and predation. Within catchments, the ground surface and soils effectively act as a labile pollutant 'store' within which pathogen strength diminishes through die-off, leading to a natural reduction in pathogen delivery to receiving waters. Opportunities for die-off are increased by processes that promote the retention of microbes on the ground surface or within soils, notably the filtering effect of vegetation on surface runoff through entrapment and deposition (as a result of reduced flow velocities) of larger mineral and organic particles to which microbes might be attached, and through absorption by soils as a result of infiltration. Sedimentation, particularly of particle-attached microbes, and die-off also occur in ponds and along watercourses, though in the latter case the bed sediments and surviving microbes will tend to be remobilised when the bed is disturbed (Muirhead, Davies-Colley *et al.* 2004, Jamieson, Joy *et al.* 2005, Wilkinson, Kay *et al.* 2006), for example, in response to increased water velocity following rainfall or physical disturbance at fording points (Davies-Colley 2004). The various BMPs designed to attenuate microbial fluxes within catchments effectively replicate and enhance these processes.

6.3 LIMITATIONS OF THE EXISTING EVIDENCE BASE

Most studies, including those of analogous natural sewage treatment systems (examples of which are included in the present database), have only monitored FIOs, principally total coliforms (TC), faecal coliforms (FC), *Escherichia coli* (EC), faecal streptococci (FS) and enterococci (EN). These are generally much easier and less expensive to determine than specific pathogens (Vymazal 2005). The evidence base for livestock-derived pathogens such as *Escherichia coli* O157, *Campylobacter* (both bacterial) and *Cryptosporidium* spp. (protozoan) remains extremely limited. While broad underlying relationships might be anticipated between concentrations of FIOs and pathogens, there are few comparative data on the survival and transport of livestock-derived FIOs and pathogens (Pachepsky, Sadeghi *et al.* 2006). For example, Reinoso *et al.* (2008) report an absence of correlation between FIOs and pathogens in the context of a constructed wetland (CW) – with different organisms being retained and eliminated at different rates. Also, while data on the effectiveness of natural

sewage treatment systems and their controlling factors (e.g. (Struck 2006)) augment the database, it should be noted that the proportions of independent and particle-attached pathogens and FIOs, the character of the particles present and, hence, their vulnerability to sedimentation and die-off, may differ significantly between livestock and sewage effluent. Boutilier *et al.* (2009), for example, report EC surviving longer in the treatment of effluent from a dairy farm than in that of domestic wastewater.

Accurate characterisation of the sources, source strengths and catchment dynamics of agriculture-derived FIOs and pathogens, and of the effectiveness of BMPs, is confounded by the sheer complexity of geographical (spatial) and temporal factors that affect them. From the global scale down to the level of individual farm units there is very marked spatial variability in environmental conditions (e.g. climate, topography, soils and hydrology) and in the nature of livestock farming systems and management practices. Consequently, the empirical data are catchment- or site/farm-specific and therefore need to be used with caution when applied elsewhere.

Equally important is the range of temporal variability, including seasonal patterns in weather conditions, housing of livestock and the application of manure to land; diurnal patterns in certain operations, notably the daily washing of milking parlours and yards on dairy farms; and the episodic and variable nature of rainfall events. High-flow conditions following rainfall may at times provide elevated microbial fluxes within catchments. In the United Kingdom, concentrations and flow volumes both typically increase by an order of magnitude, leading to a 100-fold increase in catchment export coefficients (Kay, Crowther *et al.* 2008). At such times the effectiveness of certain BMPs is likely to be compromised, for example, hydraulic residence time (HRT) within CWs will be reduced, with a greater likelihood of rapid by-pass flow, leading to diminished opportunities for die-off, predation and sedimentation (Werker, Dougherty *et al.* 2002, Edwards 2005, Kay 2005, Kay, Crowther *et al.* 2008).

Water quality monitoring data are often biased towards low-flow conditions and may provide an inadequate basis for characterising high-flow events. It should also be recognised that when longitudinal (before-and-after BMP adoption) studies are employed at a catchment or field scale, then the results are likely to be confounded by different antecedent weather conditions in the two sampling periods. The assessment of the effectiveness of certain BMPs may be further complicated by a delayed response as a result of labile pollutant stores on ground surfaces and within the soil, as reported for nutrient attenuation in Sweden (Grimvall, Stålnacke *et al.* 2000). Individual catchment- and field-scale investigations of BMPs must therefore be regarded very much as unique "case studies". In contrast, plot- and laboratory-scale (e.g. soil-box) investigations provide a better

basis for isolating the effects of different processes and controlling factors, but need to be used with care when scaling up to field and catchment situations. Some investigators (Miller, Lewis *et al.* 2007, Miller, Lewis *et al.* 2008), for example, note that there is a greater chance of channelized flow-reducing attenuation through an actual vegetated buffer strip (VBS) than through a plot- or laboratory-scale VBS.

6.4 OUTLINE OF PRESENT DATABASE

The present CREH database (which will be available in due course at http://www.ies.aber.ac.uk/en/research/groups/centre-research-environment-and-health) focuses on those BMPs (as identified in Table 6.1) for which microbial attenuation has been quantified, and includes data for the full range of microbial parameters reported in the various studies. Representative data on the influent and effluent concentrations of FIOs or pathogens, and attenuation rates (flux-based where possible) are presented in Table 6.2. In each case data have been reported for a single parameter: EC or FC where possible, since these are the most commonly reported parameters, or an alternative FIO/pathogen parameter if neither was measured.

Attenuation through an individual scheme (e.g. a CW) is conventionally expressed as either a reduction in flux (i.e. number per unit time) or concentration of organisms. Flux data provide the best measure since they take into account any reductions in water flow, for example, reductions in surface flow through a system as a result of soil infiltration (Collins 2004, Roodsari, Shelton *et al.* 2005) and/or evaporation/evapotranspiration. Summary statistics for the attenuation data are presented in Figure. 6.2. These need to be interpreted with some degree of caution since they include cases where the number of independent data sets and/or data values is very small ($n < 10$), attenuation data for all the individual FIOs and pathogens reported (not just the parameters listed in Table 6.2), rates of attenuation in either microbial flux or concentration (with the former being used where data permit), data with different levels of aggregation, and all data irrespective of the study type (empirical field- or plot-based and modelling-based) or of conditions at the time of sampling (e.g. flow or season). Nonetheless, they provide a preliminary basis for assessing the relative effectiveness of the different measures. For consistency, all microbial concentrations in the text are reported to two significant figures using the scientific notation and attenuation rates are expressed as $\log_{10}$ reductions: $\log_{10}$ attenuations of 1.0 and 3.0 correspond with percentage reductions of 90.0 and 99.9%, respectively.

Table 6.2 Summary data on influent and effluent concentrations of faecal indicator organisms or pathogens and attenuation rates for selected BMPs. [a]

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	Attenuation: f/c[e]
A. Ponds												
Farm waste stabilisation ponds (WSPs): standard two-pond (anaerobic(AP)/facultative(FP)) system												
(Craggs, Tanner *et al.* 2003)	New Zealand, dairy farm effluent: summary of published data from 4 areas based on 4 sources: Hickey *et al.* 1989; Bolan *et al.* 1996; Mason & Ellwood 1996; Selvarajah 1996 [FC]	FC	MPN/100 ml	md				70000				
(Craggs, Tanner *et al.* 2003)	New Zealand, dairy farm effluent on single farm: paired study with advanced pond system (APS) (see below) [EC]	EC	MPN/100 ml	md			24	16195				

(Sukias, Tanner *et al.* 2001)	Waikato, New Zealand, dairy farm effluent: standard pond size, 12 sites, monthly samples, 1 yr (summary, pub data) [FC, TC]	FC	MPN?/ 100 ml	md	144	35000
	Taranaki, New Zealand, dairy farm effluent: standard pond size, 2 samples from 20 sites (summary, pub data) [FC]	FC	MPN?/ 100 ml	md	40	80000
	Manawatu, New Zealand, dairy farm effluent: standard pond size, 6 sites, monthly samples, 1 yr (summary, pub data) [FC, TC]	FC	MPN?/ 100 ml	md	72	70000
	Waikato, New Zealand, dairy farm effluent: larger pond size (to meet 1996 Guidelines), 6 sites, monthly samples, 1 yr [FC]	FC	cfu/100 ml	md	72	24000

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	f/c[e]
Farm advanced pond system (APS): facultative pond is replaced by a high rate pond (HRP), algae settling ponds (ASPs) and maturation pond (MP)												
(Craggs, Tanner *et al.* 2003)	New Zealand, dairy farm effluent from anaerobic pond of WSP (i.e. partly treated): APS with HRP, ASP and MP, single farm, paired study with WSP (Craggs *et al.* 2003) [EC]	EC	MPN/100 ml	md	24	122000		146	121854	99.880	2.92	(c)
Ponds for treatment of raw municipal/domestic wastewater												
(Reinoso, Torres *et al.* 2008)	NW Spain, wastewater from small village: facultative pond, HRT 75.9 days, 10 × monthly samples, 12/04–9/05 (effluent subsequently treated by CW) [EC, FS, TC, *C.per*, Co, *Cr, Gi*, He]	EC	cfu/100 ml	am	10	2240000	10	71456	2168544	96.81	1.50	(c)

Ponds for secondary or tertiary treatment of sewage effluent												
(Kay 2005)	SE England, effluent from oxidation ditch system serving pop of c.32000: lagoon, c.100 × 600 m, 1–3 m deep: dry conditions [EC, EN]	EC	cfu/100 ml	gm	16	107152	16	289	106863	99.730	2.57	(c)
	As above: wet conditions [EC, EN]	EC	cfu/100 ml	gm	46	871836	46	304	871532	99.965	3.46	(c)
B. Vegetative treatment areas (VTAs) for feedlot runoff												
(Coyne, Gilfillen *et al.* 1998) [Koelsch *et al.* 2006]	USA, 2 VTAs treating runoff from pasture area on which poultry manure applied, simulated rainfall at 64mm/h: small VTAs [FC, FS]	FC								75	0.60	(c)
	USA, 2 VTAs treating runoff from pasture area on which poultry manure applied, simulated rainfall at 64 mm/h: larger VTAs [FC, FS]	FC								91	1.05	(c)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	Attenuation: f/c[e]
(Komor & Hansen 2003)	USA, beef cattle, runoff monitored over 7 storm events [FC]: Site 1	FC								62	0.42	(f)
	As above: Site 2	FC								18 to 79	0.09 to 0.68	(f)
(Lim, Edwards *et al.* 1997) [Koelsch *et al.* 2006]	USA, investigation of effects of VTA size, simulated rainfall [FC]: VTA:feedlot area ratio 0.5	FC								100		(f)
	As above: VTA:feedlot area ratio 1.5	FC								100		(f)
	As above: VTA:feedlot area ratio 1.0	FC								100		(f)
(Mankin and Okoren 2003) [Koelsch *et al.* 2006]	USA, dairy heifers, data presented for outlet (150 m) but almost identical reductions recorded at 30 m [EC, FC]	EC								90	1.00	(f)

(Mankin, Barnes *et al.* 2006) [based on abstract]	Kansas, USA, automated sampling of 22 runoff events at 4 sites, mostly under unstocked conditions, investigation of importance of site characteristics [EC, FC, FS]	EC	83	0.77	(c)
(Williamson 1999) [Koelsch *et al.* 2006]	Kansas, USA, beef cattle, comparison of 4 VTAs [EC, FC]: Site 1	EC	79.3	0.68	(c)
	As above: Site 2	EC	78.2	0.66	(c)
	As above: Site 3	FC	36	0.19	(c)
	As above: Site 4	EC	88.4	0.94	(c)
(Willrich & Boda 1976) [Koelsch *et al.* 2006]	USA, swine lagoon effluent, natural rainfall events [FC]	FC	31	0.16	(c)
(Young, Huntrods *et al.* 1980) [Koelsch *et al.* 2006]	Rainfall simulation, 63.5 mm/h for 71 min [FC, FS, TC]: orchard grass	FC	55	0.35	(c)
	As above: Sorghum-Sudan grass mix	FC	83	0.77	(c)
	Rainfall simulation, 63.5 mm/h for 71 min [TC]: corn	TC	71	0.54	(c)
	As above: oats	TC	70	0.52	(c)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	f/c[e]
C. Constructed farm wetlands (CFWs), including integrated constructed wetlands (ICWs) on farms												
(Carty 2008)	Waterford, Ireland, farmyard runoff: 12 demonstration ICWs (later report than Harrington *et al.* 2007 – presumed to inc additional data) [EC]	EC	cfu/100 ml	am		833396		<500	>832896	99.940	>3.22	(c)
(Duggan, Bates *et al.* 2001)	?, poultry waste: horizontal SSF reedbed [EC, Ca]: sequential loading	EC		am						99.972	3.56	(c)
	As above: continuous loading	EC		am						99.994	4.25	(c)
(Gouriveau 2008)	SE Scotland, steading runoff from mixed beef/arable farm: CFW, 5 ponds <1 m deep and intervening wetlands in series, c.0.9 ha [FC]	FC	cfu/100 ml	am	2	1800	2	75000	0	0.000	0.00	(c)

(Gouriveau 2008)	SE Scotland, steading runoff and septic tank effluent, large dairy farm, 400 cows: CFW, single pond, 0.2 ha, c.0.85 m deep, sparsely planted [FC]	FC	cfu/100 ml	am	2	>104500	2	6625	>97875	>93.7	>1.20	(c)
(Harrington 2007)	Waterford, Ireland, farmyard runoff: 12 demonstration ICWs	EC	cfu/100 ml	am?	300	200000	300	500	199500	99.750	2.60	(c)
(Kay 2005)	Waterford, Ireland, effluent from 12 sites (10 farm (dairy and beef), 1 industrial (cheese factory) and 1 domestic (septic tank effluent)): 12 ICWs, total number of samples shown [EC, EN]: dry conditions	EC	cfu/100 ml	gm	12	36069	13	39	36030	99.892	2.97	(c)
	As above: wet conditions	EC	cfu/100 ml	gm	48	136672	52	101	136571	99.926	3.13	(c)
(Kern, Idler *et al.* 2000)	E. Germany, dairy farm wastewater, single farm: CFW [FC]: summer	FC	cfu/100 ml	am?	?	460000?				99.3	2.15	(c)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	f/c[e]
(Knox, Tate *et al.* 2007)	California, US, May-Oct 2004 + 5 runoff from irrigated foothill beef cattle pasture (4.9 ha); wetland c. 0.2 ha, flow length 123 m, vegetated with *Polygonum punctatum, Veronica catenta and Leersia oryzoides*), investigation of effects of flow volumes and time since grazing [EC]: results for 14 irrigation events	EC	cfu/100 ml	nd	146	5400	146	1283		33 to 91	0.17 to 1.05	(f)
	As above: winter	FC	cfu/100 ml	am?	?	460000?				95.8	1.38	(c)
(Mantovi, Marmiroli *et al.* 2003)	Italy, dairy parlour and domestic effluent on hill farm: CFW, 2 horizontal SSF reedbeds, 75 m2 each [EC, FS]	EC	cfu/100 ml							>99	>2.00	(c)

(Mustafa 2009)	Waterford, Ireland, runoff from dairy farm steading, 0.50 ha, 77 dairy cattle: ICW, 4-cell system, 0.76 ha [EC, TC]	EC	cfu/100 ml	am	7	960000	7	30	959970	99.997	4.51	(c)
(Newman, Clausen *et al.* 2000)	Connecticut, USA, single dairy farm, milking parlour washings, 2.65 m3/d: CFW, surface flow wetland, weekly samples over 2.5 yr	FC	cfu/100 ml	gm	c.125	557378	c. 125	13700	543678	97.542	1.61	(c)
CWs for treatment of captive wildfowl waste												
(Drewe, Mwangi *et al.* 2009)	Slimbridge, Gloucester, England, runoff from captive wildlife enclosures: CW, inc reedbeds, common reed (Phragmites australis) and greater reedmace (Typha latifolia), study of M. avium (causal agent of avian tuberculosis) [M.av]	*M. avium*								100.000		(c)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	f/c[e]
CWs for treatment of raw municipal/domestic wastewater												
(Kay 2005)	Wales, effluent from combined system with population of c.300: CW, 2 reedbeds in sequence (which is subsequently treated by vertical-flow reedbed – see below) [EC, EN]: dry conditions	EC	cfu/100 ml	gm	35	8736896	34	181365	8555531	97.924	1.68	(c)
	As above: wet conditions	EC	cfu/100 ml	gm	38	11381582	39	582251	10799331	94.884	1.29	(c)
(Molleda, Blanco *et al.* 2008)	Leon, Spain: CW, only data for season(s) with greatest attenuation rates recorded here [EC, FS, TC, C.per, Cr, Gi, He]: Spring and autumn	EC								99.9	3.00	(c)

(Newman, Clausen *et al.* 2000)	USA: Synthesis of results from 154 CWs (from N. American Wetland Database) [EC]	EC	cfu/100 ml	am		53335		663	52672	98.757	1.91	(c)
(Song 2005)	Shandong Province, China: CW, 80 ha, treatment capability of 20000 m3/d [FC, TC]	FC								99.6	2.40	(c)
(Vymazal 2005)	International review: 60 sites worldwide [FC, TC, FS]	FC								98.0	1.65	(c)
CWs for secondary or tertiary treatment of sewage effluent												
(Kay 2005)	Wales, effluent from horizontal-flow reedbed serving population of c. 300: CW, single (immature) vertical-flow reedbed [EC, EN]: dry conditions	EC	cfu/100 ml	gm	34	181365	35	36545	144820	79.850	0.70	(c)
	As above: wet conditions	EC	cfu/100 ml	gm	39	582251	38	257055	325196	55.852	0.36	(c)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	f/c[e]
(Kay 2005)	SE England, effluent from trickling filter plant serving population of c.22000: CW, vertical-flow reedbed [EC, EN]: dry conditions	EC	cfu/100 ml	gm	16	200173	16	59622	140551	70.215	0.53	(c)
	As above: wet conditions	EC	cfu/100 ml	gm	67	3451025	67	2224204	1226821	35.549	0.19	(c)
(Manios, Stentiford *et al.* 2002)	England, treating primary-treated domestic wastewater: CW, horizontal SSF reedbed (*Typha latifolia*) with various underlying media: data for gravel bed (which was most effective medium) [EC, FC]	EC								99.950	3.30	(c)

(Perkins & Hunter 2000)	Yorkshire, England, treatment of secondary municipal wastewater effluent: CW, 4 horizontal SF reedbeds (*Typha*-dominated), sampled twice weekly for 10 wks, June-Sep 1998 [FC, FS]: Site 1	FC	cfu/100 ml	am	20	167145	19	17855	149290	91.4	1.07	(c)
	As above: Site 2	FC	cfu/100 ml	am	20	181325	19	30502	150823	85.4	0.84	(c)
	As above: Site 3	FC	cfu/100 ml	am	20	170943	19	27695	143248	87.4	0.90	(c)
	As above: Site 4	FC	cfu/100 ml	am	20	197040	19	22009	175031	93.9	1.21	(c)
(Reinoso, Torres *et al.* 2008)	NW Spain, treatment of secondary effluent from facultative pond: CW, SF wetland, 10 × monthly samples, 12/04–9/05 [EC, FS, TC, C.per, Co, Cr, Gi, He]	EC		am	10	71456	10	44617	26839	37.56	0.20	(c)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: $\log_{10}$	Attenuation: f/c[e]
	NW Spain (treatment of secondary effluent from facultative pond and SF wetland): SSF wetland, 10× monthly samples, 12/04–9/05 [EC, FS, TC, C.per, Co, Cr, Gi, He]	EC		am	10	44617	10	12484	32133	72.02	0.55	(c)
(Stapleton, Lowe *et al.* 2006)	NW England, effluent from rotating biological contactor, serving residential home: CW, horizontal-flow reedbed (*Phragmites*), 45 m^2, overlying stone medium, lined base [FC, TC, IE, Co]	FC	cfu/100 ml	gm	58	111000	58	9310	101690	91.613	1.08	(c)

(Stapleton, Lowe *et al.* 2006)	NW England, effluent from rotating biological contactor, serving 8 houses: CW, horizontal-flow reedbed [FC, TC, IE, Co, Ev]	FC	cfu/100 ml	gm	64	490000	64	152000	338000	68.980	0.51	(c)
(Stapleton, Lowe *et al.* 2006)	NW England, septic tank effluent from small domestic/holiday cottage complex: CW, horizontal-flow reedbed (*Phragmites*) [FC, TC, IE, Co, Ev]	FC	cfu/100 ml	gm	64	1160000	64	758	1159242	99.935	3.18	(c)
(Stapleton, Lowe *et al.* 2006)	NW England, effluent from primary settlement plastic media filter, serving 92-bed hotel: CW, horizontal-flow reedbed [FC, TC, IE, Co, Ev]	FC	cfu/100 ml	gm	54	665000	54	135000	530000	79.699	0.69	(c)
(Stapleton, Lowe *et al.* 2006)	NW England, effluent from rotating biological contactor, serving nursing home: CW, vertical-flow reedbed (*Iris* and *Phragmites*) [FC, TC, IE, Co]	FC	cfu/100 ml	gm	65	2180000	65	248000	1932000	88.624	0.94	(c)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: $\log_{10}$	Attenuation: f/c[e]
(Thurston, Gerba *et al.* 2001)	Arizona, USA, Treatment of secondary effluent via duckweed pond: CW, SSF wetland with mixed vegetation [FC, TC, Co, Gi, Cr]	FC	cfu/100 ml	gm	27	7400	27	45	7355	98.2	1.74	(c)
D. Woodchip corrals												
(McDonald, McDonald *et al.* 2008)	NE and SW Scotland, investigation of leachate from 4 woodchip corrals used for over-wintering beef cattle: data for over winter when cattle present (data also presented for periods when cattle absent (summer) and for high and low flow) [EC, TC, IE]: Site SW1	EC	cfu/100 ml	gm				162742	383178			
	As above: Site SW2	EC	cfu/100 ml	gm				281579	382913			

	As above: Site NE1	EC	cfu/100 ml	gm		31565	62130			
	As above: Site NE2	EC	cfu/100 ml	gm		2843151	1061207			
(Vinten, Donnelly *et al.* 2006)	SW Scotland, investigation of leachate from 9 woodchip corrals used for overwintering beef cattle: leachate from base of woodchips [EC, EN]: corral with highest EC attenuation	EC	cfu/100 ml	am?	3400000	80000		3320000	97.647	(c)
	As above: corral with lowest EC attenuation	EC	cfu/100 ml	am?	3400000	1000000		2400000	70.588	(c)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	Attenuation: f/c[e]
E. Vegetated buffer strips (VBSs), including riparian buffer strips (RBSs)												
(Atwill, Hou *et al.* 2002)	California, lab-scale investigation of effects of soil texture/bulk density and slope in attenuation of *Cryptosporidium parvum* through a 1 m vegetated (fescue) buffer strip with simulated low-moderate rainfall intensities. Modelled results tabulated here for conditions with high, medium and low attenuation [*C.par*]: Silty clay loam (bulk density = 0.66 g/cm3), slope 2.9°	*C.par*	oocysts/l							99.369	2.2	(c)
	As above: slope 5.7°	*C.par*	oocysts/l							99.921	3.1	(c)
	As above: slope 8.5°	*C.par*	oocysts/l							99.800	2.7	(c)

	As above: Loam (bden = 1.0–1.33 g/cm3), slope 2.9°	*C.par*	oocysts/l	99.206	2.1	(c)
	As above: slope 5.7°	*C.par*	oocysts/l	99.602	2.4	(c)
	As above: slope 8.5°	*C.par*	oocysts/l	99.645	2.45	(c)
	As above: Sandy loam (bden = 1.66 g/cm3), slope 2.9°	*C.par*	oocysts/l	96.019	1.4	(c)
	As above: slope 5.7°	*C.par*	oocysts/l	90.000	1.0	(c)
	As above: slope 8.5°	*C.par*	oocysts/l	96.019	1.4	(c)
(Collins 2004)	Ruakura, New Zealand, riparian buffer strips, in-field plot-scale experiments using dairy-farm effluent and simulated events, both surface and subsurface (to collect bypass flow through soil) outflows monitored, data for microbial numbers recovered, assessment of importance of grass height, flow volume and remobilisation [EC, *Ca*]: slow flow	EC		96 to >99	1.40 to >2	(f)
	As above: intermediate flow	EC		38 to 84	0.21 to 0.80	(f)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	Attenuation: f/c[e]
	As above: fast flow	EC								0 to 59	0 to 0.39	(f)
(Coyne, Gilfillen *et al.* 1998) (cited by Collins *et al.* 2004)	Buffer strip 3 m wide, 9° slope, sampled during 1 in 10 yr rainfall events [FC]	FC								43 to 74	0.24 to 0.59	(f)
(Goel, Rudra *et al.* 2004)	Canada, dairy slurry application to land, investigation of 2 lengths of buffer strip and 3 vegetation types, using simulated rainfall [FC, FS]: perennial ryegrass/5 m wide	FC								71	0.54	(f)
	As above: perennial ryegrass/10 m wide	FC								77	0.64	(f)
	As above: mixed grass species/5 m wide	FC								56	0.36	(f)
	As above: mixed grass species/10 m wide	FC								75	0.60	(f)
	As above: Kentucky blue grass/5 m wide	FC								91	1.05	(f)

	As above: Kentucky blue grass/10 m wide	FC	99	2.00	(f)
(Fajardo, Bauder *et al.* 2001) (cited by Collins *et al.* 2004)	Tall fescue buffer strip, 30 m wide, 2.3° slope, sampled during two high magnitude rainfall events [FC]	FC	64 to 87	0.44 to 0.89	
(Kouznetsov, Roodsari *et al.* 2007)	Lab-scale rainfall simulation of attenuation of FC removal through a 6 m VBS (grassed), used in developing a transport model [FC]	FC	99	2.00	(f)
(Larsen, Miner *et al.* 1994) [cited by Tate *et al.* 2006]	Simulation studies of effectiveness of VFS of different widths in attenuation of FC in runoff from fresh bovine faeces [FC]: 0.61-m VBS	FC	83	0.77	
	As above: 2.13-m VBS	FC	95	1.30	

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Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	Attenuation: f/c[e]
(Ministry of Agriculture and Forestry [New Zealand] (MAF) 2006)	New Zealand, modelling study using modified sediment-based CREAMS model of Cooper *et al.* (1992) for all major soil types in NZ, to investigate likely effects of soil type, slope angle, slope length and buffer strip length, range of data presented here are for high and low bacterial-particle attachment, with the greater length and lower reduction rate being for sites with low attachment (data for moderate attachment are presented in report), and are for the optimal buffer width (taking into account amount of land used in the buffer): width 1 to 9 m, flat to undulating terrain, low soil infilt cap (<4 mm/h)									80 to 95	0.79 to 1.30	(c)?

As above: width 1 to 4 m, flat to undulating terrain, mod soil infilt cap (5–64 mm/h)	80 to 95	0.79 to 1.30	(c)?
As above: width 1 to 3 m, flat to undulating terrain, high soil infilt cap (>64 mm/h)	85 to 95	0.82 to 1.30	(c)?
As above: width 2 to 15 m, rolling to mod steep terrain, low soil infilt cap (<4 mm/h)	50 to 90	0.30 to 1.00	(c)?
As above: width 1 to 11 m, rolling to mod steep terrain, mod soil infilt cap (5–64 mm/h)	55 to 95	0.35 to 1.30	(c)?
As above: width 1 to 4 m, rolling to mod steep terrain, high soil infilt cap (>64 mm/h)	60 to 95	0.40 to 1.30	(c)?
As above: width 2 to 15 m, mod to very steep terrain, low soil infilt cap (<4mm/h)	20 to 45	0.10 to 0.26	(c)?
As above: width 1 to 11 m, mod to very steep terrain, mod soil infilt cap (5–64 mm/h)	35 to 60	0.19 to 0.40	(c)?

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	Attenuation: f/c[e]
	As above: width 1 to 4 m, mod to very steep terrain, high soil infilt cap (>64 mm/h)									50 to 75	0.30 to 0.60	(c)?
(Miller, Lewis *et al.* 2007)	Tomales Bay, California, US, regression models of attenuation of *Giardia duodenalis* through VBS based on runoff data from 57 cattle lots on five dairy farms during 2002–2004 [*G.duo*]: data presented here are the modelled values for a 10 m wide VBS	*G.duo*	cysts/l							90.5	1.02	(m)
(Miller, Lewis *et al.* 2008)	As above, but for *Cryptosporidium* spp. [*Cr]*	*Cr*	oocysts/l							18.3	0.09	(m)

(Núñez-Delgado, López-Periago *et al.* 2002)	Spain, plot scale experiments using cattle slurry and simulated rainfall, lolium perenne meadow [FC, FS]: 1 day after slurry application/width 4 m	FC	MPN/100 ml	400	(c)
	As above: 1 day after slurry application/width 6 m	FC	MPN/100 ml	43000	(c)
	As above: 1 day after slurry application/width 8 m	FC	MPN/100 ml	300	(c)
	As above: 7 days after slurry application/width 2 m	FC	MPN/100 ml	8	(c)
	As above: 7 days after slurry application/width 4 m	FC	MPN/100 ml	8	(c)
	As above: 7 days after slurry application/width 6 m	FC	MPN/100 ml	28	(c)
	As above: 7 days after slurry application/width 8 m	FC	MPN/100 ml	12	(c)
	As above: 21 days after slurry application/width 2 m	FC	MPN/100 ml	7	(c)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	f/c[e]
	As above: 21 days after slurry application/width 4 m	FC	MPN/100 ml					4				(c)
	As above: 21 days after slurry application/width 6 m	FC	MPN/100 ml					0				(c)
	As above: 21 days after slurry application/width 8 m	FC	MPN/100 ml					150				(c)

(Roodsari, Shelton *et al.* 2005)	USA, vegetative filter strips, controlled plot-scale lysimeter experiments to investigate factors controlling FC removal from bovine manure through surface runoff and infiltration, only vegetated plots (orchard and fescue grass) reported here. FC removal reported as reductions of mass of organisms in runoff compared with mass in manure, and therefore largely reflect amounts of infiltration [FC]: clay loam soil	FC	99	2.00	(f)
	As above: sandy loam soil	FC	100	total	(f)
(Schellinger & Clausen 1992) [cited by Roodsari *et al.* 2005]	Removal of bacteria in water from a dairy manure retention pond through a VFS [FC?]	FC?	30	0.15	

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: log_{10}	Attenuation: f/c[e]
(Stout 2005)	USA, vegetative filter strips, controlled laboratory plot-scale experiments using dairy manure, vegetated boxes and simulated rainfall to compare FC and phosphorus retention and effects of gradient and buffer length, only data for longest buffer (3 m) reported here [FC]: slope 1.1°	FC								67.3	0.49	
	As above: slope 2.3°	FC								69.7	0.52	

(Tate, Pereira *et al.* 2004)	Sierra Nevada foothills, California, US, soil box experiments, 1.1 m flow length, investigation of effects of slope and (simulated) rainfall intensity (30 to 47.5 mm/h for 2 hr) upon attenuation of *C. parvum* within annual ryegrass VBSs [*C.par*]: slope 2.9°	*C.par*	oocysts/l	96.369	1.44	(f)
	As above: slope 6.8°	*C.par*	oocysts/l	93.543	1.19	(f)
	As above: slope 11.3°	*C.par*	oocysts/l	93.393	1.18	(f)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: $\log_{10}$	Attenuation: f/c[e]
(Tate, Atwill *et al.* 2006)	Sierra Nevada foothills, California, US, 48 enclosed field plots to investigate effects of slope (2.9, 11.3 and 19.3°) and surface litter cover on EC attenuation within 3 m wide VBSs during natural rainfall events in 2002/4 [EC] – v high proportion of rainfall is absorbed by soil: results reported here for effectiveness of 1 m VBS	EC	cfu/100 ml							49.88 to 99.92	0.3 to 3.1	

(Trask, Kalita *et al.* 2004)	Use of tilting soil chamber to investigate effects of VBS, vegetation Bome (*Bromus inermus* Leyss.), slope (0.9–2.6o) and rainfall intensity in attenuation of *C. parvum* [*C. par*]: simulated rainfall 25.4 mm/hr for 44 min	*C.par*	oocysts/l		98.3 to 99.4	1.77 to 2.22	(f)
	As above: simulated rainfall 63.5 mm/hr for 44 min	*C.par*	oocysts/l		72.8 to 99.2	0.57 to 2.10	(f)
(Winkworth, Matthaei *et al.* 2008)	New Zealand, plot-scale field experiments (2.5 m long VBSs) using simulated runoff from ground-level drip irrigation system to evaluate effect of VBS, quite sparsely vegetated with native sedge cover (cf. bare soil), on 5° slope in attenuation of *Giardia* cysts [Gi]	*Gi*	cysts/l	am	26	0.13	(f)

(*Continued*)

Table 6.2 (*Continued*).

Reference	Study description [parameters measured][b]	Parameter[b]	Units	Stat[c]	Influent conc: n[d]	Influent conc: Mean	Effluent conc: n[d]	Effluent conc: Mean	Attenuation: Actual	Attenuation: %	Attenuation: $\log_{10}$	Attenuation: f/c[e]
F. In-stream ponds												
(Kay 2005)	SE England, Holland Brook, river water ponded behind tidal sluice, flow weighted input from several sources [EC, TC, IC]: Low-flow conditions	EC	cfu/100 ml	gm		6827		110	6717	98.389	1.79	(c)
	As above: high-flow conditions	EC	cfu/100 ml	gm		22443		570	21873	97.460	1.60	(c)

(Vinten, Sym *et al.* 2008)	W. Scotland, Cessnock catchment, stream water sampled upstream/downstream of small (0.6 ha) in-stream pond (a disused reservoir) [FC, FS]: runoff event conditions	FC	cfu/100 ml	gm	9	28184	8	2512	25672	91.087	1.05	(c)

[a] For each dataset results are presented for *E. coli* (EC) or faecal coliforms (FC), or an alternative parameter if neither was measured. Data for other FIOs and/or pathogens, which are used in compiling the summary statistics presented in Fig. 2, are included in the full database. [b] Parameters: FIOs (EC = *E. coli*, EN = enterococci, FC = faecal coliforms, FS = faecal streptococci, IE = intestinal enterococci, TC = total coliforms) and pathogens (*Ca* = *Campylobacter* spp., *C.per* = *Clostridium perfringens*, Co = coliphages, *Cr* = *Cryptosporidium* spp. (oocysts), *C.par* = *Cryptosporidium parvum*, Ev = enterovirus, *Gi* = *Giardia* spp. (cysts), G. duo = *Giardia diodenalis* (cysts), He = helminth (eggs), *M.av* = *M. avium*; [c] Stat(istic): gm = geometric mean, am = arithmetic mean, md = median; [d] n = Number of samples; [e] Attenuation expressed as flux (f) or concentration (c)

6.5 BMPs TO ATTENUATE PATHOGEN TRANSFERS TO WATERCOURSES

6.5.1 Containment of farm steading/feedlot sources

Contaminated water from farm steadings (i.e. yards and associated buildings) and cattle feedlots, if not intercepted and stored as part of a manure handling system for ultimate application to land, represents a potentially significant source of pathogen transmission to watercourses. Edwards *et al.* (2008), for example, report median FC concentrations of between 1.2×10^5 and 1.4×10^7 cfu 100 ml^{-1} in runoff from three farm steadings frequented by cattle in Scotland, and note that in one case steading runoff and dairy washings are piped to an adjacent watercourse. In order to minimise the volumes of dirty water generated during rainfall (to reduce storage requirements or the flow rate through treatment systems), CoGAPs generally recommend that relatively clean runoff from roofs is conveyed away from yard areas.

6.5.2 On-farm treatment of contaminated water

In general terms, three options for on-farm treatment of dirty water from steadings and feedlots can be distinguished: ponds, vegetative treatment areas for treating feedlot runoff and constructed farm wetlands, and these are discussed in detail below.

Ponds (see: Table 6.2, Section A). As noted above, ponds provide ideal conditions for microbial attenuation. However, they can also be a source of infection where livestock and/or birds have access (Jones 2005, Oliver 2007, Edwards 2008). Since the 1970s, two-stage waste stabilisation ponds (WSPs), comprising an anaerobic pond (AP) and a facultative pond (FP), have been widely used to treat dirty water on dairy farms in New Zealand. Laboratory studies have shown UV light to be the dominant cause of disinfection within WSPs (Davies-Colley 1999). While effective in lowering BOD and suspended solids, WSPs are less effective in removing FIOs. Sukias *et al.* (2001), for example, report median outflow FC concentrations of $3.5–8.0 \times 10^4$ cfu 100 ml^{-1} for traditional WSPs, while 1996 guidelines recommend a reduced median concentration of 2.4×10^4 cfu 100 ml^{-1} in the larger ponds. To address this constraint, advanced pond systems (APSs) are being developed and tested in which the FP is replaced by a high rate pond (HRP), algae settling ponds (ASPs) and a maturation pond (MP). Many investigators (Sukias, Tanner *et al.* 2001, Craggs, Tanner *et al.* 2003, Craggs 2004) found that APS effluent has a much lower EC concentration (median, 1.5×10^2 cfu MPN 100 ml^{-1}) than a conventional WSP (1.6×10^4 cfu MPN 100 ml^{-1}) treating the same farm

influent, and that the HRP, ASP and MP components of the system caused a 2.92 $\log_{10}$ EC reduction in the influent from the FP. $\log_{10}$ rates of EC attenuation for the treatment of sewage range from 1.50 for a FP (Reinoso, Torres *et al.* 2008) to 2.57 (dry conditions) and 3.64 (wet conditions, when influent concentrations were almost 10 × higher) for a large lagoon (Kay 2005). Attenuation of pathogens recorded in the FP ranged from 0.34 (coliphages) to 1.12 $\log_{10}$ (helminth eggs). Overall, the median microbial attenuation within ponds is 1.12 $\log_{10}$ (Figure 2).

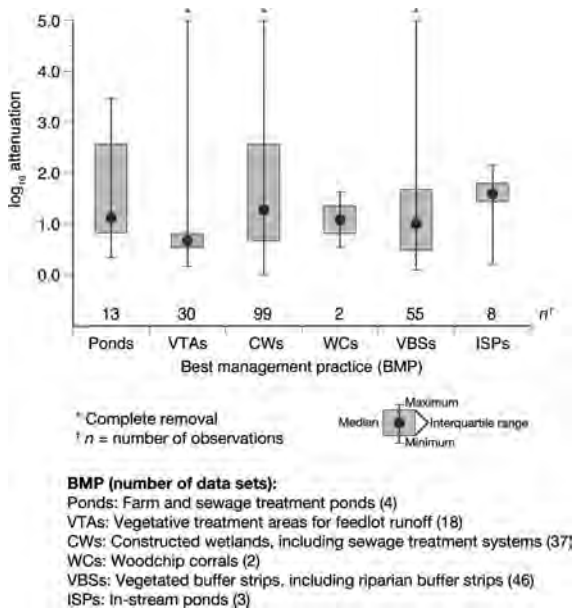


Figure 6.2 Summary pathogen/FIO attenuation data for the six BMPs for which datasets have been compiled.

Vegetative treatment areas (VTAs) for treating feedlot runoff (see: Table 6.2, Section B). VTAs are areas cropped with grass or forage through which influent, either directly from a feedlot or via a vegetative infiltration basin (VIB; see constructed wetlands, below), passes as a surface flow. Attenuation occurs as a result of filtration, sedimentation, soil infiltration and associated die-off. Gharabaghi, Rudra *et al*. (2006) report almost all particles >40 μm being captured within the first few metres of flow, whereas smaller particles generally remain in suspension and Schmitt, Dosskey *et al*. (1999) note the importance of infiltration (in relation to pesticides). Based largely on a review of >40 studies in the USA by Koelsch, Lorimor *et al.* (2006), FC attenuations of between 0.16

$\log_{10}$ and complete removal are recorded for VTAs, with lower/upper quartiles of 0.53/0.81 $\log_{10}$ (Figure 2). Often, VTAs are capable of absorbing all the runoff from individual storms – that is, there is complete attenuation of microbial flux during certain events. For example, Mankin *et al.* (2006) report zero runoff during 93% of individual runoff events from sites with a VTA:catchment area ratio (AR) of $\geq$0.5. The review identifies various factors, such AR, VTA slope, nature of vegetation cover and flow characteristics, as being critical in affecting attenuation rates – for example, Komor and Hansen (2003) report greater FC attenuation values over winter when the surface is covered by a mat of wilted grass (as compared to tall growing grass in summer). Various design recommendations are made (e.g. use of a pre-treatment sedimentation basin to minimise solid accumulation at the front end of VTAs, thereby minimising vegetation damage and the likelihood of channelized flow; and the design of inlets and headlands to initiate and maintain sheet-flow), and these have been formalised in a guidance document on VTAs (USDA 2006).

Constructed farm wetlands (CFWs) (see: Table 6.2, Section C). Constructed wetlands (CWs) are widely used as a means of municipal wastewater treatment (e.g. Kivaisi 2001, Song 2005, Vymazal 2006) and represent a sustainable, low-energy/maintenance form of treatment, particularly suited to rural areas (Kadlec & Knight 1996). Essentially, their design spectrum ranges from free water surface (FWS) systems that are either free of, or have different proportions of, emergent macrophytes (typically reeds) to subsurface flow (SSF) systems which act as a percolating filter, with wastewater being applied intermittently and seeping down through, or horizontally past, the plant rhizosphere and supporting media, such as gravel and sand (Oliver 2007). Components range from a simple pond to an engineered horizontal-flow reedbed. In practice, many multiple pond/wetland systems combine elements of this spectrum into a cascading system. However, there are few "design rules" or engineering loading calculations available for treating effluent parameters other than BOD. Observed reductions in pathogen concentrations are thought to be effected by a combination of predation (e.g. by protozoa and crustaceans, such as copepods (Song, 2008), die-off through UV disinfection and sedimentation, with HRT being an important factor affecting attenuation (e.g. Hodgson, Perkins *et al.* 2004, Vymazal 2005). Edwards *et al.* (2005) reviewed the efficacy of CWs in removing FIOs from municipal and domestic wastewaters. While attenuation rates are generally very good, they note that concentrations of FIOs in CW effluents often periodically exceed acceptable levels, especially at times of high flow when HRT is reduced.

CFWs, and analogous vegetative treatment systems (VTSs – for feedlot runoff), are used to treat flows of faeces-contaminated surface water before they are

discharged to watercourses (see: design manual for CFWs in Scotland and Northern Ireland (Carty, 2008)). They are typically used for more lightly contaminated runoff, such as drainage from roofs and yards that are used infrequently by cattle, rather than heavily contaminated, slurry-rich, daily yard and milking parlour washings associated with dairy activity. Since CFWs represent a form of wetland habitat creation or restoration, they enhance the landscape and biodiversity. Indeed, the integrated constructed wetlands (ICWs) concept, which was first pioneered in Ireland, specifically promotes a sustainable approach to natural resource management (Harrington, 2005, Harrington, 2007, Harrington, 2009). In Waterford, Ireland, 12 ICWs were established in 1999/2000, ranging from 0.3–2.2 ha in extent (approximately $1.4 \times$ the size of the associated steading). These have led to reductions in mean EC concentrations in yard runoff from 8.3×10^5 to 5.0×10^2 cfu 100 ml^{-1} (i.e. 3.22 $\log_{10}$ reduction) (Carty 2008), with a $\log_{10}$ reduction as high as 4.51 (from 9.6×10^5 to 3.0×10^1 cfu 100 ml^{-1}) in one 4-cell ICW (Mustafa 2009). Other reported rates of EC or FC attenuation in CFWs range from 1.61–3.56 $\log_{10}$ (excluding the site in SE Scotland where Gouriveau (2008) reports an increase in FC due to faecal inputs from wild birds), and Knox, Tate *et al.* (2007) report lower rates of EC attenuation at higher flow rates in irrigated beef cattle pasture in California. For municipal/domestic wastewater treatment the reported range is from 1.29–3.00 $\log_{10}$ for raw wastewater to 0.19–3.30 $\log_{10}$ for secondary or tertiary treatment. Although few data are available on pathogen attenuation in runoff from livestock units, Duggan, Bates *et al.* (2001) report $\log_{10}$ attenuation rates for *Campylobacter* spp. from poultry waste of 3.13 and 2.96, respectively, for sequential and continuous loading to a SSF reedbed. Drewe, Mwangi *et al.* (2009) report complete removal of *Mycobacterium avium* (causal agent of avian tuberculosis) within a reedbed treatment system for captive wildfowl waste. Wider ranges of attenuation have been reported for the treatment of municipal/domestic wastewaters: coliphages (0.67–2.64 $\log_{10}$), enterovirus (0.33–1.82 $\log_{10}$), *Clostridium* (0.28 $\log_{10}$–complete), *Giardia* (0.43 $\log_{10}$–complete), *Cryptosporidium* (0.28 $\log_{10}$–complete) and helminths (complete). Overall, the median microbial attenuation for CWs is 1.27 $\log_{10}$ (Figure 2).

6.5.3 Control of livestock on farmland

Streambank fencing and bridging. Where livestock have access to watercourses, faecal contamination can occur as a result of direct voiding and contaminants being washed from their hooves and lower legs. The flux of FIOs and pathogens will also be enhanced by disturbance of bed sediments, which are often contaminated with faecal material (Muirhead, Davies-Colley *et al.* 2004).

Detailed monitoring of EC concentrations downstream of a fording site on the Sherry River, New Zealand, revealed increases in EC concentrations from a background concentration of 3.0×10^2 cfu 100 ml^{-1} to a peak of 5.2×10^4 cfu 100 ml^{-1} as a result of a herd of 246 cows crossing during an 11-minute period (Davies-Colley 2004). With four crossings of the herd per day, the estimated daily release of EC to the stream was 8.9×10^{11} cfu, leading to an increase in the daily mean concentration from approximately 3.0×10^2 to 1.3×10^3 cfu 100 ml^{-1}. In this particular instance, bridging the stream would lead to a 0.62 $\log_{10}$ attenuation in the daily mean EC concentration and flux. Exclusion of livestock from watercourses through a combination of streambank fencing and, where necessary, replacement of fords by bridges, clearly eliminates direct pollution. Where fencing is set back from the bank to create a riparian vegetated buffer strip (VBS), then it will serve a dual function (Collins 2007). A New Zealand Ministry of Agriculture and Forestry report recommends that livestock also be excluded from areas of wetland and parts of slopes that are subject to intermittent channelized flow following rainfall (MAF 2006).

Minimising livestock congregation areas and soil poaching/Exclusion from areas and at times of high risk. Fluxes of pathogens from faeces voided by livestock on pastureland will be attenuated by reducing the likelihood of surface runoff, for example, minimising soil compaction and poaching by eliminating permanent congregation areas (e.g. through regular movement of water and feed troughs), and excluding livestock from areas of, and at times of, high runoff risk. Using a modelling approach, (McGechan, Lewis *et al.* 2008) have shown that the benefits of reducing soil compaction are potentially considerable, though they note that in reality it is difficult to prevent some degree of livestock congregation in fields.

Woodchip corrals (see: Table 6.2, Section D). Woodchip corrals (WCs) for outdoor over-wintering of beef cattle have recently been adopted quite widely in Great Britain and Ireland, with the number in Scotland thought to be at least 500 (McDonald, McDonald *et al.* 2008). They typically comprise a fenced and bunded enclosure with a 20–40 cm deep covering of woodchips. While providing significant benefits in terms of livestock management and welfare (Hickey, French *et al.* 2002), by removing cattle from fields they have the added benefit of reducing soil poaching and providing a means of containing faecal pollution. They produce two types of manure: a liquid fraction, which soaks through the woodchips, and a solid mixture of woodchips and slurry. The liquid fraction, in particular, poses a significant pollution risk (Edwards, Campbell *et al.* 2003), especially to groundwater where a corral overlying a permeable substrate is not lined. For example, in a study of leachates at the base of four corrals in Scotland, McDonald *et al.* (2008) recorded a maximum flux of $8.6 \times$

10^6 cfu m^{-2} h^{-1} during high-flow conditions following rainfall. Detailed monitoring of drain flow from the base of six corrals on a farm in SW Scotland showed a $\log_{10}$ attenuation in EC concentration of 0.53–1.63 through the woodchip fill (Vinten, Donnelly *et al.* 2006). Where the drain slots were backfilled with subsoil or topsoil, a very marked increase in $\log_{10}$ attenuation was recorded (range, 2.99–4.53), which presumably reflects the filtering and die-off of microbes within the soil fill. The study also indicated that EN tend to survive longer than EC within the woodchip matrix, and may possibly show signs of growth.

6.5.4 Control of manure application to land

Location and timing of application/Incorporation of manures in soil. CoGAPs generally include recommendations on the location and timing (e.g. avoidance of steep slopes, wet conditions and frozen ground) of manure applications, and on the incorporation of manure. While these undoubtedly contribute to the attenuation of microbial transport within catchments, there are few empirical data on attenuation rates.

6.5.5 Vegetated buffer strips (see: Table 6.2, Section E)

Vegetated buffer strips (VBSs), or "vegetative filter strips" (VFSs), are strips of land located either along river banks ("riparian buffer strips" (RBSs) or on slopes, designed to attenuate surface fluxes of sediments and other pollutants. While most attenuation occurs within the VBS, (Hussein, Ghadiri *et al.* 2008) report some retention of sediment-adsorbed *C. parvum* in the backwater zone created upslope of the buffer. Where land is grazed, RBSs need to be fenced to prevent direct faecal contamination, thereby also precluding livestock access to watercourses. CoGAPs usually include restrictions on the application of manure within a certain distance of watercourses, thus effectively creating or re-enforcing existing RBSs. As with CFWs, VBSs enhance landscape/habitat diversity and provide nesting/breeding sites and migration corridors for wildlife (Bradbury & Kirby 2006).

Reported microbial attenuation rates range from zero to complete removal, with lower quartile, median and upper quartile values of 0.48, 1.00 and 1.68 $\log_{10}$, respectively (Figure 2). It should be noted that these data will often overestimate the impact of RBSs, since significant numbers of FIOs and pathogens may enter watercourses via subsurface flow generated within the RBS. This is especially likely where there is rapid bypass flow through a soil, notably in naturally well-structured soils with continuous macropores or artificially drained soils (Collins 2004). Also, some microbes that become trapped at the surface may

survive temporarily in the moist and shady environment of a VBS and be remobilised in subsequent rainfall events. The FIO attenuation figures are similar to the much more extensive data available on the retention of sediments. In a review of over 40 studies covering 79 sites, Liu, Zhang *et al.* (2008) report median $\log_{10}$ trapping efficiencies for both RBSs and VFSs (on slopes) of 0.87, with lower/upper quartiles of 0.63/1.15 and 0.62/1.52, respectively, and the results of regression analyses that reveal a $\log_{10}$ sediment reduction of 1.32 for the most effective combination of buffer strip width (10 m) and gradient (5.14°).

Inevitably, the efficiency of a VBS will vary according to local, site-specific factors such as slope angle (both upslope and within the VBS), the size distribution of transported particles, and soil and vegetation characteristics within the VBS. The key VBS design variable is strip width (i.e. length of flow). Data relating to FIOs are very limited and while several studies have reported increases in attenuation with wider strips (e.g. Young, Huntrods *et al.* 1980, Collins, Ross *et al.* 2002, Goel, Rudra *et al.* 2004, Liu, Zhang *et al.* 2008, Hay 2006, Tate, Atwill *et al.* 2006), others have found no clear relationship with width (e.g. Entry, Hubbard *et al.* 2000, Núñez-Delgado, López-Periago *et al.* 2002). The underlying importance of buffer width is, however, clearly confirmed by the statistically significant correlation ($p < 0.001$) between sediment removal and $\log_n$ buffer width reported in an analysis of published data from 79 sites (Liu, Zhang *et al.* 2008). The latter study also revealed that VBS efficiency peaks at a slope angle of 5.26°. Below this optimal angle, diminishing lateral flow rates limit sediment trapping, whilst at steeper gradients sedimentation is more likely to be compromised by increased channelization of flow and shorter retention times. Attenuation rates for an individual VBS will also vary temporally, depending on factors such as antecedent weather conditions and the magnitude of runoff events. Several studies have reported reduced attenuation of FIOs and/or pathogens at times of greater runoff/higher rainfall intensity (e.g. Collins 2004, Trask, Kalita *et al.* 2004, Tate, Atwill *et al.* 2006).

Because of the inadequacy of the FIO/pathogen database, the few models that have been developed for microbial attenuation within VBSs rely on the adaptation of models developed for other parameters such sediments, nutrients and pesticides. While laboratory experiments have demonstrated that rates of nutrient and FIO attenuation are quite closely correlated (e.g. Stout 2005), erosion-based sediment models are more commonly used. Examples include VFSMOD developed by Muñoz-Carpena, Parsons *et al.* (1999) to model runoff and sediment transport within VFSs, and the Universal Soil Loss Equation (USLE), which is used by the US Natural Resources Conservation Service to set standards for buffer width:catchment area ratios for different *R* factors (rainfall amounts and intensities).

In a recent study the Chemicals, Runoff and Erosion from Agricultural Management Systems (CREAMS) model (Cooper, Smith *et al.* 1992) for sediment attenuation has been adapted for FIOs, and applied to the major soil groups of New Zealand (MAF) 2006. In this study particular attention was given to the influence of particle size, with free-floating bacteria being assumed to behave as clay particles, and attached microbes as larger particles (i.e. silts and sands). Although a lack of understanding of the form in which microbes are transported in surface runoff limits the veracity with which the model might be applied in specific circumstances, the model outcomes highlight some of the key factors affecting attenuation rates. The results generated optimum RBS widths (taking into account the amount of land given over to the buffer) and associated efficiencies for the following combinations of site characteristics: slope, soil drainage characteristics and degree of bacterial attachment. Overall, the modelled rates of FIO attenuation range from 0.10–1.30 $\log_{10}$, with wider buffer strips needed on steeper slopes, where soil infiltration rates are lower, and/or where a low proportion of bacteria are attached to particles. The model presented does not take into account very small microbes (e.g. viruses of typically 25–350 nm) for which entrapment efficiencies are likely to be lower than for those microbes modelled, nor of the possibility of the survival and re-mobilisation of microbes during subsequent runoff events. The model is based long-term average rainfall conditions (efficiencies are likely to be lower during events of higher intensity). Despite these limitations, the model provides a good basis for developing design guidelines.

Since the function of the VBS is to filter out pollutants from surface runoff, the measure is likely to be relatively ineffective in two circumstances (MAF) 2006. First, on steeper slopes there is likely to be greater convergence of surface (and subsurface) flow, resulting in channelized flow with greater velocity (cf. diffuse sheet flow), much of which will pass rapidly through and have little contact with the VBS (Collins 2005). Where channelization does not occur, Atwill, Hou *et al.* (2002) found in soil-box experiments that for VBSs with slopes of 3–11° attenuation may increase with gradient as a result of the reduced depth of flow. Secondly, where the soils have a high infiltration capacity, then some flow will again bypass the VBS. Where vertical flow is rapid, for example in well-structured clay soils, quite high proportions of microbes are likely to be carried down through the soil and, ultimately, into adjacent watercourses. For example, Collins (2004) reports considerable volumes of subsurface flow bypassing RBS study plots on Hamilton clay loam soils in New Zealand, and Núñez-Delgado, López-Periago *et al.* (2002) found quite high concentrations of FIOs, especially FS ($>1.1 \times 10^3$ MPN 100 ml^{-1}), in soil waters at depths of 31–51 cm within a VBS. In contrast, porous soils

that lack a well-developed structure will be effective in filtering microbes (e.g. certain allophonic and volcanic soils (McLeod, Aislabie *et al.* 2001).

6.6 BMPs TO ATTENUATE PATHOGEN CONCENTRATIONS WITHIN WATERCOURSES

Once pathogens/FIOs enter a watercourse there will some degree of natural attenuation as a result of sedimentation of particle-attached microbes, die-off through exposure to sunlight and predation. Two BMPs are currently employed to increase the effectiveness of these attenuation processes:

Grassed waterways ("swales"). Grassed waterways function very much as VBSs (Liu, Zhang *et al.* 2008). They may be either natural features on slopes, along which surface runoff may occur following rainfall (i.e. a natural extension of the drainage network), or constructed features, such as those connecting different components of a water treatment system. Contaminated runoff from cattle tracks might also be diverted through grassed waterways before entering a permanent watercourse. In their review of published data Liu, Zhang *et al.* (2008) report a median $\log_{10}$ sediment attenuation rate of 1.15 for three grassed waterway sites.

In-stream ponds (see: Table 6.2, Section F). Large bodies of water are very effective in the attenuation of FIOs. Crowther *et al.* (2010), for example, report high-flow GM FC and EN concentrations of 8.3×10^1 and 1.6×10^1 cfu 100 ml^{-1}, respectively, in effluent waters from five upland lakes and reservoirs in England and Wales, compared with GM inputs from areas dominated by rough grazing of 8.6×10^3 and 1.2×10^3 cfu 100 ml^{-1}, respectively (Kay, Crowther *et al.* 2008) – that is, attenuation rates of *c.* 2.00 $\log_{10}$. For smaller ponds on streams, rates of attenuation are likely to be generally lower, and quite variable, depending on local factors such as the ratio of flow/pond volume, retention time and water depth, and again there are relatively few published data. Vinten, Sym *et al.* (2008), for example, report a 1.05 $\log_{10}$ attenuation in GM FC concentration during rainfall events through a small, 0.6 ha, in-stream pond (a disused reservoir) in a dairy farming-dominated catchment in Scotland. Attenuation of FS was much less (0.20 $\log_{10}$), which is likely attributable to inputs from nesting/roosting birds. Somewhat higher rates of attenuation have been reported where river water from a semi-urbanised arable catchment in SE England is temporarily impounded behind a tidal sluice gate. In the case of GM EC, for example, the $\log_{10}$ attenuation rates reported are 1.79 during dry weather conditions and 1.60 under high-flow conditions following rainfall.

6.7 EVIDENCE OF THE EFFECTIVENESS OF CATCHMENT-SCALE BMP IMPLEMENTATION ON WATER QUALITY

Hitherto, the adoption of BMPs to address concerns about livestock-derived faecal contamination of watercourses has generally been sporadic and typically confined to a small proportion of farms and/or land area within catchments, thereby precluding meaningful empirical investigation of changes in catchment-scale microbial fluxes. One notable exception was a 10-year (1987–96) longitudinal study in the Piedmont region of Virginia, USA, in which a 11.63 km^2 catchment monitored for two years prior to the introduction of BMPs (manure storage, streambank fencing, use of water troughs, etc.) and for seven years afterwards (Inamdar, Mostaghimi *et al.* 2002). While reductions in both FC and FS concentrations were recorded at the main watershed outlet, no improvement was detected in certain subcatchments. Similarly equivocal results were reported for longitudinal study undertaken of stream water quality in four catchments in north and west Scotland (Kay, Wilkinson *et al.* 2005, Kay, Aitken *et al.* 2007). Here there was quite intensive targeted implementation of measures (streambank fencing or control of steading runoff) in selected sub-catchments, with a series of nearby 'control' subcatchments in which no measures were introduced. The data, which were seasonally adjusted to allow for the fact that the pre- and post-remediation sampling had to be undertaken at different times of the year, indicate reductions in high-flow GM EC and IE concentrations of *c.* 0.52 $\log_{10}$ where the extent of new streambank fencing is >40% of streambank length (CREH 2006). In contrast, in the sub-catchments where the steading measures were implemented, no improvement in water quality was detected.

In other studies, models have been developed to predict the effects of particular BMPs upon water quality at the catchment outlet. Collins & Rutherford (2004), for example, developed a spatially distributed model using the Watershed Assessment Model (WAM; Bottcher, Hiscock *et al.* 1998) in which fluxes and die-off of EC are simulated on a cell-by-cell basis across a catchment. Scenario modelling, which was applied to steep hill-country catchments grazed by beef cattle and sheep (as compared to lowland pastoral landscapes in which dairy cattle are dominant), predicted $\log_{10}$ reductions in median EC concentrations of 0.11 and 0.19 in two simulations of the effects of RBSs. Also, (McGechan, Lewis *et al.* 2008) have combined the MACRO model (to simulate transport from fields by surface runoff and macropore flow to field drains; (Jarvis 1994) with a channel network model to simulate EC fluxes from catchments. The resulting model, which was calibrated and tested on a dairy farm in West Scotland, has been used to investigate the effects of a range of BMPs during the summer grazing season:

avoidance of lagoon overflow, elimination of steading runoff, prevention of cattle access to streams, prevention of soil compaction in cattle congregation areas – each of which was shown to reduce the output load of EC from the catchment.

6.8 SYNTHESIS AND CONCLUSIONS

Ideally, agencies responsible for the design and implementation of POMs to attenuate the transport of livestock-derived pathogens and FIOs within catchments require accurate empirical data characterising the rates of attenuation associated with each BMP. Unfortunately, the effectiveness of certain measures is overwhelmingly site-dependent. For example, the reductions in microbial fluxes resulting from the containment of steading or feedlot runoff that was previously transmitted to a watercourse, or from streambank fencing/bridging, are both critically dependent upon levels and patterns of usage by livestock, which will vary from farm-to-farm and field-to-field. In other cases, discrete influent and effluent waters associated with a particular BMP cannot readily be identified and monitored (e.g. practices relating to the location and timing of grazing and manure applications to avoid areas and times of high pollution risk). The situation is further complicated by the fact that a BMP may eliminate one source of faecal contamination, yet create a potential secondary source(s), for example, containment of steading runoff creates a need for treatment of the contaminated water, with resultant effluent fluxes, or disposal to land with attendant risks of microbes being transported to watercourses via surface runoff and/or bypass flow through soils.

In the present chapter, datasets presented for six BMPs have shown each to be effective in attenuating microbial fluxes within catchments. As would be anticipated from the nature of the data reported, all six measures exhibit very marked variability (Figure 2). The median values also differ quite markedly, with values ranging from 0.67 (VBSs)–1.59 $\log_{10}$ (ISPs). Interestingly, somewhat higher median rates of attenuation ($\geq$1.12 $\log_{10}$) are recorded for the three BMPs that include ponding of water and/or wetlands. A median reduction of 1.08 $\log_{10}$ is recorded for WCs, though this is based on just two data values. By comparison, a lower median $\log_{10}$ attenuation is recorded for the two systems based on dry-land vegetative systems for attenuating microbial fluxes in surface flows: VTAs (0.67) and VBSs (1.00). In addition, a range of other measures identified in the chapter needs to be considered in the design of POMs. Some, such as streambank fencing/bridging and the containment of steading and feedlot runoff that would otherwise discharge to streams, will clearly have a direct and immediate impact, whereas the effectiveness of others is less easily quantified.

Clearly, much more empirical work on microbial attenuation is needed to augment the datasets presented here and to address the various data gaps identified. Two areas in particular merit detailed attention. First, a much better understanding is needed of the spatial and temporal variations in microbial source strengths and pollution risk within different livestock farming systems in order that pollution "hot spots" can be targeted for remedial action. One important development in this field is the pioneering modelling work currently being undertaken by ADAS UK Ltd to quantify the proportions of FIOs generated by different livestock (e.g. dairy cattle, beef cattle, lowland sheep, upland sheep and broiler chickens) that are released at different points within the landscape: steadings, fields, streams, tracks, spreading of manure, and so on. (Steven Anthony, ADAS, pers. comm.). Secondly, very little is known of the effectiveness of BMPs in reducing microbial fluxes at a catchment scale. Longitudinal studies are fraught with difficulties and hitherto the results have proved somewhat equivocal (Inamdar, Mostaghimi *et al.* 2002), (CREH 2006). Modelling-based approaches, such as those developed by Collins & Rutherford (2004) and McGechan, Lewis *et al.* (2008), supported by empirical data, perhaps provide the most effective way forward.

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7

Exposure

Will Robertson and Gordon Yasvinski

7.1 INTRODUCTION

A variety of microorganisms (viruses, bacteria and parasites) have the capacity to transmit disease through contact with contaminated water (US EPA 2009). Waterborne infectious diseases caused by these microorganisms can be fatal. Globally, over 1.5 million people die each year from unsafe water, inadequate sanitation and insufficient hygiene as a result of diarrhoeal diseases, schistosomiasis and others (Prüss-Üstün *et al.* 2008). In the USA alone, between 1971 and 2000 43 outbreaks involving almost 1800 cases associated with untreated recreational surface waters were attributed to zoonotic pathogens such as pathogenic *E. coli*, *Leptospira*, *Giardia*, *Cryptosporidium* and *Schistosoma* (dermatitis) (WHO 2004). Of these outbreaks, ten were attributed to animal and avian sources.

Catchment area loading with livestock wastes and the transport of this contamination to water bodies are of growing concern for their potential effects

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on the quality of natural waters used for a variety of human contact activities. The question of the magnitude of these impacts on waters is one of significant importance to those involved in ensuring the safe use of these waters. The focus of this chapter is to provide discussion on the places that can be contaminated by livestock waste and the factors influencing the potential for human exposure, and possible infection and illness.

7.2 RELEVANT WATER TYPES

The water types of interest for these discussions are surface waters that are impacted by contamination from livestock waste (either directly or through transport) and that also support activities that can lead to human exposures to contaminated water. Contamination inputs within a catchment area can include both point and diffuse sources. Examples of point source inputs are intensive livestock operations (dairy/swine/poultry barns and feedlots) and animal processing facilities (slaughterhouses). Diffuse sources are pasturelands or grazing ranges, which can vary in size from large farms to households having a small number of domestic animals.

Specifically, the types of surface water encompassed by this definition include traditionally recognized water bodies such as coastal marine waters, and freshwater rivers, lakes and streams. Also certain less conventional water types such as rice paddies or flooded fields are covered by the definition, as are standing waters that have been created by flood events. In general these waters are located primarily in rural areas, but transport and weather-related pressures affecting pathogen movement can also contribute to the occurrence of exposures in urban areas.

The type of water use plays a large role in dictating which water areas may have an associated contamination exposure risk. This can be strongly influenced by geographical, social and economical factors. Uses for persons in developed countries where water is abundant, can be entirely different from uses for those living in developing countries or impoverished areas where water resources are far more scarce.

7.3 FACTORS INFLUENCING HUMAN EXPOSURE, INFECTION AND ILLNESS

In assessing the potential for the risk of human exposure to waterborne pathogens at the point of water contact, it is important to consider issues influencing the probability of human contact, infection and illness. Three

main components influence the probability of human infection by pathogenic organisms: the environment, the pathogen and the host. The principle behind each component and the considerations for contributing to exposures are the following:

- Environment: The environment must be favourable to permit the pathogen's survival and pathogen-host interaction.
- Pathogen: The pathogen must be present and possess the specific virulence factors required for it to cause infection; and these factors must be successfully expressed in the host environment.
- Host: The host must take up the pathogen and be susceptible to infection.

If a pathogen is to be successful in initiating infection, all three of these components must be satisfied. Should infection be established, the resulting disease can range in severity from asymptomatic, through mild and severe to lethal.

The interaction between these factors is depicted in a simplified illustration in Figure 7.1. The full impact of the pathogen on the risk of exposure and illness can be expected to occur when environmental interaction, host susceptibility and pathogen virulence are considered high. Similarly, the pathogen's impact is reduced if one or more of these determinants are lowered.

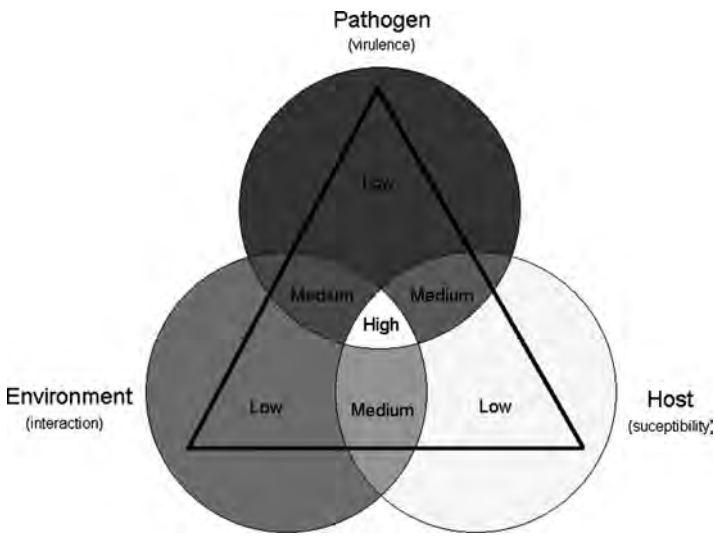


Figure 7.1 Risk triangle: Factors influencing exposure, infection and illness from zoonotic pathogens.

7.3.1 Environment

Preceding chapters have discussed the steps and conditions required for the transmission of viable zoonotic pathogens from livestock wastes to surface waters, and the factors required for understanding transport within these water bodies. Continuing this discussion as it relates to human exposure, it is recognized that various pressures within the water environment can influence the ability of a pathogen to reach points where human contact is possible. Different water types possess different intrinsic properties (sizes, depths, flow or circulation rates) that can affect pathogen movement. For example, coastal waters are affected by tides, inflows from discharging rivers, deep and shallow currents, and up- and down-welling effects caused by temperature and salinity differences. Inland lakes, especially smaller lakes can be considered relatively static water bodies. Inflows from rivers and streams can contribute to transport and mixing in these waters, and thermal stratification in stagnant lakes can lead to vertical movement of contaminants through up- and down-welling events. Larger lakes (for example, the North American Great Lakes) can be more comparable to coastal waters, having similar mixing pressures. Rivers, by comparison are very dynamic water bodies, where the primary mixing factors are the flow rate or mechanical speed and the turbulence effects (e.g. eddies) created by flow obstructions.

Sedimentation and resuspension are additional processes that operate alongside flow and circulation processes in determining microbial transport and distribution (Brookes *et al.* 2004). Attractive forces or the presence of substances such as extracellular polymers can allow microorganisms to attach to particles in the water column. Microorganisms attached to particles will settle more quickly than free, unattached microorganisms (Brookes *et al.* 2004, Characklis *et al.* 2005). Reciprocally, bottom sediment disturbances can result in the resuspension of settled microorganisms back into the water column. Jamieson *et al.* (2004) demonstrated that water quality in rural streams could be significantly influenced by faecal microorganisms in stream bed sediments. Disturbances can be the result of flow changes (currents, wave action or weather-induced flow changes) or due to human or animal activities within the watershed (motorized watercraft, canoeists, dredging activities or animals). This phenomenon of microorganism resuspension can create unexpected pathogen presence scenarios (Brookes *et al.* 2004).

Some transport and mixing pressures, like weather-related influences are common to all water types. Circulation and flows for coastal waters, lakes and rivers alike are aided by the action of winds.

Rainfall is an extremely important factor influencing the contamination of water bodies with faecal contamination from non-point sources. Faecal pathogen events in rivers, lakes and reservoirs have been shown to be associated with

rainfall events (Atherholt *et al.* 1998, Kistemann *et al.* 2002, Dorner *et al.* 2007). Krometis *et al.* (2007) observed that average stream water concentration of the faecal indicators thermotolerant coliforms and *E. coli* were highest in the earliest stages of a storm (referred to as the "first flush" phenomenon). The researchers additionally estimated that wet-weather events of short-term duration contribute to a much greater fraction of microbial loading of a waterway than lengthier dry weather events (Krometis *et al.* 2007). In an investigation of the effects of wet weather on pathogen and indicator concentrations in an agriculture-intensive watershed, Dorner *et al.* (2007) observed that during storm events, the peak *Campylobacter* concentration arrived earlier than the peak turbidity level. The authors speculated that this was because pathogens are generally in limited supply within a watershed and are therefore more likely to be flushed out of the stream before the turbidity level declines.

Research has recently highlighted evidence of a connection between extreme wet-weather events, microbial transport from non-point faecal sources and elevated risks of waterborne illness (Curriero *et al.* 2001). Flooding caused by heavy rains or disaster events such as hurricanes or tsunamis can create vast areas of standing water and with it new areas for potential pathogen exposures. It is expected that effects linked to climate change such as elevations in environmental temperatures and increases in extreme hydrological events may further contribute to water quality impairment by faecal pollution of livestock origin and thus further increase the potential for human exposures and illness.

Various physical, chemical and biological pressures can also have an effect on pathogen survival in the water column, including sunlight intensity (solar radiation), water temperature, salinity, pH, nutrient availability, the presence of toxic substances, predatory grazing by protozoa and other invertebrates, and competition from native microorganisms.

Numerous studies have examined the effects of temperature on the survival of faecal microorganisms in the aquatic environment. Research suggests that *Cryptosporidium* oocysts can survive for periods of weeks to several months at temperatures frequently encountered in the environment (4–25°C) (Robertson *et al.* 1992, Medema *et al.* 1997, King *et al.* 2005) and are capable of maintaining a high level of infectivity at temperatures below 15°C (Fayer *et al.* 1998). Data have also been generated (Fayer & Nerad 1996) indicating that oocysts frozen for short periods of time (several days) at low freezing temperatures (−10°C) are able to maintain viability and infectivity. The capacity for oocysts to persist for very long periods in the environment at low temperatures remains uncertain. A recent study designed to replicate winter survival conditions in a northern aquatic environment (Robertson & Gjerde 2006) determined that no viable *Cryptosporidium* oocysts or *Giardia* cysts could

be detected (dye uptake method) after 20 weeks and one month, respectively. For faecal bacteria, data from temperature exposure experiments conducted with *E. coli* (Rhodes & Kator 1988, Sampson *et al.* 2006), *Campylobacter* (Obiri-Danso *et al.* 2001), *E. coli* O157:H7 (Wang & Doyle 1998) and *Salmonella* (Rhodes & Kator 1988) suggest these organisms are capable of surviving for days to several weeks at temperatures above 15°C. Similarly, water temperatures below 10°C can permit longer survival times. Thomas *et al.* (1999) reported that a *C. jejuni* population of greater than 4 log (initial concentration >6 log cfu/mL) could be maintained for more than 60 days in sterile river water at a temperature of 5°C. Sampson *et al.* (2006) observed less than a 1 log decline from a 5 log population of *E. coli* after storage for 30 days at 4°C in lake water. Information resulting from studies comparing the temperature-related survival of faecal indicator bacteria to bacterial faecal zoonotic pathogens has often been conflicting, with some studies observing prolonged survival for the pathogens (Roszak 1984, Rhodes & Kator 1988) and others showing equivalent or greater survival for the indicator species (McCambridge & McMeekin 1980, Korhonen & Martikainen 1991).

Sunlight intensity is another significant factor affecting the survival of faecal microorganisms in aquatic systems. Experiments with faecal zoonotic pathogens (*Cryptosporidium, Campylobacter*, *Salmonella*) and indicators (*E. coli*, faecal coliforms, enterococci) have demonstrated that inactivation is strongly influenced by the intensity of sunlight, with significantly greater die-off rates occurring under sunlight exposure than under dark conditions (Fujioka *et al.* 1981, Johnson *et al.* 1997, Obiri-Danso *et al.* 2001, Sinton *et al.* 2002, King *et al.* 2008, Nasser *et al.* 2007, Schultz-Fademrecht *et al.* 2008). Similarly, data have been provided to suggest more rapid inactivation under solar irradiation levels more typical of summer as compared to winter sunlight conditions (Noble *et al.* 2004, Sinton *et al.* 2002). Studies in which the survival ability of different faecal microorganisms have been directly compared have demonstrated a more rapid die-off for the bacterial species (*E. coli*, enterococci, *Salmonella*) as compared to the faecal protozoa *Giardia* and *Cryptosporidium* (Johnson *et al.* 1997, Medema *et al.* 1997. Nasser *et al.* 2007).

Also important in the consideration of the exposure environment are the activities which bring the human population in contact with the areas of pathogen presence. In those developed countries where water resources are in abundance, the primary human activities expected to facilitate contact with livestock-contaminated waters are recreational uses and potentially through drinking-water consumption.

For recreational water uses, it has been proposed that activities can be separated into two categories as defined by the degree of water exposure expected: primary-contact and secondary-contact (WHO 2003). In primary or whole-body

contact activities, the whole body, including the head is intentionally immersed in the water or wetted by spray. Hence, it is likely that some water will be swallowed (WHO 2003). The most common primary-contact activity is swimming, which includes related actions such as diving or jumping into the water. Other common examples are wading, surfing, water-skiing, windsurfing, scuba-diving and snorkelling. White-water sports such as canoeing, kayaking, tubing and rafting are also considered to fit in this category. In secondary or incidental contact activities only the arms and legs are intentionally exposed, and greater contact is unusual (WHO 2003). Examples of secondary-contact activities would be rowing, canoeing or kayaking; power boating and fishing. Another important consideration for the discussion of recreational contact relates to the location of potential exposures. Swimming activities occur near shorelines and thus users may be located in closer proximity to contamination inputs. Other activities like waterskiing or surfing can have immersion exposures that occur at significant distances from shore. Secondary contact activities such as recreational boating or canoeing may allow bathers to access possibly more polluted water closer to the source of contamination.

Drinking-water exposures in developed countries would be largely restricted to instances where livestock-contaminated source waters were untreated or incompletely treated prior to human consumption.

In developing countries, contact circumstances can be expected to be entirely different from the developed world. Numbers published by the WHO and UNICEF suggest that as of 2000, 1.1 billion people lacked basic access to a water supply within 1 kilometre of their home (Howard & Bartram 2003). In poor rural communities water resources can be shared by multiple households and would support a variety of sanitary or hygienic and household uses. Expected water contact activities would be bathing and laundering, and other uses such as drinking or handwashing may also occur if there is inadequate household access to water (Howard & Bartram, 2003). It is also expected that in certain countries a greater number of exposures may arise through occupational circumstances. Examples include domestic farmers working in rice paddies, and military personnel on manoeuvres in rural areas. Rescue workers providing relief in floodwaters produced by natural disasters are a noteworthy example of occupational exposure created by unpredictable circumstances. This scenario would have importance in both the developing as well as the developed world. In interpreting the degree of water exposure associated with this range of activities, it is possible that the WHO contact definitions can also be applied here. For the examples described, bathing might be considered a primary-contact activity, while laundering and occupational exposures may be more representative of secondary contact.

An area of emerging interest that perhaps can be considered a subcategory of water contact relates to contact with pathogens that may be present in contaminated sands or sediments directly adjacent to surface waters. Sands and soils have been noted as a potential medium for human contact with microorganisms (including faecal microorganisms) that have been transported to this environment (Bolton *et al.* 1999, Obiri-Danso & Jones 1999, WHO 2003, Whitman & Nevers 2003, Edge 2008). Numerous studies have demonstrated the extended persistence of faecal microorganisms in sand and sediments of environmental waters (Davies, *et al.* 1995, Byappanahalli & Fujioka 1998, Desmarais *et al.* 2002, Brookes *et al.* 2004, Byappanahalli *et al.* 2006). Greater nutrient availability and increased protection from predation in this environment have been suggested as explanations for this effect. Contact activities important for this issue would be those occurring near shore to contaminated surface waters or in shallow water areas. Potential areas of relevance could include contaminated beach sands, rice paddies and flooded agricultural fields.

7.3.2 Pathogen

Various waterborne zoonotic bacteria and protozoa can cause gastrointestinal illness in humans. Yet for a variety of reasons it is not practical to routinely monitor them in faecally-contaminated water including those impacted by livestock wastes and used for various water contact activities (WHO 2003). Instead, these waters, particularly those used for primary contact recreational use, are routinely tested for faecal indicator organisms The presence of faecal indicator organisms (FIOs) in water indicates that it has been subject to recent faecal contamination and may therefore contain zoonotic pathogens and present a risk to bathers. Other studies have measured FIOs or zoonotic pathogens in surface waters usually as part of research programmes directed towards microbial risk assessments or the development and evaluation of best management practices. Our knowledge of their presence, persistence and infectivity is limited. A number of notable studies where zoonotic pathogens and waterborne FIOs have been measured in waters with known livestock impacts are presented in Tables 7.1 and 7.2.

In general Tables 7.1 and 7.2 illustrate that FIOs are definite indicators of the presence of livestock wastes in surface waters and that livestock wastes can contribute zoonotic pathogens to surface waters. In addition, there does not appear to be a good correlation between FIOs and the presence of waterborne zoonotic pathogens, although the data are limited (Dorner *et al.* 2007, Till *et al.* 2008). There may be several reasons for this discrepancy. For example, FIOs are present in much greater concentrations in livestock wastes and in contaminated

Table 7.1 Notable studies: Areas with livestock impacts – pathogen monitoring.

Location	Organism	Results	Comments	Reference
Japan	*C. parvum*	Overall: 50% of sites Dairy farming areas: 88% of sites Horse-rearing areas: 0% of sites	Rivers – significant dairy farming and horse rearing.	Tsushima *et al.* 2001
USA	*Cryptosporidium spp.*	0–40% of sites 36% of samples	Watershed with small, concentrated dairy industry; drinking water source.	Sischo *et al.* 2000
Japan	*C. parvum*	37–100% of sites 1.4–2.4 oocysts/20 L	Rural sites, varying livestock populations (cattle, swine, poultry)	Ono *et al.* 2001
Swaziland	*E. coli* O157	12 of 81 (15%) of samples	River – Heavy rains, cattle faeces, outbreak from untreated drinking water.	Effler *et al.* 2001
New Zealand	*Campylobacter*	(3 beaches) 85, 74, 52% of samples Median: 0.84, 0.36, 0.12/100 mL	Predominantly agricultural catchment, levels higher during summer months.	Eyles *et al.* 2003
Malaysia	*Giardia spp.* *Cryptosporidium spp.*	4–23% of samples; 1.3–9.0 cysts/L 12–21% of samples; 0.7–240 oocysts/L	Rivers near cattle farms with potential bathing/swimming uses.	Farizawati *et al.* 2005
Canada	*Campylobacter* *E. coli* O157:H7 *Giardia* *Cryptosporidium spp.*	50% samples; Med: 63 /100 mL ($1.2–1.2 \times 10^6$) 6.7% samples; Med: 100/100 mL (100 to 110) 35% samples; Med: 22/100 L ($2–1.0 \times 10^4$) 37.9% samples	River watershed – Site with high livestock density. Weak correlations between pathogens and faecal indicators reported.	Dorner *et al.* 2007

(*Continued*)

Table 7.1 (*Continued*).

Location	Organism	Results	Comments	Reference
USA	*Cryptosporidium* spp.	Median: 70 oocysts/10 L	Lake with significant agricultural communities; cattle grazing.	Keeley & Faulkner 2008
	Giardia spp.	Median: 5 cysts/10 L		
New Zealand	*Campylobacter* spp.	58% of samples	Dairy cattle predominant impact at five freshwater recreational water sites	Till *et al.* 2008
	Salmonella spp.	7% of samples		
	Giardia spp.	8% of samples		
	Cryptosporidium spp.	5% of samples		
New Zealand	*Campylobacter* spp.	66% of samples	Sheep/pastoral predominant impact at six freshwater recreational sites. Poor correlations reported between *E. coli* and all pathogens except *Campylobacter*.	Till *et al.* 2008
	Salmonella spp.	21% of samples		
	Giardia spp.	6% of samples		
	Cryptosporidium spp.	2% of samples		
Kenya	*C. andersonii*	25% of sites	River site surrounding pasture used for herded animals	Muchiri *et al.* 2009
	C. parvum,	0% of sites		
	C. hominis	0% of sites		
Spain	*Cryptosporidium* spp.	93% of sites; 2–1350 oocysts/L	River basin – dairy farming; drinking, recreational water uses.	Castro-Hermida *et al.* 2009
	Giardia duodenalis	100% of sites; 2–722 cysts/L	*C. hominis* most frequent at rec. areas; *C. parvum* and *C. andersonii* in river samples	

Table 7.2 Notable studies: Areas with livestock impacts – indicator monitoring.

Location	Organism	Results	Comments	Reference
Hong Kong	*E. coli* Enterococci	Geo. mean: 978 cfu/100 mL Geo. mean: 144 cfu/100 mL	Two beaches impacted by livestock wastes (mainly swine)	Cheung *et al.* 1990
USA	Faecal coliforms	Day 2 (leading edge): $>1 \times 10^6$ cfu/100 mL Day 14: 100–1000 cfu/100 mL	River – Accidental swine waste spill	Burkholder *et al.* 1997
UK	*E. coli* Enterococci *E. coli* Enterococci	River 1: 56–8300 cfu/100 mL River 1: 9.5–810 cfu/100 mL River 2: 120–19000 cfu/100 mL River 2: 17–3300 cfu/100 mL	Two rivers: Livestock predominate in catchments; outflows near bathing beaches. High flow samples statistically higher than low flow samples.	Crowther *et al.* 2003
USA	Faecal coliforms	Monthly samples: 50–17,400 cfu/100 mL Spring high flow: 30–3480 cfu/100 mL Autumn low flow: 30–4480 cfu/100 mL	Watershed – Pasture 60% of land use; cattle have free access to stream at many locations.	Graves *et al.* 2007
Canada	*E. coli*	Daily geo. mean: 14–2189 cfu/100 mL Seasonal geo. mean: 32–186 cfu/100 mL	Bathing beach – located at mouth of river draining mainly agricultural area. 1999–2008 seasonal data.	Huron County Health Unit 2008

(*Continued*)

Table 7.2 (*Continued*).

Location	Organism	Results	Comments	Reference
USA	Enterococci	Site 1: Geo. mean 2600 cfu/100 mL (95% C.I. 1011–6685) Site 2: Geo. mean 1423 cfu/100 mL (95% C.I. 795–2547)	Stream – Two sites where cattle have free access.	Lee *et al.* 2008
UK	Faecal coliforms Enterococci	D: Geo. mean: 1.9×10^3 cfu/100 mL D: Geo. mean: 2.2×10^2 cfu/100 mL	15 Rivers – 11-year monitoring period during summer bathing season. D: Dairy land use areas, P: Pasture land use areas (cattle, sheep)	Kay *et al.* 2008
	Faecal coliforms Enterococci	P: Geo. mean: 3.6×10^2 cfu/100 mL P: Geo. mean: 4.7 cfu/100 mL		
Scotland	Faecal coliforms Faecal streptococci	Mean 95th %ile: 1804 cfu/100 mL (500–3400) Mean 95th %ile: 1083 cfu/100 mL (171–4498)	Coastal bathing beach impacted by grazing livestock. 2000–2008 seasonal data.	SEPA 2009
India	Enterococci	Median 32 cfu/100 mL (23–44)	River Site 1 – Rural area with agricultural farms; drinking water, hygienic uses.	Lata *et al.* 2009

water than zoonotic pathogens; some zoonotic pathogens do not always occur in livestock wastes, but exhibit a seasonal presence, and some tend to survive much longer than FIOs in the aquatic environment. This data scarcity highlights significant gaps in our understanding of the occurrence of zoonotic pathogens in different water types impacted by livestock wastes. Additional empirical data could also help better characterize their relationships to FIOs and to validate transport models described in Chapter 5. Nevertheless, zoonotic pathogens are responsible for human illness and although the risks from waterborne exposures are not well known, measures to control their presence in water and reduce human exposures should be taken.

Factors affecting the ability of livestock faecal pathogens to reach areas within a watershed where the potential for human exposure through water contact activities can occur have been discussed. If an adequate number of pathogens survive the stresses existing in the aquatic environment, they may be present in sufficient numbers to cause infection and illness. The independent action, or single-organism premise is a generally accepted hypothesis that suggests that a single infectious organism is sufficient to cause infection (Haas *et al.* 1999). However, it is further believed that the likelihood of a single organism surviving the entire battery of defences encountered within a host is small, and that in general, a number of organisms are required for infection (Haas *et al.* 1999). Epidemiologically-derived estimates of the number of cells required to be ingested to lead to infection have ranged from as many as 10^3–10^4 cells for less infective species and strains of enteric bacteria and protozoa to as low as 10–100 cells for more highly infective species and strains (Percival *et al.* 2004, Pond 2005).

The biological properties exhibited by the individual pathogen types themselves are also important to survival. It is well recognized that *Giardia* cysts and *Cryptosporidium* oocysts are extremely resistant to environmental stresses and can survive for long periods in the environment, whereas vegetative bacterial cells are more sensitive and die off more quickly.

Specific virulence mechanisms possessed by the faecal pathogens of relevance have been discussed in Chapter 2. In general, these are products or mechanisms which facilitate a pathogen's ability to cause disease through invasion and colonization of the host, the impact on host cell functions and the evasion of the host's immune defences. These take on different forms for different pathogens, but have the similarity that they must be expressed in order to be capable of causing illness. Also, as addressed in Chapter 2, particular species and strains of these pathogens can vary in their virulence potential. Different genotypes of *Cryptosporidium parvum*, and assemblages of *Giardia duodenalis* (*lamblia*) are known to have unique virulence capabilities (Fayer

2004, Nichols 2008). Strains and serotypes of a range of virulence levels have also been demonstrated for the waterborne bacterial pathogens: *Campylobacter*, pathogenic *E. coli*, *Salmonella* and *Yersinia* (Lightfoot 2004, Molbak & Scheutz 2004, Percival *et al.* 2004).

7.3.3 Host

Aspects that influence the susceptibility of the host include the type (degree) of water exposure, the duration of the contact period and the strength of the defences or immunity exhibited. The nature of water activity also impacts the means through which pathogens may gain entry into the human host. Three main routes exist for the uptake or entry of pathogens during water-related activities: inhalation, direct body contact and oral ingestion.

7.3.3.1 Inhalation

Airborne pathogens in droplet form can enter the human respiratory tract as a result of direct inhalation through the mouth or nasal passages. A number of natural and human-based actions can result in the production of aerosols containing microbes (Haas *et al.* 1999). These include waves, white-water spray and spray from engine-driven water activities. In these contexts, inhalation can be a conceivable route of entry for any primary or secondary-contact activity.

7.3.3.2 Direct body contact

For direct body contact, small cuts or abraded skin, as well as prominent access points like the eyes or ears are potential entryways for microbial pathogens. These routes would be considered of relevance for all primary-contact activities. Skin contact is the most frequent type of exposure during contact with near-shore sands, soils or sediments. For secondary-contact recreational pursuits, exposed arms and legs constitute the most frequent points of contact; but it is also important to consider that splashing can lead to additional exposure scenarios, and spills or falls can result in whole-body immersion. Schistosomes, whose infectious larvae are water-based, actively penetrate the skin of suitable hosts in contact with contaminated water.

7.3.3.3 Oral ingestion

Faecal microorganisms that are transmitted via the faecal-oral route and which infect the gastrointestinal tract are referred to as enteric pathogens (Haas *et al.*

1999). As suggested by the exposure definitions, swallowing water constitutes a significant route for potential pathogen entry during primary-contact activities. This would encompass swimming and bathing as well as any drinking uses of untreated or inadequately treated water. Recent reports indicate that playing in beach sand may expose children to pathogenic microorganisms through ingestion (Whitman *et al.* 2009). This route would be of particular importance for children during beach sand play. Water ingestion is regarded to be less likely during secondary contact exposures. Still, as with direct body exposures, inadvertent immersion can lead to whole body contact, and brings with it the potential for water ingestion.

Based on the discussions in Chapter 2, the routes of human entry for the livestock faecal pathogens of concern are illustrated in the following table (Table 7.3).

Table 7.3 Primary faecal pathogens of livestock origin and their routes of entry for infecting a human host.

	Pathogen name	**Route(s) of entry**
Bacteria	*Campylobacter spp.*	Oral ingestion
	Pathogenic *E. coli*	Oral ingestion
	Salmonella spp.	Oral ingestion
Protozoa	*Cryptosporidium (parvum, hominis)*	Oral ingestion
	Giardia duodenalis (lamblia)	Oral ingestion

According to the principles of risk assessment (Haas 1999), the development of an exposure estimate requires knowledge of the concentration of organisms in the medium and the amount of medium that the individual may come in to contact with. For the primary livestock zoonotic pathogens, it is apparent that oral ingestion is the exposure route of most significance. The probability of human infection and illness occurring through other exposure routes can be considered insignificant. Consequently, the amount of water potentially ingested represents the critical contact factor when assessing exposure for these pathogens. Estimates of the quantities of water ingested during water activities are difficult to obtain, and as a consequence, research in this area has been limited. Approximations have largely focused on swimming-type exposures of the kind seen in freshwater environments. Values proposed for the amount of water that may accidentally be swallowed by an adult or a child during a swimming episode have ranged from 250 mL (WHO 2003), to 100 mL (Haas 1983, Mena

et al. 2003) to 50 mL or less (Evans *et al.* 2006). These values may not hold for other types of water exposure, however, as different assumptions may be necessary for different activities and water types.

For all waterborne pathogens, the potential for exposure is also influenced by the frequency and duration of water contact activities. The probability of coming in contact with pathogenic microorganisms increases with repeated water visits or prolonged exposures (Pond 2005). In addressing livestock zoonotic pathogens specifically, certain behavioural, demographic, socioeconomic and climate factors can have affects on this component. With recreational water activities, children are likely to have more frequent exposures and spend more time in the water than adults. In countries in warmer climates, individuals may see more opportunities for recreation due to higher water temperatures and longer seasons. It has also been put forward that equipment advances, for example, wetsuits can extend the length of a contact season or session (Pond 2005). In developing countries, exposures are likely to be out of basic necessity for sanitary purposes or occupational requirements, as opposed to leisure pursuits. Gender factors may also play a comparatively larger role in these countries. An example may be communities where women shoulder the burden for chores involving water contact such as laundering and bathing of children.

Box 7.1 Additional pathogens of interest: *Leptospira* spp., *Schistosoma japonicum*

Two additional pathogens, *Leptospira* spp. and *Schistosoma japonicum* are worth mentioning in the context of this chapter. These organisms are not among those thought to be of primary significance from a global perspective. However, both are major public health concerns in developing countries and represent interesting examples of disease transmission stemming from human contact with livestock wastes.

Leptospira spp

Leptospirosis is most significant in developing countries located in tropical and subtropical climates. The disease is considered endemic to most countries of Southeast Asia, and outbreaks have been recently reported in Nicaragua, Brazil and India (Vijayachari *et al.* 2008). In waterborne transmission, humans become infected as a result of direct body contact with water contaminated with leptospires, with the organisms gaining entry through mucous membranes or broken skin. Cattle and swine are considered the primary livestock reservoirs for *Leptospira* (Vijayachari *et al.* 2008). Rodents and dogs are also recognized carriers, and these animals may have more relevance in urban exposure scenarios. In developing countries

leptospirosis is primarily regarded as an occupational disease. Rice or sugar cane farmers and military personnel are among the more significant groups affected through water contact. Additionally, a number of outbreaks have been linked to disaster events and persons exposed to wet environments created by flooding (Barcellos & Sabroza 2001; Gaynor *et al.* 2007). Incidence of leptospirosis as reported in developed countries have been most commonly linked to recreational water activities, including both domestic exposures and those occurring during travel to endemic countries (Pond 2005).

Schistosoma japonicum

Incidence of *S. japonicum* have largely been reported in China, the Philippines and certain areas of Indonesia. Human exposures occur primarily among rice farmers working in marshland areas. These marshlands serve as the natural habitat for water buffalo, the primary reservoir for this organism. Increasing use of these animals as work animals has also been reported in these countries (Gray *et al.* 2008).

Infection with *S. japonicum* is acquired through direct contact with contaminated waters. The organism is capable of gaining access to the human body by penetrating through intact or broken skin; it then travels to the bloodstream to develop and cause further illness. Data specific to rates of *S. japonicum* infection in affected countries has been limited. Globally, however, schistosomiasis (from all sources) is estimated to affect 207 million people, with an additional 779 million being considered at risk of infection (Steinmann *et al.* 2006).

A final element of consequence in the discussion of host uptake and susceptibility is the issue of strength of immunity. The status of a host's immune system has a significant role in determining the susceptibility to infection and the severity of illness (Pond 2005). Groups recognized as having reduced immune functions include persons in different vulnerable life stages (pregnant women, children, the elderly), undernourished individuals, and persons with compromised or suppressed immune defences either as the result of disease (HIV/AIDS, cancer, liver disease) or medical interventions (chemotherapy, immunosuppressive medications). Tourists as a group may be comparatively more vulnerable than resident populations – a consequence of lacking prior exposure to the types of pathogens in new environment. Other considerations can have a positive effect on the host's immune status. The overall gains to health from repeated physical activity may improve and individual's ability to resist disease. As well, repeated exposures to, or past infections with certain waterborne pathogens may confer a degree of immunity to the user (Dangendorf 2004).

7.4 ADEQUACY OF TOOLS FOR ASSESSMENT AND MONITORING; REGULATORY CHALLENGES

Various voluntary programmes, guidelines, policies, standards and regulations based on sound science can be used in parallel at the source of contamination and at sites of human exposure, to proactively manage risks and limit exposures to zoonotic pathogens from livestock. The relevance of these tools as they pertain specifically to exposure assessment are addressed in the following Chapter, with their potential role as exposure interventions.

Monitoring activities specific to water exposures chiefly involve measurements of faecal indicator organism levels as an indication of the potential risk of exposure, and waterborne illness surveillance as an indication of the burden of illness potentially attributable to the hazards.

In terms of the adequacy of faecal indicator monitoring practices it is clear that, worldwide, measurements have been restricted to bathing beach areas for the protection of recreational-type exposures. Efforts for other water types and user activity combinations have been comparatively limited. Additional challenges impacting the effectiveness of the current methods include:

- Significant spatial-temporal variability of faecal indicator concentrations in natural waters;
- Substantial delays between the time of testing and the acquisition of the information; and,
- Noted poor correlations between faecal indicators and individual pathogens.

A significant hindrance to establishing effective regulation and monitoring is that the issue of livestock contamination of natural waters traverses multiple subject areas (agriculture, environment, health), as well as jurisdictional and geographical boundaries. Regulation and monitoring at bathing beaches have been reasonably well established in most developed countries. Certain jurisdictions have defined specific regulatory requirements (US EPA, EU), while in others (Canada, Australia, New Zealand) enforcement falls under the broader scope of public health legislation. In contrast, regulatory coordination of monitoring relevant to other areas of potential human contact has not received much attention.

As for surveillance, all countries have mechanisms in place for infectious disease surveillance and reporting; however, reporting of data specific to waterborne illness has been limited. Fundamental reasons for this include lack of resources and technical capacity, preferential focus on diseases of epidemic or pandemic importance and absence of a rigorously coordinated reporting framework. Even in developed countries recognized for their robust surveillance mechanisms, it is widely believed that the rates and causes of waterborne illness

are severely underreported. As with monitoring, the chief obstacle interfering with regulation or improved management in this area is the challenge that the responsibilities for reporting, detection and communication cut across multiple sectors, jurisdictions and geographical boundaries. Examples of specific challenges to provide context to this problem:

- Responsibilities for disease reporting lie with the patient, the diagnosing physician, the medical laboratory and coordinating health agency, with the potential existing for information to go unreported or undetected at each level.
- In most countries, gastrointestinal illness is not a reportable illness in and of itself. At the most basic level, coordinated detection and identification steps are required in order to positively identify cases of waterborne infection and illness.
- Water exposures of relevance for these discussions (natural water exposures) often involve travel to other jurisdictions, which can increase the difficulty of case tracking and outbreak detection.

Advancements are being made that may permit some of these challenges to be overcome in the future. Gains in the area of monitoring include the development of rapid detection and predictive tools, as well as the gradual betterment of our understanding of pathogen-indicator relationships through continued research in this area. Challenges facing the improvement of surveillance mechanisms may be somewhat more daunting, but advances in information technology and electronic communications are expected to be of great benefit to this area. Considerable investments in terms of time, funding and cooperation between multi-jurisdictional stakeholders will be required to see these expectations fulfilled; however, the increasing global attention to the issue of waterborne zoonosis may serve as the catalyst for progress.

7.5 SUMMARY

The subject of human exposure to zoonotic waterborne pathogens from livestock wastes is of a considerable complexity. Environmental, biological, behavioural, geographical, political and cultural pressures all combine to form a complex web dictating the potential for human contact, infection and illness. In general, the risks that livestock pathogens present to humans during activities in relevant aquatic environments are insufficiently understood. Science has advanced our understanding of interrelationships between livestock waste contamination, water impairment, zoonotic pathogens and human infection and illness. However, echoing a conclusion of Chapter 5 – there are still many areas where

our basic knowledge is deficient. The lack of data pertaining to pathogen occurrence for numerous source and water environment combinations, and an evidence base for accurate measures of exposure for various water contact activities are but two examples.

Continued research is needed to advance our understanding in those areas where knowledge is lacking. One important challenge in developing tools to facilitate exposure assessments with a broad applicability is the role and relative importance of local factors in influencing the true nature of host-pathogen-environment interactions. Despite the advancement of the tools and knowledge, significant efforts will still be required to fit this information to the specific scenarios, contexts and circumstances. An additional challenge relates to the aforementioned mismatch between the strength of scientific resources and the burden of global illness seen in developed and developing countries. This raises the question how to identify opportunities or solutions for utilizing science to better comprehend the risks of livestock waterborne pathogen exposure with a more global focus.

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8

Exposure interventions

Julie Kinzelman and Calum Mcphail

8.1 A CONCEPTUAL FRAMEWORK FOR EXPOSURE INTERVENTIONS

Evidence from epidemiological studies (Chapter 11) supports clear associations between faecal indicator organism density and exposure risk in waters contaminated with human sewage, but the utility of these pathogen indicators in bodies of water contaminated with animal waste and other non-point source pollution has been questioned (Kay *et al.* 1994, WHO 2001, Shuval 2003, Wade *et al.* 2006, Colford *et al.* 2007, US EPA 2009a). In this context, therefore, the results of such studies may be inconclusive, yet the reality of risks to human health associated with waterborne disease transmission remains irrefutable (Cotruvo *et al.* 2004, Craun *et al.* 2004, US EPA 2009b). Diffuse sources of contamination are commonplace; impacting both urban and rural settings in developed and developing nations alike. By some estimations, 99 per cent of all faecal contamination reaching surface waters is derived from the excreta of

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poultry, cattle, hogs and birds, but the nature and magnitude of these risks is largely unknown (Steven Esrey, UNICEF, pers. comm.). Therefore, the utility of traditional faecal indicators may be rightly questioned when assessing water quality throughout much of the world (Scott *et al.* 2002). The diversity of potential inputs necessitates a multi-tiered approach to identify the pollutant sources and to establish a framework for the assessment of the risks that these source may pose in exposed populations (Molina 2007).

Multi-tiered approaches such as those combining sanitary surveys with expanded monitoring programmes, including assessments of traditional faecal indicator organisms and tracking of microbial sources (source attribution), may provide this conceptual framework for risk assessment (Ruecker *et al.* 2007). Identification of pollution sources and quantifying the plausibility of illness from each source are necessary steps in the development of effective exposure or management interventions. Trends in animal husbandry and agricultural practices have increased the potential for large scale contamination events and the socio-political context in which these serious incidents occur is often outside the realm of formal public health and epidemiological investigations (Ali 2004, Hubbard *et al.* 2004).

Large scale contamination events may also be exacerbated by climate change effects such as projected increases in average precipitation (WHO 2009). The impacts of climate change on the epidemiology of waterborne diseases are through increased flooding, heavy rainfall and increased temperatures which can expand the range and geographic distribution of known pathogens (WHO 2003). Scientific studies have linked substantial increases in microbial loads in watercourses and drinking-water reservoirs as a result of extreme rainfall and the resulting runoff (Kistemann *et al.* 2002). This was the case in 2000 when Canada had one of the largest outbreaks of waterborne disease in North America. Over 2300 people became ill and seven died in Walkerton, Ontario as a result of consuming contaminated drinking-water following heavy rains (Auld *et al.* 2004). In a 2005 study Schuster and colleagues analyzed information on Canadian waterborne outbreaks occurring between 1974 and 2001 (Schuster *et al.* 2005). They found that severe weather events and close proximity to animal populations were correlated with disease outbreaks that could be tracked down to drinking-water supplies. The geographic distribution of climate change will show significant variation and human exposure will be "location-specific and path-dependent" (Yohe & Tol 2002, Hess *et al.* 2008). Therefore, in addressing potential environmental or behavioural human exposure interventions, the historic relationships with land use and (agri-)cultural traditions must be considered (Hess *et al.* 2008).

The advent of climate change is not the only development that may increase the likelihood of direct and indirect waterborne disease transmission originating from

domesticated animal populations. Increased human exposures may occur as a result of increased leisure travel to regions where water-related diseases such as schistosomiasis, leptospirosis and others are endemic. The global nature of the marketplace also provides opportunities for the spread of disease: from wild populations to closely-associated domestic populations and ultimately a wider geographic spread through the transport of goods (Fouchier *et al.* 2005). Changes in human demographics at regional, national and global levels will be another confounding factor, again possibly exacerbated by climate change and effects resulting from displacement or changes to human migration patterns (Hess *et al.* 2008).

In spite of limitations imposed by current faecal indicator assessments and the difficulties in relating pathogen burden to human exposure risk in a definitive way, the need for intervention (management of the physical environment and of human behaviour) still exists. Adequate protection of public health necessitates the mitigation or elimination of the pollution source or the prevention of exposure whenever real human health risks exist. Early hazard detection and risk attribution are necessary and relevant to evidence-based interventions, ranging from herd management (both farmyard and field measures), to downstream best management practices, to regulation and policy development, and the implementation of public education campaigns. The standardization of a step-wise investigative framework may support the targeting of these interventions and a logical progression may include the following:

- Step 1 – develop an investigative framework for waterborne disease exposure interventions requiring the identification of credible microbial pathogens in the aqueous environment capable of causing disease in humans (see: Chapter 2).
- Step 2 – identification of a conveyance mechanism (water, sediments, intermediary hosts) capable of delivering the pathogen (see: Chapter 5, Haack *et al.* 2003); establishing an exposure route connecting the human host to the source (see: Chapter 7).
- Step 3 – determine an attributable practice (i.e. agricultural, animal husbandry, recreational, or cultural) which may be managed with the anticipated outcome being the reduction of the transmission risk and, ultimately of the disease burden.

Management of pollutant sources or the affected populations does not come without cost and any intervention must weigh the expenditure against the direct and indirect economic benefits (see: Chapter 12) and feasibility of a sustained positive outcome. The carbon cost of different mitigation options may also be considered, comparing, for example, the pouring of concrete and pumping

(e.g. animal excreta or storm water runoff storage) with passive natural purification (e.g. reedbeds or constructed wetlands).

8.2 A STEP-WISE APPROACH TO MEDIATING WATERBORNE ZOONOSES

8.2.1 Weight of evidence

The disease cycle of certain microbial pathogens leaves no room for doubt as to their mode of transmission. A good example of this is malaria, a common infectious disease caused by protozoans of the genus *Plasmodium* which are transmitted to humans by females of mosquito vector species belonging to the genus *Anopheles*. Intimate knowledge of the parasite's life cycle provides evidence as to which interventions may prove successful in interrupting transmission. Options include the reduction of mosquito population densities, a reduction of the vectorial capacity or the prevention of human/vector contact. A reduction of mosquito population densities can be achieved by environmental management (drainage and other methods of eliminating mosquito breeding places), biological control (predators and parasites of anophelines) or chemical measures, or a combination of these. This will only interrupt the malaria transmission in situations where there is a linear relationship between density and transmission level. Such situations may exist in fringe areas (altitude boundaries, forest fringes, latitudes where temperature becomes a limiting factor) or man-made situations (urban areas or irrigation schemes). In large areas where malaria currently continues to be a public health problem, densities would have to be reduced by several orders of magnitude in order to have an impact on transmission levels.

Spraying with residual insecticides, the intervention on which a partial success towards malaria eradication was based in the 1950s and 1960s, reduced the life span of female mosquitoes so their capacity to complete a full cycle of the parasite is significantly curtailed and, hence, the associated entomological inoculation rate. This leads to sharp falls in levels of endemicity, but the approach is highly vulnerable to the development of insecticide resistance.

The use of barriers (mosquito nets, screening of windows and eaves) to separate mosquitoes from humans at peak biting times) has a history going back to the discovery of the transmission of malaria by mosquitoes, but has gained new momentum with the development of insecticide-impregnated nets in the 1980s and their large scale deployment since the late 1990s. In the context of this book, the use of cattle as a specific barrier separating human communities from the breeding places and resting sites of zoophilic anophelines (i.e. *Anopheles*

species that prefer to take blood meals from animals rather than from humans) deserves mentioning. This zooprophylaxis approach implies management of the spatial distribution of livestock and therefore also has relevance to the management of animal waste to avoid contamination of water bodies. An extensive literature on zooprophylaxis for vector-borne disease control exists (Bogh *et al.* 2001, Kawaguchi *et al.* 2004, Mathys 2010).

Integral to all these malaria control options is the need for public education such that local populations understand the relationship between certain behaviours (be they personal, cultural or linked to agricultural practices) and transmission of disease. In the case of waterborne microbial pathogens the load, transport and exposure mechanisms may not be as clearly defined as for malaria, necessitating an investigative approach to identify the pollutant source prior to the development of exposure interventions.

8.2.2 Faecal indicator organisms as a gauge of water quality

Global water quality standards and analytical methods to detect water contamination may differ in some aspects, but they are primarily or exclusively based on percentage compliance with faecal indicator bacteria levels (total coliforms, faecal coliforms, enterococci, and *E. coli*) (WHO 1999). Regardless of the indicator employed, all imply faecal contamination has, at least in part, a human origin and none explicitly address the specific contribution of faecal contamination of non-human origin to any risk assessments, based on past and recent epidemiological studies (Gleeson & Gray 1997). However, the etiologic agents implicated in the transmission of waterborne disease may originate from a variety of direct and indirect sources (Calderon *et al.* 1991, Bartram & Rees 2000, Solo-Gabriele *et al.* 2000, Noble *et al.* 2003). Among these sources, a clearly defined association exists between human exposure to water of poor quality resulting from agriculture and/or animal husbandry and the transmission of zoonoses.

Faecal indicators used to gauge water quality may be loosely correlated with pathogen presence dependent on the source of pollution (Kueh 1995, Marino *et al.* 1995, Schiff *et al.* 2007). While direct pathogen analysis would be preferable, the array of organisms capable of transmitting disease to humans via exposure to the water environment make individual analysis impractical for a variety of reasons including the cost and technical expertise necessary to execute the assays. Therefore, despite the fact that current binary indicator schemes leave room for confounding factors, compliance with best management practices need not be inhibited and may move forward with available data being translated into useful information for intervention.

8.2.3 Alternative or secondary indicators of faecal contamination

Survival rates of microbiological pathogens vary from organism to organism and are influenced by environmental conditions (see: Chapter 7). This paradigm also holds true for pathogens associated with manure from domestic animals and livestock (Pachepsky *et al.* 2006). As a result, a single indicator organism is unlikely to reliably predict the presence of all enteric pathogens for all types of waters and faecal contamination associated with different hosts (Hörman *et al.* 2004). The application of combined conventional and alternative indicators and/or methods of detecting faecal contamination increases sensitivity and specificity and may enhance the ability to predict and reduce health risk associated with the use of surface waters (Savichtcheva & Okabe 2006). Alternative or secondary indicators (see: Chapter 9) may include faecal anaerobes (*Bacteriodales/ Bacteroides* spp., *Bifidobacterium* spp. and *Clostridium perfringens*), viral indicators (*Bacteroides fragilis* bacteriophage and F-specific RNA coliphage), chemical compounds (caffeine, optical brighteners and faecal sterols) and many other anthropogenic markers associated with human activity (Leeming *et al.* 1996, Calci *et al.* 1998, Gardinali & Zhao 2002, Scott *et al.* 2002, Hörman *et al.* 2004, Savichtcheva & Okabe 2006, Schulz *et al.* 2006, Sobsey *et al.* 2006, Cao *et al.* 2009). Source attribution employing the combined use of traditional and secondary or alternative faecal indicators is often referred to as microbial source tracking (MST) (Bitton 2005).

8.2.4 Source attribution (microbial source tracking, MST)

In addition to alternative faecal indicators, MST is increasingly informed by the use of modern techniques taken from molecular biology, such as DNA fingerprinting by polymerase chain reaction (PCR), developing assay, or antigen electrochemical rapid methods (Jones & Smith 2004, Ruecker *et al.* 2007). The aim of MST is to identify the original host animal group(s) of faecal bacteria found in water. For example, this could be at the human or animal level (ruminant, non-ruminant), or to elucidate the contributions from specific animal groups for example, cow, sheep, dog, or avian species. A variety of faecal bacteria exist. Those typically used for regulatory purposes (e.g. *E. coli* or intestinal enterococci) have at least some properties of indicators, and as such allude to the possible presence of pathogens. The only way to be certain that MST data relate directly to the faecal bacteria used for regulation would be to attribute those same bacteria to a host source. However, to date, methods to obtain MST information directly from

these organisms have provided limited or uncertain information, and are not always suited for routine laboratory use (Johnson *et al.* 2004).

Due to current limitations, MST methods may be better applied to specific site and catchment investigations. For example, MST may be applied to understanding specific water quality issues and provide useful information on diffuse sources of pollution. Data must, however, be interpreted with caution and sampling strategies need to be robust and relevant. The impacts of point sources can be readily monitored using traditional bacteriological testing; MST is not always needed (though it can provide evidence of specific effects). The use of ultraviolet (UV) disinfection can complicate detection of bacteria using the polymerase chain reaction, as the bacteria are killed, yet the DNA may persist (Bae & Wuertz 2009). However, it may be used to suggest qualitative changes resulting in water quality degradation following rainfall events, which can impact shellfish and bathing waters, where increases in faecal bacteria may originate from agricultural run-off or from raw sewage through a discharging CSO (Stapleton *et al.*, 2009).

The application of source attribution techniques such as MST to at least some routine monitoring may be operational in many countries. However, it is still in its infancy, which limits the number of case studies available for detailing remedial action based on source tracking data. Additionally, it may not always be clear to a regulator what action to take once a source has been identified. Frequent spills from a CSO can lead to discussions with site operators about possible solutions, but diffuse pollution and the complexities of environmental variables, even when assessed with MST and quantitative source apportionment, may still be challenging (Kay *et al.* 2007). The utility of MST as a source attribution technique is further discussed in Chapter 9.

8.2.5 Sanitary surveys

Tools of a non-microbial nature may also prove beneficial in identifying principal sources of faecal contamination. They include sanitary surveys (Kinzelman & McLellan 2009). A sanitary inspection or survey is an assessment tool designed to evaluate the principal sources of faecal pollution impacting water quality (Bartram & Rees 2000). Comprehensive sanitary inspections should categorize all potential direct and indirect contamination sources in order to provide the best estimation of health risk as part of a suite of assessment tools including faecal indicator organism assessments and source attribution techniques (see: Chapter 9). Proper sanitary surveys are indicative of contamination sources but they lack the capacity of definitively identifying the source of faecal indicator organisms or pathogens.

8.2.6 Mathematical modeling

In additional to categorizing direct and indirect contamination sources, a proper sanitary survey also provides the framework for the collection of environmental conditions which may be associated with influxes of faecal indicator organisms and pathogens. Correlations between environmental conditions such as rainfall, turbidity, and stream direction/speed may be explained through statistical analysis (Kay *et al.* 2005). The development of statistical models capable of forecasting or 'nowcasting' surface water quality based on a predetermined set of environmental conditions is known as predictive modeling (Nevers & Whitman 2005, Francy 2009).

Predictive models, although conceptually simple, are a powerful statistical tool developed for the assessment of rivers at various scales (from site-specific to a national scale) and coastal waters which are gaining prevalence in global assessment schemes (Olyphant *et al.* 2003, Feio *et al.* 2009). Once operational, these tools are of value to water users, allowing them to make informed choices about behaviours to reduce exposure risk (Boehm *et al.* 2007, McPhail & Stidson 2009). Coastal beaches in the United States and Scotland successfully run predictive models for the protection of public health (Nowcast, http://www.ohionowcast.info/index.asp; Swimcast, http://www.lakecountyil.gov/Health/want/SwimCast.htm; and Beachline, http://www.sepa.org.uk/water/bathing_waters/bathing_signs.aspx).

8.2.7 Limitations of current methodologies

Although there have been many successes in the development and standardization of compliance monitoring schemes, the present approaches still suffer from limitations due to sampling reliability and source identification (Whitman & Nevers 2004). These limitations may influence their capacity to protect public health from waterborne zoonotic disease, especially when regulation is based primarily, or exclusively, on the detection of faecal indicator organisms, whose reliability in reflecting the presence of pathogens has been questioned (Haack *et al.* 2008).

8.3 OPPORTUNITIES FOR EXPOSURE REDUCTION

Routine monitoring (based on faecal indicator organisms), sanitary surveys and spatial distribution studies, complemented by research initiatives (microbial source tracking, modeling based on environmental parameters) can provide adequate information to initiate targeted remediation efforts within the

boundaries of their limitations. Within the context of local, political and public health frameworks, remediation schemes may be developed for the reduction of microbial contamination to adjacent surface water. These coordinated efforts are further supported by the management of human behaviour, such as citizen engagement and public education campaigns, designed to increase awareness regarding surface water management.

8.3.1 Eradication and control measures

Chapter 4 addresses the management of livestock herds at the direct level: controlling inter-animal pathogen transmission, culling of infected cattle, the use of antibiotics, bacteriophage treatment, conservation issues, bio-security and vaccination. Chapter 6 discusses the management of farms, where animal excreta are generated. Farm management measures to prevent or reduce the transport of microorganisms include riparian filter strips, animal exclusion (fencing, providing alternative watering points), waste retention or re-use, management of animal wastes (slurry storage or biogas systems), farms ponds and constructed wetland buffering systems. Chapters 5 and 7 describe the fate and transport of micro-organisms and emphasize the potential utility of microbial source tracking techniques as a surveillance and evaluation tool to judge the possible success of on-site preventative measures by assessing the ambient water environment. In Chapter 8 "downstream" preventative or mitigation measures are addressed. These are measures that may be taken to improve water quality and prevent the transmission of disease as a complement to herd management or on-site management measures or as a substitute where such measures are impractical, socially unacceptable, or where the definitive source remains unidentified. The design of downstream mitigation measures tends to be based on various monitoring techniques and may include physical alterations to the environment but also encompass managing human behaviours through regulatory or educational means.

8.3.2 Physical alterations

Physical alterations to the natural environment that have proven successful in reducing faecal contamination of surface water include: vegetated swales, natural or constructed wetlands, riparian buffer systems, barriers (berms, dams, curtain technologies), exclusion measures (fencing, wiring), and harassment techniques. While wetlands, vegetative swales, and riparian buffer systems have proven useful in improving water quality in agricultural settings (see: Chapter 6) (Gathumbi *et al.* 2005, Kay *et al.* 2005), these same measures are also effective

in mitigating downstream contamination impacting recreational bathing waters in urban settings (Kinzelman *et al.* 2009). Barrier technologies have also proven successful in reducing faecal indicator organism density at coastal waters impacted by rivers and streams, in rural settings and populated areas impacted by urbanized waterfowl populations, or locations which retain an agricultural profile within their watersheds (Charlton *et al.* 2006, Heagy 2006, Yeung-Cheung & Melendez 2007). Exclusion and harassment techniques have been documented to reduce urbanized waterfowl populations which degrade water quality and carry organisms capable of causing infection in humans such as *Campylobacter* and *Salmonella* (Petti 2006). Another important physical amenity that may reduce the transmission of waterborne disease is proper sanitation. The construction of latrines can prevent transmission of diseases such as schistosomiasis, whose cycle is perpetuated through the release of organisms in the faeces of infected individuals into freshwater rivers, lakes, and streams (Febles 1964).

8.3.3 Regulatory measures

Regulatory measures are another way of managing human behaviour (Rostier & Hastie 1996). While mandatory rather than voluntary, ordinances can elicit the desired response with respect to exposure interventions. In the USA several pieces of legislation and programmes exist which regulate commercial operations generating animal waste, drinking-water producers, and dischargers to surface waters. Examples of US federal regulations include: the Federal Water Pollution Control Act of 1972 (Section 402, National Pollutant Discharge Elimination System Permit), the Safe Drinking Water Act (similar in scope to the Ontario Clean Water Act), and the Coastal Zone Management Act. The Department of Food, Agriculture, and Rural Affairs (DEFRA) for England and Wales and the Scottish Government have similar regulations, policies, as well as guidelines for agricultural practices within the United Kingdom. Global water quality guidelines are also in place for recreational waters, for example, United States Environmental Protection Agency (US EPA) Ambient Water Quality Guidelines (US EPA 1986) and, across European Union member states, the EU Bathing Water Directive (CEC 2006, Mansilha *et al.* 2009). The Australia and New Zealand Guidelines for Fresh and Marine Water Quality present a combined approach, addressing both recreational water uses and industry (2000, Online: http://www.mincos.gov.au/publications/australian_and_new_zealand_guidelines for_ fresh_and _marine_water_quality). While these guidelines or mandates are applied to designated bathing waters, recreational activities (canoeing, kayaking, surfing, jet skiing, and windsurfing for example) frequently occur at sites or seasonal periods

that fall outside of these designations. Regulations, policies and guidelines, while protective and a necessary component of exposure intervention, therefore, must be complemented and strengthened by public education initiatives to provide the greatest positive impact.

8.3.4 Public health education

Managing people via educational measures should be complementary to managing resources (farms and livestock) and physical environmental management. Managing human behaviour is crucial to the reduction or elimination of waterborne disease. Public education can take many forms. Printed materials such as fact sheets, brochures, signs, billboards, and informative pieces in the popular press are frequently employed. Other successful public education initiatives sometimes utilize mass media: radio, television, dramatizations, and computer-based applications. Classroom presentations and one-on-one counseling may also prove effective in eliciting the desired change in personal or professional practices. Specific campaigns can be initiated by national or local authorities, community or environmental interest groups and annual performance tables can provide platforms for continual assessment of progress and repeat messages.

Local authorities often combine efforts with environmental interest groups or academic institutions to provide public education. The University of Rhode Island Cooperative Extension has developed, jointly with authorities of the town of North Kingstown, Rhode Island (US), a series of fact sheets, posters, and face-to-face trainings to educate owners of livestock on small acreages about protecting water quality and human health (Burdett and Sullivan 2005, Online: http://www.uri.edu/ce/healthylandscapes/livestock/livestock_publications.htm; Burdett *et al.* 2009).

Academic institutions may also have a role to play by offering multi-disciplinary undergraduate and graduate coursework on the impact of waterborne zoonoses. Adequate preparation (work place training and education) of those entering professional positions will increase awareness amongst qualified veterinary personnel, public health and human health care workers in order to facilitate inter-disciplinary discussions, collaborative ventures and adequate primary prevention and health care to both human recipients and animal hosts (Cripps 2000). The development of educational campaigns must also be couched in terms of the norms of the population in which sustainable habitual changes are desired. The prevention of waterborne zoonoses is frequently hampered by cultural traditions of long standing such as slaughtering and cooking, religious, and personal hygiene activities. The inability to address

these issues may results in failure to manage human behaviour (Robinson 2003). Participatory action on the part of those being educated may be of benefit: until people desire change no improvements will be seen. In developing nations, attempts have been made to provide human and animal health services, including education, simultaneously. In Chad, this technique was used to engage nomadic people because often animal health services, such as vaccination campaigns, had better coverage than health initiatives (Schelling & Berneck 2002). Joint animal sector and public health interventions may also be of economic benefit in resource limited and transitioning countries (Zinsstag *et al.* 2007).

8.3.5 Confounding factors: political, cultural, monetary, access, and climate change

There is always a political dimension (local, regional, and national) to the issue of exposure interventions, for example, political will/support/leadership, input from commercial and tourist interests and overall provision of funding for monitoring to reduce exposure risks with respect to recreational waters; particularly at high profile beaches. Outbreaks of waterborne disease at these high profile venues would result in negative press and loss of utility and, therefore, the willingness to expend time and resources is greater. On the other hand, political unrest may disrupt public health programmes and lead to municipal services being suspended, thereby possibly causing a decline in the quality of life, and frequently forcing individuals into unsanitary conditions. Fortunately, with respect to the control and elimination of waterborne zoonoses, the involvement of political leaders and celebrities, media, interest groups, charitable organizations and a general increase in public awareness of environmental issues are stimulating an unprecedented level of interest in global health issues, including previously neglected tropical diseases such as schistosomiasis and dracunculiasis (Hotez 2009).

Effective water quality management requires addressing not only the physical attributes of the environment but also the behavioural decisions of the people who impact that environment (Hurlimann & Dolnicar 2009). The ability to implement adequate exposure interventions can be confounded by the conventions and customs of the people whose behaviour one seeks to modify, as water quality problems are generally grounded in historic personal and land use practices. Incremental improvements then require a measure of social change (influencing people's awareness, skills, attitudes, capacity, or constraints related to water quality) (Genskow & Prokopy 2008). Confirming that awareness and attitudes are changing and improved behaviours are being adopted in a

catchment is one way to demonstrate progress toward water quality goals (direct water quality improvements and reduction in incidence of illness). Social indicators provide consistent measures of change within a watershed and can be used by managers to estimate the impacts of their efforts and resources (Genskow & Prokopy 2008).

Survey tools have been used successfully to gauge citizen acceptance to change through documentation of issues of socioeconomic importance. Collection of data on issues such as how residents earn their livelihood, how natural resources within a catchment are utilized (land use), how natural resources impact those living within the catchment, resident expectation for environmental services, landowners' opinions about best management practices and environmental attitudes may provide useful information when devising and implementing water-based management plans (McDermaid & Barnstable 2001) Landowner perception of environmental and health issues, self-reported concerns, and their willingness to change can contribute to the overall success of mitigation measures. Realizing that effective management of indirect water pollution requires addressing both environmental conditions and the choices people make that impact the environment, the USEPA (Region 5) has developed a Social Indicators Planning and Evaluation System (SIPES) for state agencies endeavoring to change people's behaviour. Monitoring social indicators, in addition to monitoring indicators of ecosystem health, will yield valuable information on the success or failure of management strategies in the protection of human and environmental health (Genskow & Prokopy 2008).

Capacity for change may not only be influenced by the willingness to change behaviours but also by access to resources: intellectual, technological and monetary. Sound science is needed to drive the political decision-making process as well as target remediation measures. Access to the latest knowledge and technological advances can increase the probability of success but this frequently requires a significant capital outlay. The best and most sustainable exposure interventions will be those that maximize the resources of the target area, balancing utilization of assets against direct and indirect economic benefits, taking a stakeholder approach (regulators, policy/decision makers, health care providers, practitioners and the interests of the general populace), and recognizing the limitations placed on interventions that can arise from climate change or other changes to the environment.

In the USA, the Great Lakes Regional Collaboration process demonstrates the holistic approach necessary to elicit change with respect to water quality improvements and public health (GLRC 2005). This is not always the case. In Australia, a referendum related to implementing water conservation measures

was voted down due to lack of political will, vested interests by industry, and information manipulation (Hurlimann & Dolnicar 2009).

8.4 EXAMPLES OF EXPOSURE INTERVENTIONS

The remainder of this section demonstrates the potential for positive impact, improved water quality, and the protection of public health from remediation initiatives. In some instances these actions were derived from the distribution of faecal indicator organisms and pathogens in the environment frequently in response to transport mechanisms such as the influence of environmental conditions and riverine/coastal processes (see: Chapter 5) (Haack *et al.* 2003, Touron *et al.* 2007). Other times intervention programmes have been developed based on knowledge of the life cycle or the clinical disease state (see: Chapter 2). In either instance, the measures often resulted in the revamping of traditional cultural or agricultural practices.

8.4.1 *Schistosoma japonicum* (schistosomiasis)

Schistosoma spp. are trematode parasites with a lifecycle of adult worms living in the veins of mammals and birds and larval stages in the aquatic environment with an obligatory passage through aquatic or amphibious snails. The disease they cause (schistosomiasis) is of significant global public health importance: and estimated 779 million people are at risk globally (Steinmann *et al.* 2006). In East Asia *Schistosoma japonicum* is distributed in China, the Philippines and Indonesia, and closely associated with irrigated rice production systems. Excreta from infected humans and other infected mammals, containing parasitic eggs, are released into surface water. The parasitic eggs in turn infect intermediate hosts (in the case of *S. japonicum*: the amphibious snail *Oncomenalia hupensis quadrasi*) (Carabin *et al.* 2005, Riley *et al.* 2005). Schistosomiasis in humans occurs when the larvae released from the intermediate host penetrate the skin of persons washing, bathing, or participating in recreational activities (wading, swimming, or rafting) in contaminated water (MMWR 1993, Schwartz *et al.* 2005). The nature of disease transmission results in high incidence of infection or exposure risk in communities where humans are in close proximity to a livestock or other mammalian source. Multiple challenges exist with respect to exposure interventions: case detection and treatment, mass drug treatment, installation/use of properly built latrines, eradication of the intermediate host, or prevention of contact with contaminated water (Febles 1964, Yi-Xin & Manderson 2005, Tallo *et al.* 2008). The eradication of the intermediate host

may not be an effective intervention measure due to the extent of the habitat and its ecological characteristics. Prevention of contact with infested waters may also prove difficult in regions where there are few alternatives with respect to sanitation, agricultural practice and recreational venues. In spite of these challenges, effective eradication campaigns have been mounted in endemic countries in other parts of the world where the intermediate host is strictly aquatic, like sub-Saharan Africa. For example, using school-based public education (essay writing, video recorded dramas, and household sanitation observations), public health officials in Tanzania have successfully broken the cycle of transmission using children to change or improve personal practices within their communities (Freundenthal *et al.* 2006). A survey of respondents from Guangxi, China, noted eradication of schistosomiasis provided clear benefits in terms of agricultural outputs and improved farming conditions through increased work capacity and that long term maintenance strategies needed to continue (Sleigh *et al.* 1998). On the other hand, a study from Osun State, Nigeria, determined that neglect of environmental effects of development projects, such as dam construction, were thought to counteract eradication efforts and allow transmission to remain unabated in the absence of national control programme (Oladejo & Ofoezle 2006).

8.4.2 Leptospirosis

Leptospirosis is found throughout the world, as an endemic condition in tropical climates and as a seasonal illness in more temperate climates. It is a true zoonosis with infection being maintained in the animal host and transmission occurring only when there is direct contact with the animal reservoir (Bolin *et al.* 2004). Passed to the environment in urine, the pathogen can survive outside the host for several weeks (e.g. *L. interrogans* survives in surface waters and moist soil for months) presenting a significant exposure risk to those individuals coming in contact with contaminated water through agricultural practices, aquaculture, sanitation, or leisure time pursuits (CDC 1993, Narita *et al.* 2005). The prevalence of leptospirosis is highest in those in contact with domesticated animals (agriculture, butchers and animal handlers) or the excreta of infected animals and humans (sanitation workers) (Sharma *et al.* 2006). While the ubiquitous nature of this organism in the environment contributes to its success as a zoonotic pathogen, that attribute also presents challenges for intervention strategies.

Testing for the presence of leptospires in water is not practical, considering their fastidiousness and time required for growth in laboratory media. A high probability of animal contact with water and a high density of rodents in alkaline water bodies

results in a greater likelihood of isolating this organism. In endemic/enzootic regions, new molecular epidemiological approaches have proven to be useful tools for directing public health actions. Definitive identification of significant numbers of microorganisms should prompt public education targeted at prevention strategies, such as use of footwear for contact exposure and chlorination of drinking-water (Haake 2006). In urban settings, garbage collection and other rodent control measures would be effective in reducing the concentration of pathogens in surface water. In rural settings, improvements in animal husbandry, such as antibiotic treatment of infected animals and routine vaccination, could reduce leptospiral carriage and shedding (Haake 2006).

Such a model for exposure intervention has been formulated in Kerala State, India. The plan of action was prepared by the Kerala State Institute of Virology and Infectious Disease; outlining personal protective and public health measures in an attempt to prevent disease transmission (John 2005). The Kerala State leptospirosis control programme is four-pronged involving: 1) a broad-based infectious-disease control policy, priority, and programme in the government health system, 2) public health training for all officials (home visits), 3) a functional disease surveillance programme (epidemiological surveys), and 4) a diagnostic laboratory capable of monitoring the success of the leptospirosis control programme (including the ability to participate in the survey of local fauna including rodents, wild, and domesticated animals) (John 2005).

8.4.3 *Salmonella*

Salmonella infection transmitted via a water source has historically focused on typhoid (*S. typhi*) and paratyphoid fever (*S. parathyphi*). Reductions in typhoid fever have occurred as a result of advanced sanitation practices, yet the existing estimate of the global burden of typhoid and paratyphoid fever is 27 million illnesses and 216,000 deaths annually (Crump *et al.* 2004). Typhoid fever can be avoided through vaccination, adequate treatment of drinking-water, proper food handling, and exclusion of disease carriers from food preparation.

Other species of *Salmonella* may also be of public health concern. *Salmonella* serotype Saintpaul has been isolated from irrigation water used to produce alfalfa sprouts (CDC 2009). A study of the Seine River in France (2000–2005) revealed that the highest density of culturable *Salmonella* was to be found in upstream portions of the estuary and urban areas (Touron *et al.* 2007). Elevations in *Salmonella* occurred in response to a variety of environmental conditions with upstream (agricultural) locations having greater densities during high flow situations and downstream (urbanized areas) under low flow (Touron *et al.*

2007). This research infers that *Salmonella* loading is likely a result of animal excreta as well as discharge from wastewater treatment works.

Excreta from domesticated poultry such as ducks and geese have been noted to carry *Salmonella* (Yu *et al.* 2008). Migratory populations of shorebirds, such as Canada geese and gulls, have become resident populations and could be viewed in the same light as domesticated populations. Ishii *et al.* (2006) noted frequent *Salmonella* isolates in mats of filamentous green algae. The likely source was hypothesized to be gulls. In a 2008 Great Lakes study, the carriage rate of *Salmonella* among urbanized gull populations in Lake County, Illinois, USA was estimated at ten percent (Kinzelman, unpublished). Although there have been rare instances of salmonellosis contracted from recreational waters (Pond 2005) the presence of pathogen strains cannot imply zero effect and, therefore, exposure interventions such as fencing and wiring may be appropriate where there is indication that animals are likely to come in contact with bathing waters. Behaviour modification such discouraging feeding is also of benefit although difficult to enforce both from a regulatory standpoint and as the presence of shorebirds is felt to add to the ambience of a coastal visit by some.

8.4.4 *Campylobacter*

Campylobacter spp. are adapted to the gut of warm blooded animals (including ruminants, avian populations and humans), hence animal-to-animal and animal-to-human infection is most likely to occur at water bodies impacted by excreta (Pond 2005). A New Zealand study (McBride *et al.* 2005) done in response to high rates of campylobacteriosis found that a large proportion of *Campylobacter* in rivers and streams originated from sheep and dairy cattle; this affirmed the findings of a previous study (Baker *et al.* 2002). Other investigations have found that wild birds, poultry and urbanized avian populations (ducks, geese, and gulls) excrete a variety of human gastrointestinal pathogens in their droppings. These included the bacteria *Campylobacter, Listeria, Salmonella, Vibrio cholerae, Yersinia spp.* and *E. coli* O157, the protozoa *Giardia* and *Cryptosporidium*, as well as the bacterial indicators of pollution, faecal coliforms and enterococci (Fallacara *et al.* 2001, Jones 2004, Ishii *et al.* 2006, Kinzelman *et al.* 2008). Therefore, wild birds, urbanized birds and poultry may serve as expansion environmental reservoirs of infection for *Campylobacter* (Jones 2004).

Based on this information, intervention measures must be considered for drinking-water, recreational activities, contact with livestock or other human activities resulting in exposure. Intervention measures might include the

exclusion of animals from the aquatic environment, an option difficult to achieve with avian species but likely successful for ruminants. Signage designating impacted water bodies as unsuitable for swimming would aid the reduction of exposure through recreational contact. Enhanced public education can induce better sanitation practices: safe handling of live poultry and livestock, safe handling of carcasses (food chain model), and adequate management of the drinking-water supply. The results of the New Zealand *Campylobacter* transmission route (CTR) study (Baker *et al.* 2002) identified several potential risk management options for residents of, or visitors to, rural communities. Among these were public awareness about the presence of *Campylobacter* in ruminant faeces, the need for adequate hand-washing after animal handling and prior to "hand-to-mouth" activities, and other intervention messages such as avoided consumption of untreated water (such as roof-collected water due the prevalence of *Campylobacter* in avian species). Adequate treatment is also necessary for public drinking-water supplies derived from surface waters in agricultural areas. Several instances of waterborne disease outbreaks have been attributed to insufficient, untimely or inadequate treatment of potable water supplies (Stehr-Green *et al.* 1991, Inkson 2002, and O'Connor 2002).

8.4.5 *E. coli* O157:H7

The Canadian Walkerton Inquiry highlights the dangers of waterborne transmission of pathogens such as *E. coli* O157:H7. Significant morbidity and seven fatalities occurred when Walkerton's municipal water supply became contaminated with *Campylobacter* and *E. coli* O157:H7. It was presumed that the contamination arose from farm animal run-off into a shallow well, from which the water supply was taken (O'Connor, 2002). In 1996, an outbreak of *E. coli* O157 infection in central Scotland resulted in over 500 confirmed cases and 17 deaths (Cowden *et al.* 2001). Scotland continues to report higher incidence rates of infection with verotoxin-producing *Escherichia coli* (VTEC), such as *E. coli* O157:H7, than elsewhere in the United Kingdom (Figure 8.1). In addition to potable water supplies, disease outbreaks have also been associated with consumption of vegetables irrigated with contaminated water (Islam *et al.* 2004; CDC 2006, online: http://www.cdc.gov/foodborne/ecolispinach/100606.htm, Jay *et al.* 2007) and at recreational waters (Levy *et al.* 1998, Proctor & Davis 2000, Samadpour *et al.* 2002, Keene *et al.* 2004, CDC 2008).

Characteristics of *E. coli* O157:H7 such as the ability to enter a viable but non-culturable state and to survive under conditions of low pH and temperature lend to its ease of transmission (Pond 2005, Nwachuku & Gerba 2008). Multiple mediation measures are required which should address load interventions

(Chapter 3), transport interventions (Chapter 6), public health education regarding the risks associated with the consumption of unwashed/uncooked vegetables, personal hygiene, and exclusion of animals and individuals with symptoms of gastrointestinal illness from recreational water venues.

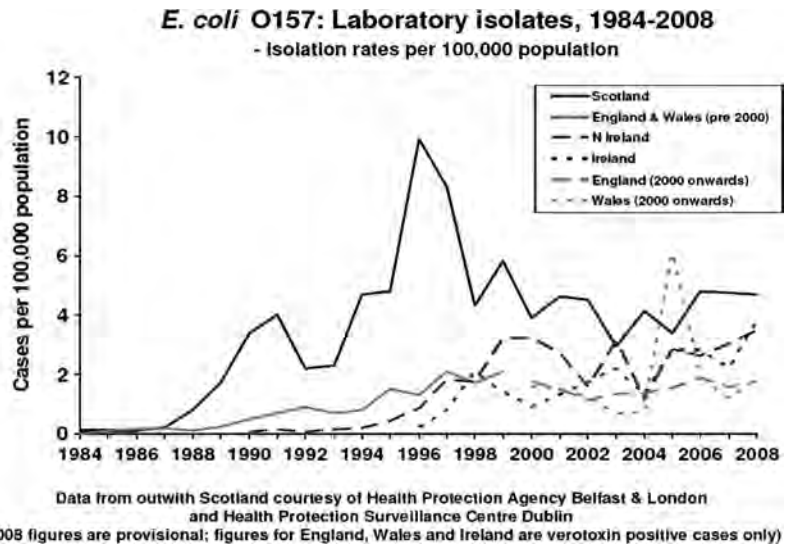


Figure 8.1 Confirmed *E. coli* O157:H7 isolates from the United Kingdom and Republic of Ireland, 1984–2008.

8.4.6 *Cryptosporidium*

In Scotland, 600–900 laboratory-confirmed cases of cryptosporidiosis are reported to Health Protection Scotland (HPS) each year. The infection is typically associated with bloating, abdominal pain, nausea and prolonged diarrhea. While the illness is normally self-limiting, a recent study has shown it can lead to serious health sequelae and may even be fatal (Hunter *et al.* 2004, Caccio *et al.* 2005). Infection is frequently disseminated by person-to-person transmission, by animals, and indirectly through the environment (particularly by water). Indeed, drinking-water contaminated by oocysts is an internationally recognized risk factor for human disease (McAnulty *et al.* 2000, Goh *et al.* 2005).

Within the past ten years, two large outbreaks of waterborne cryptosporidiosis have occurred in Scotland: 90 confirmed cases in Glasgow in the year

2000 associated with unfiltered Loch Katrine water and 140 cases in Aberdeen in 2002 associated with suboptimal filtration of River Dee water. Previous evidence has also suggested an association between consumption of unfiltered water from Loch Lomond and cryptosporidiosis (Smith *et al.* 1993). Data were analyzed from laboratory-confirmed cases of cryptosporidiosis collected from 1997 through 2003 to determine risk factors. An association was identified between the incidence of cryptosporidiosis and consumption of unfiltered drinking-water. This association supports public education efforts with respect to exposure risks and the exclusion of animals, domestic or wild, from the vicinity of water supplies as a means of controlling outbreaks of cryptosporidiosis.

8.4.7 Mitigation in the absence of a definitive host attribution

Even in the absence of a clearly defined host, one can imagine potential risks and possible actions to assess and reduce those risks. A multi-barrier approach may enhance protection of human health from source to water contact exposure (Robertson & Yasvinski 2006). As a first step, a sanitary or environmental health and safety survey will assist in cataloging all potential sources of contamination, safety hazards, and user activity patterns which will create a blueprint on which to base management strategies for exposure interventions. The second step involves applying or implementing barriers to reduce risk (either reducing sources or preventing human contact during periods of elevated risk) (Robertson & Yasvinski 2006). In these cases monitoring water quality via primary or secondary water quality indicators is only part of the investigative process. Results of any analytical tests must be taken in the proper context and in light of historical evidence of disease. Weight of evidence may serve as a proxy for indicators when direct monitoring of the water body is not feasible.

8.5 CASE STUDIES

In this section three case studies are presented which illustrate the efficacy of various human exposure intervention measures. The studies utilized various assessment schemes to implement interventions as presented in this chapter: source attribution leading to improved drinking-water treatment, physical alterations and disinfection to reduce bathing-water quality failures, and informative signage based on predictive models to reduce exposure risk at Scottish bathing beaches. While by no means comprehensive, they are good

examples of how recognition of a problem has resulted in initiatives that have led to concrete improvements.

Case Study 1: Cryptosporidiosis and unfiltered drinking water, Scotland, UK[1]

Background

Cryptosporidiosis may be caused by multiple species of the genus *Cryptosporidium*; the most important human pathogens being *Crypto. hominis* and *Crypto. parvum*. *Crypto. hominis* infection is mainly restricted to humans but *Crypto. parvum* infects a variety of mammals (especially neonatal cattle and sheep) as well as humans. Persons at risk of infection include those participating in recreational activities (swimming as well as the drinking of unfiltered water), and travelers to endemic regions.

Problem

In Scotland, 600–900 laboratory-confirmed cases of cryptosporidiosis are reported to Health Protection Scotland (HPS) each year. Infection is frequently disseminated by person-to-person transmission, by animal reservoirs such as sheep and cattle, and indirectly through the environment (particularly by water). Indeed, drinking-water contaminated by oocysts is an internationally recognized risk factor for human disease (McAnulty *et al*. 2000; Goh *et al*. 2005, Hunter & Thompson 2005). The disease state is typically associated with bloating, abdominal pain, nausea and prolonged diarrhoea. While the illness is normally self-limiting, a recent study has shown it can lead to serious health sequelae and may even be fatal (Hunter *et al*. 2004; Caccio *et al*. 2005).

Within the past ten years, two large outbreaks of waterborne cryptosporidiosis have occurred in Scotland: 90 confirmed cases in Glasgow in 2000 associated with unfiltered Loch Katrine water and 140 cases in Aberdeen in 2002 associated with suboptimal filtration of River Dee water. Previous evidence has suggested an association between consumption of unfiltered water from Loch Lomond and cryptosporidiosis (Smith *et al*. 1993). Loch Lomond supplies water to ~34% of the population of central Scotland.

Improvement Initiative

Loch Lomond. Before November 1999, Loch Lomond water was only micro-strained (only particles >23 μm were filtered out) and disinfected with chlorine, and the risk of transmitting *Cryptosporidium* spp. oocysts (4–6 μm) to consumers of this water was relatively high. In November 1999, enhanced physical treatment (coagulation and

[1] Contributed by Kevin G.J. Pollock, Colin N. Ramsay, and David Young (Health Protection Scotland).

rapid gravity filtration), designed in part to reduce the number of oocysts, was introduced. This treatment of the Loch Lomond supply was expected to reduce the oocyst load in the final supply. To determine risk factors for cryptosporidiosis, including in drinking-water, data were analysed of laboratory-confirmed cases of cryptosporidiosis collected from 1997 through 2003. An association between the incidence of cryptosporidiosis and unfiltered drinking-water supplied to homes was identified. Data strongly suggested that drinking unfiltered tap water from Loch Lomond transmitted *Cryptosporidium* spp. at the population level. The association supports the view that adding a filtration system to minimally treated water can substantially reduce the number of confirmed cryptosporidiosis cases.

Loch Katrine. Water originating from Loch Katrine is used to supply part of the population of Central Scotland. Prior to September 2007, this water was unfiltered and therefore posed a relatively high risk of transmitting viable *Cryptosporidium* oocysts to consumers. After September 2007, rapid gravity filtration treatment was introduced, designed to reduce the number of oocysts passing into the final distribution supply. In theory, if a proportion of the sporadic cases of illness were attributable to drinking unfiltered Loch Katrine water, then there should be a reduction in the number of such cases following the introduction of filtration treatment.

Lessons for the future

The association of outbreaks linked to drinking inadequately treated tap water supports the view that adding a filtration system to minimally treated water can substantially reduce the number of confirmed cryptosporidiosis cases.

Case Study 2: Bathing Water Quality: UV light disinfection system to reduce beach postings, Fanshawe Beach, Ontario, CA[2]

Background

In the 1950s the Fanshawe Dam was built on the Thames River, Ontario, Canada to control flooding in the watershed. The Upper Thames River Conservation Authority (UTRCA) subsequently developed the surrounding land and reservoir including a swimming area and beach for outdoor recreational use. Low flow and high nutrient loads from urban and rural areas often resulted in unacceptable levels of *E. coli* in surface water.

[2] Contributed by Steven Musclow (Upper Thames River Conservation Authority) and Gordon Yasvinski (Health Canada).

Problem

High levels of *E. coli* in bathing waters have been linked to increased human exposure risk due to the likelihood of pathogen presence via epidemiological studies. In addition to poor circulation within the beach area and nutrient loading the following pollutant sources were identified: faulty septic systems, wet weather urban and rural surface runoff, and industrial discharge. As a result of the influx of contamination, the bathing beach was frequently closed for lengthy periods of time during the recreational swimming season.

Improvement initiative

The Upper Thames Region Conservation Authority (UTRCA) in conjunction with the Ontario Ministry of Environment and Trojan Technologies installed a polyvinyl curtain and UV light disinfection system to improve water quality in the enclosed swimming area. Water was pumped through intake screens to remove debris, passed through UV lamps in a facility onshore to disinfect the water and returned to the enclosed swimming area through two diffusers. The curtain was anchored to the bottom and enclosed some 1600 m^3 of water which was re-circulated every four hours. The UV system consisted of two reactors, each consisting of a concrete channel housing five modules. Each module contained four lamps for a total of 40 lamps in the system (20 lamps per reactor). Water was analyzed weekly for *E. coli* at seven sites within the enclosed area. UV disinfection resulted typically in a greater than 97% reduction in levels of *E. coli*, even following rainfall events (Figure 8.2).



Figure 8.2 Enclosed bathing area and intake for on-shore UV system at Fanshawe Beach, Ontario, Canada.

Lessons for the future

The UV system described can improve water quality and reduce the number of beach advisories in a bathing season. The system could be applied to similar small fresh water recreational bathing areas. System efficacy is reduced when contaminants are washed into the enclosed bathing area, during high bather loads and when untreated water from the reservoir flows over the curtain into the enclosed area. Ways to minimize these impacts are being investigated.

Case Study 3: Bathing Water Quality – prediction and signage: Providing bathers with up-to-date advice on water quality[3]

Problem

The classification and management of recreational bathing waters has traditionally relied on monitoring health-related microbiological parameters, often referred to as faecal indicator organisms (FIOs). Because results were only available some time after sampling (24 to 48h) these did not reflect relevant conditions when bathers were actually using the bathing water. It was recognized that systems were required for daily use that predict, protect and inform more timely (World Health Organization 2002). Bathers require information at the time they were considering bathing regarding potential risks to their health and with adequate warning given of any short term pollution events such as wet weather direct or diffuse faecal pollution from human or animal sources.

Improvement initiative

To address this, the Scottish Environment Protection Agency (SEPA) and Scottish Government decided to develop bathing water quality predictions which provided real-time public information and advice for bathers as they arrive at the bathing site. Daily bathing water quality predictions (forecasts) were posted on electronic variable message signs at beach locations networked to a central communication centre with simultaneous updates made on the website, and via a phone and text message service (http://www.sepa.org.uk/water/bathing_waters/bathing_signs.aspx). The electronic text message displays show either: “Excellent water quality is predicted today”, “Good water quality is predicted today” or, “Bathing not advised today; risk of poor water quality”. Functionality of the electronic signs is enhanced by including multi-pages to allow additional messages to be interspersed between the main messages. Examples include: “Welcome to the beach”, “Please take your litter home” or “Please do not feed gulls in this area”.

[3] Contributed by Calum McPhail (Scottish Environment Protection Agency).

Weather related FIO pollution events have been studied in considerable detail (Crowther *et al*. 2001 and 2003). SEPA used its extensive national on-line hydrometric network of rain and river monitoring across Scotland, and developed "nowcast" models using preceding rain/river flow to issue "live" site specific bathing water quality predictions (McPhail & Stidson 2009). The prediction tool (an Excel spreadsheet-based model) derived site-specific trigger limits for rainfall and river flow to predict bathing water quality based on historical correlations between hydrology and faecal coliform concentrations. Rainfall depth-duration thresholds used previous 24, 48 and 72 hours amounts, together with current-day rain forecast to midday (on the day the message is posted). River flow information was also included in the regression relationships. This was a simple and effective means of predicting bathing water quality where pollution was predominantly wet weather-driven and elevated microbiological levels occurred during such events. The prediction forecasts were validated annually and performance in 2008, as confirmed by water quality testing, was correct on 82% of days. To date, the overall daily signage has been correct or protective on 98 to 99% of occasions over the last seven years of operation. A public awareness survey was carried out and beach users, organizations and media continue to provide positive feedback on the signage system (Figure 8.3).



Figure 8.3 On-line electronic bathing water quality message sign; Prestwick, Scotland.

Lessons for the future

Prediction and signage systems have been shown to work and can provide timely advice to beach users. The system can be extended to suitable bathing waters where water quality prediction relationships can be modelled and validated.

Multi-parameter statistic tools, such as decision trees, are currently being tested and are looking promising for future use as they provide more sophisticated detection of combined influences such as rain, wind and tide together. In addition, data inputs from catchment rain radar will provide better spatial coverage in tandem with more accurate weather forecasts allowing in-day adjustments of the predictions. Market research, carried out during the initial development to gauge public perception and feedback, was positive. It is worth repeating this at suitable intervals to assess trends and obtain feedback from beach users to ensure that public information tools are relevant.

SEPA are planning to extend the system by adding this "nowcast" risk assessed for about 15 more beaches to be fully operational in the course of 2012. In addition to providing public information at appropriate locations this will be an operational tool to facilitate beach management of short term pollution events as required for the revised EU Bathing Waters Directive (2006/07/EC).

8.6 FUTURE DIRECTIONS IN RISK ASSESSMENT CAPABILITIES

Application of molecular techniques and multiple water quality indicators with variable environmental persistence and fate may yield greater confidence in faecal pollution assessment and may better inform remediation decisions (Haack *et al.* 2008). In a study by Haack *et al.* (2008) bacterial genes indicated potential pig or cattle sources in eight of 18 samples from mixed or agricultural land-use watersheds where pigs and cattle were present. Molecular epidemiological studies were also applied in endemic areas of the Peruvian Amazon to examine the density and diversity of *Leptospira* spp. in urban and rural environmental water samples. The outcome was the discovery of a new branch of the leptospiral phylogenetic tree, an important step in further identifying potential sources so that further virulence and general characterization studies can be performed (Haake 2006). Another significant advantage of some molecular techniques, such as real-time quantitative PCR (QPCR), is the ability to enumerate pathogens in environmental samples. This is critical for the determination whether or not an organism is present in an infectious dose. In a Racine, WI, USA study the carriage rate for *Campylobacter* spp. in urbanized gull populations at popular bathing beaches was noted to be 14 percent but this was not quantified with respect to

human infectious dose and, therefore, the health risk remained undetermined (Kinzelman *et al.* 2008).

Future developments in molecular biology detection capabilities, rapid methods, and species identification (including pathogen) will greatly enhance the information used for risk assessments and support improved intervention tools. Other tools currently under development but reaching end-user operational status include biosensors and miniaturized techniques using nanotechnology. The arrival of easily accessible DNA fingerprinting and web-based genomic libraries could revolutionize the science of bio-monitoring and this will benefit the understanding of zoonotic pathogens prevalence and better inform exposure and mitigation strategies. Quantitative Risk Assessment (RA) is another technique, to be discussed in Chapter 10, which characterizes the risk of infection in the context of faecal indicator burden and pollutant source. Risk Assessment tools can aid in crafting effective mitigation/eradication measures by leveraging data generated through routine monitoring and identification of exposure routes.

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9

Indicators, sanitary surveys and source attribution techniques

Julie Kinzelman, Katharine G. Field, Hyatt C. Green, Valerie J. Harwood and Calum McPhail

9.1 FAECAL INDICATOR ORGANISMS (FIOs) – AN HISTORICAL PERSPECTIVE

Many epidemiological studies have linked exposure to contaminated surface waters with infection and disease (Cabelli *et al.* 1975, USEPA 1976, Cabelli 1978, Cabelli 1981, Cabelli 1982a, Cabelli *et al.* 1982b, Cabelli1983a, Cabelli *et al.* 1983b, Dufour 1984b, USEPA 1986, Dufour 1992, Prüss 1998, Bartram & Rees 2000a, Georgiou & Langford 2002, Kay *et al.* 2004). Chapter 11 of this book reviews four studies with a specific focus on water contaminated with animal waste. Worldwide, regulatory schemes for the protection of human health through classification of microbial quality of recreational waters differ in some

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aspects (based on national, provincial, state or local water quality guidelines), Yet, they are primarily or exclusively based on compliance with faecal indicator organism (FIO) levels. It is not feasible to test for every type of human pathogen that may be present in an aquatic environment. This is due to their great diversity, spanning the phylogenetic spectrum from viruses and bacteria to protozoa and worms, and due to the fact that detection methods are often difficult and costly (WHO 1999). Therefore, FIO are used as proxy-indicators of increased probability of pathogens' presence (US EPA 1986, WHO 1999, WHO 2003). The ideal characteristics of microbial water quality indicators are outlined below:

- their population density (without proliferation) should give a reasonable estimate of the likelihood of pathogen presence and should be positively correlated with the potential health risks associated with exposure;
- their presence should be exclusively and consistently associated with the source of the actual pathogens;
- compared to the most resistant pathogens that may be present at significant levels they should demonstrate a similar level of resistance to environmental stress;
- their characteristics should support accurate quantification which can be achieved through simple and inexpensive detection methods;
- their presence should be harmless to humans and animals; and,
- they can be detected by laboratory methods in a short time period (i.e. hours) and provide consistent results (WHO 1997).

In spite of their global usage, several constraints have been noted with respect to current standards and guidelines based on FIO. For example:

- management actions are retrospective and take place after the exposure has occurred (due to the time delay in obtaining results of bacteriologic tests) (Whitman *et al.* 1999, US EPA 2007);
- highest health risks are attributed to human faecal contamination, followed by high-density cultivation of certain livestock, while the indicators may be derived from other, multiple sources (Chapter 11) and (Stavros 2003, Nevers & Whitman 2004, US EPA 2005);
- there is a lack of inter-laboratory and global standardization with regard to analytical data;
- beach classification using indicator organisms alone results in a designation of either safe or unsafe with no provision for incremental increases in health effects (WHO 1999, Rees 1999);
- faecal indicator organisms may not correlate well with the presence of some pathogenic organisms (Lund 1996, Lemarchand & Lebaron 2003); and,

- bacterial concentrations may change abruptly, both spatially and temporally, so that singular microbial assessments become mere snapshots of water quality captured at a single moment in time (Whitman *et al.* 2004a).

Furthermore, conclusions drawn from epidemiological studies conducted at beaches in proximity to potential point sources of faecal contamination (i.e. sewage outfalls) do not address the relative risk of exposure in the instance where bacterial contamination is predominately from diffuse or animal sources (Kay *et al.* 1999, Colford *et al.* 2007).

9.2 FIOs – ALTERNATIVE/SECONDARY INDICATORS AND NEW APPROACHES

Problems remain in correlating water quality, as identified by these FIOs, to adverse health outcomes like gastrointestinal illness, many related to the suitability of indicator species used to set standards (Godfree *et al.* 1990, Kay *et al.* 1996). In addition to faecal sources (human or animal), indicators may be autochthonous in the environment in association with sediments (Byappanahalli *et al.* 2003a, Francy & Gifford 2002, Francy *et al.* 2003, Shively *et al.* 2003, Whitman *et al.* 2003a) or plant material (Byappanahalli *et al.* 2003b, Whitman *et al.* 2003b). They may also be resuscitated from a non-culturable state, even in the Polar regions (Smith *et al.* 1994, Pommepuy *et al.* 1996). Studies have indicated that FIOs such as total and faecal coliforms, *Clostridium perfringens, Escherichia coli,* and enterococci, are associated with humans and a variety of animal hosts and may be subject to environmental replication and/or persistence, challenging their ability to support the discrimination between recent contamination events (Davies *et al.* 1995, Anderson *et al.* 1997, Alm *et al.* 2006, Kon *et al.* 2007, Ksoll *et al.* 2007, Pote *et al.* 2009, Yamahara *et al.* 2009).

9.2.1 Alternative or secondary indicators

Research evaluating the suitability of organisms other than *E. coli* and enterococci as indicators of recreational water quality began over 30 years ago due to concerns such as inconsistencies in the relationship between pathogens and current pathogen indicators (Bisson & Cabelli 1980, Lund 1996, Lemarchand & Lebaron 2003, Nevers & Whitman 2004a). Some studies have suggested alternative bacteria or viral indicators such as coliphages (Toranzos 1991, Beaudeau *et al.* 2002, Lovelace 2004), *Pseudomonas* (Cabelli *et al.* 1976), enterovirus (Gersburg 2004, Noble *et al.* 2004, Fuhrman *et al.* 2005), *Bacteroidales* (Walters *et al.* 2007), and *Clostridium perfringens* (Bisson & Cabelli 1980, Fujioka & Shizumura 1985, Fujioka 1997, Rose 2004) on the premise that they may have a

better correlation to swimmer-related illness than current bacterial indicators under certain conditions (Table 9.1). Chemical tracers (faecal sterols, caffeine, and optical brighteners) have also been considered as alternative or secondary indicators of surface water quality (Isobe *et al.* 2004, Ferreira 2005, Cao *et al.* 2009, Hussain *et al.* 2010) (Table 9.1).

Table 9.1 Traditional and alternative indicators of faecal contamination.

Indicator	Method	Reference
E. coli	Membrane Filtration	US EPA Method 1603 ISO 9308
	ONPG-MUG	APHA Method 9223/2005
Enterococci	Membrane Filtration	US EPA Method 1600 ISO 7839
	MPN/Enterolert	ASTM D6503-99 (2009)
Pseudomonas spp.	Membrane Filtration/MPN	APHA 9213E, 9213F
	Membrane Filtration	CYS EN ISO 16266:2008
Bacteroidales	PCR	Bernhard & Field 2000
	Real-time PCR	Seurinck *et al.* 2005
Clostridium perfringens	Membrane Filtration	m-CP Medium, 98/83/EC
	Membrane Filtration	ISO 6461/2:1986
F+ RNA/DNA Coliphages	Single Agar Layer	US EPA Method 1602
	Presence-Absence	US EPA Method 1601
Enterovirus	Membrane Filtration/RT-PCR	Gilgen *et al.* 1997
Faecal sterols	Gas-Liquid Chromatography	Murtaugh & Bunch 1967
Caffeine	GC/MS	Buerge *et al.* 2003 Peeler *et al.* 2006 Verenitch & Mazumder 2008
Optical Brighteners	Fluorometry	Hartel *et al.* 2007

At one time, a comparison of the faecal coliform/faecal streptococci (FC/FS) ratio was thought to provide a reasonably precise indication of recent faecal contamination by warm-blooded animals; humans if the ratio was four or greater and other animals if the ratio was less than one (Geldreich & Kenner 1969, Hai & Hongdao 1982). Opinion regarding this technique has changed due to the differential die-off rates of faecal streptococci species and this assessment method is no longer recommended for gauging surface water quality (Pourcher *et al.* 1991, APHA 1989). While the FC/FS ratio is no longer recommended as a stand-alone source tracking method, traditional and/or alternative indicators,

employed in tandem with this technique, may provide more useful information (Savitcheva & Okabe 2006). As part of a source tracking study, researchers in Idaho examined both faecal streptococci and *E. coli* ratios in dry and wet manure, alongside other source tracking methods, as a means of assessing stream loading, with favourable results (Weaver *et al.* 2005).

It has further been suggested that factors other than measured levels of FIOs should be taken into account when monitoring ambient water quality (WHO 1999). Non-human sources, such as avian species, are known to carry enteric pathogens whose genus/species have strains known to infect and cause disease in humans (Ferns & Mudge 2000, Newell 2002, Haag Wackernagel & Moch 2004, Tizard 2004, Kinzelman *et al.* 2005) but their ability to transmit these organisms to humans within the context of ambient water exposure is as yet untested by epidemiological studies. Alternative approaches would ideally be able to take into account the source of the contamination, providing a better indication of health risk. The feasibility of a health-based monitoring approach was proposed as the result of an expert consultation sponsored by the WHO and USEPA (WHO 1999). A classification scheme for recreational waters based on health risk was suggested. Identification of the pollution source and recognition of factors such as precipitation and wave action, which can influence the condition of bathing water quality, should be part of assigning a water body classification. The collection of environmental data may also be useful in the development of real-time assessment (Haugland *et al.* 2005, Griffith *et al.* 2009, Heijnen & Medema 2009) and water quality prediction methods.

9.2.2 New approaches – predictive modeling

Predictive models have been suggested as a means of utilizing site-specific data that can be collected on a real-time basis as an alternative or in conjunction with microbial indicator testing (Bruesch & Biedrzycki 2002, Breusch & Biedrzycki 2003, Olyphant *et al.* 2003, Collins & Rutherford 2004, Olyphant 2004, Whitman 2004). Predictive modelling a surrogate for laboratory analyses, uses ambient conditions (temperature, precipitation, insolarity, wave height, wind speed/direction and other parameters) and hydrodynamic variables at the local or regional level to predict the outcome of bacteriologic analyses before they occur. For example, the Scottish Environment Protection Agency (SEPA) and Scottish Government have used their extensive national on-line hydrometric network of rain and river monitoring across Scotland to develop "nowcast" models which provide real-time public information and advice for bathers (see: Chapter 8) (McPhail & Stidson 2009). Varying degrees of success have also been demonstrated by these models in marine waters and the Great Lakes region of the

United States (Francy & Darner 2003, Olyphant & Whitman 2004, Nevers & Whitman 2005, He & He 2008, Nevers *et al.* 2009). The USEPA has developed a freely available software package designed to construct site-specific Multiple Linear Regression (MLR) models for the prediction of FIO levels at recreational beaches. (Virtual Beach, online: http://www.epa.gov/ceampubl/swater/vb2/). Since 2009, the Wisconsin Department of Natural Resources (WDNR) and the Ozaukee County Public Health Department (Ozaukee, WI, US) have partnered to implement an operational "nowcast" system in Port Washington, WI, using Virtual Beach which has proven more accurate than traditional monitoring (Mednick *et al.* 2011). Local operation of the "nowcast" model is tied to routine monitoring and notification, and requires less than five minutes of Health Department staff time per day (Mednick *et al.* 2011). Because beaches are unique, however, the predictive ability of "nowcast" models varies considerably from beach to beach and is not necessarily dependent on the number or quality of observations available for model-building (Mednick 2009). Another Virtual Beach case study site (Red Arrow Park Beach, Manitowoc, Wisconsin) had only 40 data points, yet the model FIO estimation was still more accurate than the persistence model (binary open/closed decision based solely on FIO counts). Similarly, results of experimental models built for North Beach in Racine, Wisconsin, showed that beaches with very few exceedances can still be successfully modelled (Mednick & Watermolen 2009, Kinzelman 2011).

Ultimately, a model's predictive power – as measured by model sensitivity and specificity, in addition to various goodness-of-fit measures – will determine whether input data were adequate or the beach was a good candidate for modeling (Mednick & Watermolen 2009). With respect to MST, predictive models, by virtue of associating increases in FIO density to prevailing environmental conditions, may provide site-specific estimates on when, and potentially where, faecal loading to surface waters is likely to occur.

9.3 SANITARY SURVEYS

Empirical evidence drawn from environmental observations may provide clues as to the "when" and "where" of contamination events, as well as inform associations between ambient conditions and FIOs. Measured ambient environmental conditions provide useful data for the development of tools such as predictive models. Well-crafted models have the ability to estimate FIO loading in real time, but in order to execute a classification scheme for recreational or other surface waters there must be a suitable mechanism to confirm pollutant sources; sanitary inspections are one such tool. A sanitary inspection, or survey, is an assessment tool designed to evaluate potential sources of faecal pollution

(Bartram & Rees 2000b). A sanitary survey may be used to identify probable sources of FIO, such as enterococci and *E. coli*, which are quantified as the basis for assessing health risk. Sanitary surveys can be conducted by local authorities when attempting to classify a water body for uses such as recreation or shellfish harvesting, when conducting an annual assessment, or in response to the isolation of high FIO levels from bathing waters as a result of compliance monitoring.

While the identification of direct sources may be undertaken fairly easily, a good sanitary inspection will aid in the identification of less obvious, indirect (nonpoint), sources of contamination. Sanitary surveys can identify possible pollution sources, yet they lack the capability to definitively identify the host source of FIOs and should be combined with indicator assessments and/or other source attribution techniques to provide the maximum benefit. The use of a combined approach, sanitary inspection plus FIO assessments, forms the basis of the health risk-based approach the beach management framework put forth in the Guidelines for Safe Recreational Water Environments (WHO, 2003). Field studies combining extensive sanitary surveys with FIO measurements and PCR-based analysis for host-specific markers of faecal contamination have been used successfully in Florida total maximum daily load (TMDL) implementation programmes (Staley *et al.* 2009, Wapnick *et al.* 2008).

In Europe, the EU Bathing Waters Directive (CEC 2006) establishes a statutory requirement for Member States to prepare profiles for each EU bathing water. These profiles are a form of sanitary survey which were to be in place by early 2011, and shall be maintained by a stipulated programme for review and update. They shall contain elements which describe the physical, geographic and hydrological characteristics of the bathing water (including, if appropriate, nearby surface waters), identify and assess causes of pollution which may impair bathers' health, and, if relevant, indicate other potential risks (e.g. cyanobacteria, macro algae and phytoplankton). The authorities in each EU Member State must assess the risk and anticipated nature, frequency, and detail of short–term (event-based) or remaining (persistent) pollution and provide management measures for improvement. They must also indicate what measures shall be undertaken during any such short-term pollution event. Information from the profiles is to be provided and disseminated to the public by appropriate communication channels and technologies, such as the internet, along with the provision of summary information in non-technical language at easily accessible places near bathing waters (e.g. by maps, posters or signage).

The Canadian Recreational Water Quality Guidelines (3rd edition, Health Canada 2010) recommend the use of sanitary inspections as a component of an integrated, multi-barrier approach to protect water users in Canada. Working

within a preventative framework, these surveys are one of a suite of procedures, actions and tools collectively designed to reduce exposure risks. Benefits of the multi-barrier approach are stated to be: more effective protection of public health, enhanced water quality management, improved public communication and informed hazard management (Health Canada 2010). In this capacity, the Canadian Environmental Health and Safety Survey (EHSS) serves as a flexible, site-specific blueprint for designing and implementing a recreational water quality management framework which includes compliance monitoring, public notification and mitigation. A properly performed annual EHSS would identify and assess all potential threats to health and safety (physical, chemical, biological/microbiological) leading to prioritization of intervention measures.

An EHSS should be conducted prior to the start of the bathing season and include (Health Canada 2010):

- a review of historical data/trends/problems; water body characteristic and usage observations;
- cataloguing of site-specific physical attributes;
- an assessment of potential pollution sources (especially those likely to contribute human or animal wastes);
- an evaluation of current monitoring programme effectiveness; and,
- an intervention measures performance appraisal.

The final assessment report should culminate in the development of best beach-management practices via improved operational plans, including site-specific monitoring schemes, which function as a feedback loop for continual quality improvement.

While used to assess operational quality (reduced closures due to elevated FIO levels) of shellfish beds in the United States,[1] the push for adoption of a standardized beach sanitary survey for bathing beaches was the result of the Great Lakes Regional Collaboration (GLRC) process (GLRC 2005). Previous legislation standardized national monitoring protocols employing FIOs and public notification, but made no provision for pollution source identification (United States Congress, 2000). The lack of a mechanism by which to identify contamination sources impacting bathing beaches resulted in 5,104–11,951 (2003–2005) water quality failure action days nationally, of which 84 per cent, on average, were attributed as (source unknown) (Kovatch 2006). Recognizing that identification and mitigation are necessary to reduce risk to human health through contact with surface waters and to provide the maximum personal, commercial, and recreational benefit, the GLRC Coastal Health Strategy Team recommended

[1] http://www.buzzardsbay.org/shellclssuccess.htm

that the US EPA standardize, test and implement a risk-based approach to manage recreational water (GLRC 2005). This approach builds on current water quality monitoring programmes, incorporating a standardized sanitary survey tool and expanding the programme to a holistic watershed assessment.

In 2006, a standardized sanitary survey tool was drafted.[2] In 2007 a trial of this tool was carried at 61 Great Lakes beaches within the USA and Canada. Prior to the trial all participants were asked to identify pollution sources or events which resulted in water quality failures in the previous year (2006). Study participants identified storm water discharge (16%), sanitary sewer overflows (<1%), and "unknown" sources (84%) (Rockwell & Wirick 2008). After completion of the pilot beach sanitary survey project, the participants were again asked to identify pollution sources or events which resulted in water quality failures during the study period (2007). Several relationships to environmental conditions were noted (wet weather-related discharges, re-suspension or transport of faecal matter via wave action, increased turbidity, avian sources and algal blooms) and the number of "unknown" sources dropped to 24 per cent, an improvement of 60 per cent over the previous year when no sanitary surveys were conducted (Rockwell & Wirick 2008). The identification of point or nonpoint pollutant sources, the recognition of transport mechanisms and the association of FIOs to ambient conditions are all crucial to the development of exposure interventions such as engineered storm water controls or naturalized mitigation measures (Kinzelman & McLellan 2009).

Physical condition assessments are also appropriate for assessing pollutant loading to rivers and streams (Abbott 2008). A wide range of methods are available and provide a tangible resource for decision making, management and restoration plans (Johnson *et al.* 2001, Roper *et al.* 2002). The Bank Erosion Hazard Index (BEHI) and near-bank stress (NBS), developed by Rosgen (1996, 2001) are some of the most common methods used (Somervielle & Pruitt 2004). These field monitoring methods involve focusing in on the most significant sources of sediment burden (which frequently carry faecal contamination and potentially pathogens) and estimating bank erosion. When sedimentation is an issue, problem areas in rivers and streams can be identified by undertaking stream bank erosion assessments such as the BEHI method. This method consists of quantifying sediment loading from bank sources, as well as locating key areas for implementing restoration efforts and management controls. The BEHI analysis is based on channel morphology and successful application of this method has been applied in many instances (Jones, *et al.* 2007, Ulrick & Nieber 2008). The unified stream assessment (USA) is a continuous stream-walk

[2] http://www.epa.gov/waterscience/beaches/sanitarysurvey/

method which systematically evaluates conditions to identify restoration opportunities within the stream corridor of a small watershed (Kitchell & Schueler 2005). The unified stream assessment provides a comprehensive overview of the condition of a river corridor and is based on nine components (storm water outfalls/discharge pipes, erosion, lack of vegetated buffer, leaky sewer/exposed pipes, trash/debris, channel modifications/dams, access and any unusual conditions).

9.4 SOURCE ATTRIBUTION TECHNIQUES

While *E. coli* can be useful for predicting the possible presence of faecal contamination in water via spatial/temporal distribution studies (Geldreich 1966), it does not provide any indication as to the source of pollution. In order to apply effective remediation practices for water bodies impaired by faecal contamination, the sources must be identified (USEPA 2005). This has led to much research and investment in recent years into the field of source attribution, a suite of discriminatory methods which have the potential to distinguish host sources (Scott 2002, Scott *et al.* 2002, Simpson *et al.* 2002, USEPA 2005, Field & Samadpour 2007). Other commonly used terms to describe these efforts are microbial source tracking (MST) and faecal source tracking. It is important to note that MST is a limiting term in that it is not technically inclusive of chemical methods used for source attribution. Used as part of an expanded monitoring programme along with primary and secondary FIO assessments, modelling, and sanitary surveys, source attribution techniques can provide the level of discrimination necessary to effectively identify water quality impairments due to faecal contamination.

While health risk from human sewage has been well established (Wade *et al.* 2006), the risk associated with domestic, agricultural or wild animal faeces is less clearly defined (USEPA 2007). Outside of additional epidemiological studies at water bodies solely impacted by animal sources, a direct approach for monitoring and identifying pathogens in water would be of benefit in safeguarding public health (Bertrand and Schwartzbrod 2007, Ruecker *et al.* 2007). Pathogen distribution in the aquatic environment is, however, uneven, the assembly size necessarily large, and pathogens are difficult and costly to analyze. Furthermore, the great variety of potential pathogens at any given location effectively precludes testing for even a representative subset with currently available methods. For those reasons, the combined use of FIOs and host-specific markers is important as part of a toolbox of assessment techniques allowing pathogen exposure to be predicted and reduced.

Source attribution includes several methods which may have the ability to determine whether faecal pollution is from human or non-human sources. Source tracking methods have successfully been applied to identify non-point source pollution responsible for the faecal contamination of water systems (reviewed in USEPA 2005). Methods for source attribution can be separated into three groups: molecular, biochemical and chemical. Many of the molecular and biochemical techniques have been applied or suggested for use in watershed studies and have been summarized (Simpson *et al.* 2002, Meays *et al.* 2004, Field & Samadpour 2007, Sadowsky & Santo Domingo 2007, Santo Domingo *et al.* 2007, Stoeckel & Harwood 2007, Yan & Sadowsky 2007). One of these approaches focuses on detecting host-specific molecular markers using the 16SrDNA gene of *Bacteroides* and the larger taxon, the *Bacteriodales* (Allsop & Sticker 1985, Fiksdal *et al.* 1985, Bernhard & Field, 2000a, 2000b, Dick *et al.* 2005). *Bacteroidales* constitutes one of the most numerous members of the human colonic flora. Known representatives are restricted to the gastrointestinal tract of warm-blooded animals and, unlike coliforms, this taxon makes up a significant portion of faecal bacteria (Finegold *et al.* 1983, Sghir *et al.* 2000), comprising approximately 30 percent of the flora in the human gut (Sears 2005). The application of this method has been used to detect and differentiate human and ruminant sources of faecal pollution in the environment (Bernhard *et al.* 2001, Lee *et al.* 2008, Reischer *et al.* 2008, Shanks *et al.* 2010).

In addition to distinguishing between human and ruminant sources, DNA markers may also be employed to distinguish avian from human sources. In the upper mid-western United States, one library-independent study (see section 9.4.2 below) used pooled genomic tester and driver DNAs in suppression subtractive hybridizations to enrich for host source-specific DNA markers for *Escherichia coli,* originating from locally isolated geese (Hamilton *et al.* 2006). While successful, this study results were applicable only to regional sources. Library-dependent MST efforts are also subject to regionalization for the best results (Stoeckel & Harwood 2007). A combination of both library-independent and library-dependent techniques may be necessary to provide the temporal and spatial acuity necessary to determine pollution sources (Edge *et al.* 2010).

9.4.1 Chemical analysis (source tracking/water quality indicators)

In addition to MST techniques, chemical parameters have been used to identify faecal sources (reviewed in Scott *et al.* 2002, Meays *et al.* 2004, Hagedorn & Weisberg 2009). Caffeine and coprostanol are two markers which can be used to confirm the presence of human sewage contaminated water. Caffeine is

present in a number of beverages, including coffee, tea, soft drinks and many pharmaceutical products (Scott *et al.* 2002). It is excreted in the urine of those who have ingested it. Although it requires expensive equipment to detect, degrades fairly rapidly within the environment and is naturally occurring in some plant species, caffeine has been suggested as an indicator of human sewage in surface waters due to its sensitivity (Burkhardt 1999, Hagedorn 2001). A recent study in Singapore demonstrated the usefulness of caffeine as a chemical marker for detecting human-source contamination (Wu *et al.* 2008).

Coprostanol is a faecal stanol, formed during the catabolism of cholesterol, present in the gut of humans and other animals. It is the primary stanol detected in domestic wastewater (MacDonald *et al.* 1983). It has also been proposed as a chemical indicator of sewage due to the presence of human specific variants (Chan *et al.* 1998, Edwards *et al.* 1998, Nash *et al.* 2005, Hagedorn & Weisberg 2009, Sullivan *et al.* 2010), in spite of its detection costs, sensitivity issues and non-anthropogenic sources.

Optical brighteners are chemical whitening agents added to many dishwashing and laundry detergents. Detection of these compounds in surface waters, using their ability to fluoresce under ultraviolet light, is simple, fast, and low-cost in comparison to caffeine and coprostanol. Although the detection of optical brighteners is complicated by fluorescence from humic acids and other organic materials that are naturally present in certain waters (Dixon *et al.* 2005, Harwood *et al.* 2005, Hagedorn & Weisberg 2009), this test has been successfully employed in determining the presence of human wastes in several environmental studies (Hagedorn 2001, Hagedorn *et al.* 2003, McDonald *et al.* 2006).

Tracking dry weather discharges from stormwater outfalls and other sources may require the use of field surveys, source attribution techniques or the chemical tracers previously mentioned due to the likelihood of a multi-source composition. Chemical observations can be made to support the quantification of the approximate components of a mixed discharge, using a variety of physico-chemical indicator parameters including: chlorine, conductivity, detergents, fluoride, pH, turbidity, copper, phenols, and others (Brown *et al.* 2004, Pitt 2001). While all may be employed, it is often necessary to utilize only a small subset when investigating the potential for illicit discharges, realizing that not one is perfect (Brown *et al.* 2004). Each community must determine which combination of chemical indicators works best, based on local conditions and potential discharge types. Although they are not chemical parameters, testing for indicator bacteria such as *E. coli* and enterococci in conjunction with chemical testing is likely to enhance the overall capability to track sources of sanitary sewage (Pitt 2001).

An ideal source attribution technique would be as easy and inexpensive as monitoring for FIOs, rapidly providing reliable, quantitative and easy-to-interpret differential diagnoses of faecal sources. The results of an ideal method should reflect the probability of the presence of human pathogens. In order for faecal source tracking methods to meet these criteria, the following should ideally be met (US EPA 2005, Harwood *et al.* 2007):

- each identifiable host source must possess unique characteristics that unequivocally distinguish it from other related or non-related sources (host-specific marker);
- the even distribution of host-specific markers must be predictable among hosts and have a similar environmental survival profile as host-specific markers from other species;
- the identifying trait (host-specific marker) must be detectable in water at varying concentrations;
- the host-specific traits should have a similar distribution and environmental survival profile as the FIO used to monitor that water body; and,
- the host-specific markers should correlate well with pathogen presence, fate and transport in the aquatic environment.

9.4.2 Limitations and challenges of faecal source tracking methods

Faecal source tracking, or MST, refers to the assignation of a pollutant source by tracing (fate and transport) or identifying a variety of microorganisms and/or chemicals back to their host (faecal source). Microbial source tracking may employ typical water quality indicators such as *E. coli* and enterococci as well as secondary or alternative indicators as previously described (Table 9.1). Analytical methods vary widely and may include DNA "fingerprinting", which refers to the analysis of patterns generated from the DNA of the organism and are generally performed on cultured isolates. DNA fingerprinting of faecal coliforms, *E. coli* and faecal streptococci (enterococci) forms a subset of MST, one tool in a toolbox of potential methods (USEPA 2005). Other MST methods that do not require culturing are also discussed below.

A variety of faecal bacteria, including traditional FIOs and other microbes, may lend themselves well to source attribution techniques and are the subject of an extensive body of recently published literature. In order to estimate relative contributions to a waterbody from an array of possible faecal sources, host-specific markers should have a distribution within their host and environmental survival profiles that mimic those of pathogens (USEPA 2005,

Harwood 2007). These ideal conditions do not hold true for traditional FIOs and, potentially, for many of the alternative organisms employed in source attribution techniques. It is, therefore, very important to understand the assumptions and limitations associated with each faecal source tracking tool and its application. The issues of host-specific marker survival, correlation with FIOs and pathogens, and quantitative source detection are interrelated in these assumptions.

Many source attribution methods have relied on library-dependent or library-independent methods utilizing FIOs used for regulatory purposes such as *E. coli* and enterococci (reviewed in Simpson *et al.* 2002, Scott *et al.* 2002, Genthner *et al.* 2005, Field & Samadpour 2007, Stoeckel & Harwood 2007). Library-dependent methods are based on the development of a database of isolates (usually bacterial) from known host sources. The isolates are typed ("fingerprinted") by phenotypic or genotypic methods, and the patterns generated make up the library. Isolates from the environmental samples are subsequently typed and compared to the library by a variety of statistical matching methods (Wiggins 1996, Hagedorn *et al.* 1999, Parveen *et al.* 1999, Harwood *et al.* 2000, Harwood *et al.* 2003, Ritter *et al.* 2003, Stoeckel *et al.* 2004). The database of DNA fingerprints from known sources is frequently constructed through the collection of geographically-related isolates, that is, local human sewage, ruminant manure, and wild or urbanized faecal samples from a variety of animal populations. Therefore, by their very nature, library-dependent methods are limited by the strength of the library and its relevance to the water body under investigation (Wiggins *et al.* 2003, Moore *et al.* 2005). While employed with some success, libraries based on phenotypic or genotypic characteristics of FIOs (such as antibiotic resistance) are subject to cross-reactivity between co-habitating species. Therefore, while library-dependent MST methods may be useful in determining pollutant sources within a suite of monitoring techniques, quantitative assumptions and the costs associated with assembling a library with sufficient assembly size and scope to assign a host source with some degree of certainty must be considered (Stewart *et al.* 2003).

Because of the limitations imposed by library-dependent methods that rely on typing cultured FIOs, alternative methods focusing on different faecal (bacterial) groups, such as the *Bacteroidetes* (or *Bacteroidales*), have been explored (Bernhard & Field 2000b, Field *et al.* 2003, Shanks *et al.* 2010). Other alternative targets include *Brevibacterium* for poultry faeces (Weidhaas *et al.* 2010) and viruses such as polyomaviruses (McQuiag *et al.* 2006, Hundesa *et al.* 2009). The use of MST targets other than regulatory FIOs introduces an additional level of uncertainty due to the already uncertain relationship between the marker-carrying organisms, FIOs and pathogens. The ecology of many

host-specific *Bacteroides* is not well-understood, as they have not been cultured, although some information is available on their survival and persistence (Kreader 1998, Walters & Field 2006, Okabe & Shimazu 2007) and on their host range and specificity (Bernard and Field 2000a, Gawler *et al.* 2007, Shanks *et al.* 2010). Other characteristics are less well understood at present (e.g. typical concentrations in natural samples, movement through catchments, relationships with enteric viruses). However clear source tracking may be conceptually, the actual application of the techniques and data interpretation is still under development.

Data interpretation must be approached with care and sampling strategies, in particular, need to be robust and relevant to the question(s) at hand. The impacts of point sources can be readily monitored using culture-based FIOs; source attribution techniques are not always needed (though they can provide weight of evidence). The use of ultraviolet (UV) disinfection can complicate detection of host-specific markers using methods such as PCR, as the microorganisms are killed yet their DNA may persist (Bae & Wuertz 2009). However, source tracking may be applied to understanding specific issues in water quality, often providing very useful information on diffuse sources of pollution. For example, it may be useful in explaining a decline in water quality following rainfall events, which can impact on shellfish and bathing waters, where increases in faecal bacteria may originate from agricultural run-off or from raw sewage through a discharging sanitary or combined sewer overflow (CSO) (Stapleton *et al.* 2009). Although these associations are informative, correlating the results of source attributions studies to pathogen presence and/or human health outcomes would represent a more robust means of public health protection.

9.4.3 Correlation of host-specific markers with FIOs and pathogens

There are conflicting study results regarding the correlation of host-specific markers to elevated levels of FIOs. For example, a field study by Vogel *et al.* (2007) reported an absence of coliphages during periods when high concentrations of *E. coli* were noted. In contrast, another study found a positive correlation between coliphages and FIOs, and also between F-RNA coliphages and human adenoviruses (Ogorzaly *et al.* 2009). One field study found a poor correlation of bacterial and viral markers of human sewage with FIOs; however, the human-associated markers correlated well with each other (McQuaig *et al.* 2006). Multiple studies have reported detection of human-associated *Bacteroidales* molecular markers when FIOs are low or absent ((Bernhard & Field 2000a, Boehm *et al.* 2003, Bower *et al.* 2005); such findings can

complicate interpretation from the regulatory standpoint, which focuses on FIO density-based classification. And while a recent field study reported a low correlation between quantitative *Bacteroidales* markers and FIOs, there was a good correlation between indicator and *Bacteroidales* delivery flux (Stapleton *et al.* 2009). Other studies report a good correlation between FIOs and *Bacteroidales* markers directly (Shanks *et al.* 2006). These differences are likely to be caused by some combination of differential survival between host-specific markers and FIOs, low/varied levels of faecal contamination, or geographic variation in the prevalence of markers.

Since the purpose of recreational water quality regulation is primarily to protect human health, the extent to which host-specific markers are able to predict the occurrence of human pathogens may be of greater importance than their correlation to FIOs ((Field & Samadpour 2007). A study in Alberta, Canada, compared the occurrence of human, ruminant and pig-specific *Bacteroidales* markers with the pathogens (*Campylobacter*, *Salmonella*, and *E. coli* O157:H7) and found a positive relationship between a *Bacteroidales* general marker and pathogens (Walters & Field 2006). The detection of ruminant-specific *Bacteroidales* markers also predicted *E. coli* O157:H7 occurrence and there was a significant increase in the probability of detecting *Salmonella* when ruminant markers were also present. Another Canadian study which compared the occurrence of human-, ruminant- and porcine-specific *Bacteroidales* markers in water samples to the occurrence of FIOs, thermotolerant *Campylobacter, Salmonella*, and shigatoxin genes yielded similar results (Fremaux *et al.* 2009), with elevated *E. coli* counts related to the presence of human and ruminant markers. Furthermore, ruminant marker CF128 was predictive of the presence of *Salmonella spp* (Fremaux *et al.* 2009). In addition to *Bacteroidales*, some studies have reported good predictive value between coliphages and the presence of human viruses (Ogorzaly *et al.* 2009) and between FIOs, coliphages, and enteroviruses (Costan-Longares *et al.* 2008). Neither *Bacteroidales* nor coliphages as a single indicator proved suitable for predicting *Cryptosporidium* oocysts through the wastewater treatment chain (Costan-Longares *et al.* 2008).

It has been suggested that a combination of bacterial and phage indicators is necessary to ensure the microbial quality of reclaimed water (Costan-Longares *et al.* 2008). In a New York river system study (examining microbial fate, transport and partitioning) *Cryptosporidium* and *Giardia* demonstrated a greater association to particulate matter than FIOs, although settling rates were similar (Cizek *et al.* 2008). The correlation between these organisms and FIO was, however, weak in this study as well as in others (Savichtcheva & Okabe 2006, Field & Samadpour 2007, Cizek *et al.* 2008). Faecal indicators organisms, such

as *E. coli* and enterococci sometimes demonstrate a lack of correlation to each other as well as to pathogens such as *Salmonella* spp., *Campylobacter* spp., human viruses and coliphages (Savichtcheva & Okabe 2006, Field & Samadpour 2007). These examples are likely to reflect differential survival between pathogens and FIOs, and their different distributions in host populations. A study by Weaver *et al.* (2005) of *E. coli* and enterococci in fresh and dry cow, sheep and horse manure demonstrated this phenomenon and confirmed the results of a previous source-tracking proficiency study targeting *E. coli* and enterococci levels in faecal samples from a number of host species (Field 2004).

9.4.4 Quantification of source specific markers

Advancements have been made in the field of source attribution and the ability to not only assign a host source but to quantify those host-specific markers using techniques such as nucleic acid sequence-based amplification (NASBA) and quantitative real-time PCR (QPCR) (Dick & Field 2004, Choi & Jiang 2005, He & Jiang 2005, Gregory *et al.* 2006, Layton *et al.* 2006, Ogorzaly & Gantzer 2006, Reischer *et al.* 2006, Khan *et al.* 2007, Kildare *et al.* 2007, Kirs & Smith 2007, Okabe *et al.* 2007, Rajal *et al.* 2007a, Wolf *et al.* 2007, Caldwell & Levine 2009, Hundesa *et al.* 2009, McQuaig *et al.* 2009, Mieszkin *et al.* 2009a). For example, a QPCR method for detecting poultry (chickens and turkeys) pollution was developed based on a *Brevibacterium avium*-like 16S rRNA gene sequence (MacBeth *et al.* 2008, Weidhaas *et al.* 2010). The marker was detected in poultry faeces, poultry litter, soil on which litter had been spread and in surface waters close to areas where poultry litter was applied to the land. As with qualitative techniques, result interpretation may not be straightforward. Assumptions underlying quantitative source detection include (1) host-specific markers have similar environmental survival rates, fate and transport, (2) each species sheds a similar amount of its host-specific marker, and (3) each host-specific marker has a similar prevalence and proportional distribution among individuals within the species.

Leach *et al.* (2008) used a modelling approach to test the effects of multiple variables on the quantitative abilities of source tracking including: prevalence or amounts of host-specific markers among individuals of the same species; specificity of markers; proportions of markers shed by different species; proportion of faecal contamination in the environment; decay rates; and evenness of environmental distribution on the ability of quantitative source tracking to correctly estimate relative contributions from different sources and the percentage error likely to result from each of these factors in quantitative

versus qualitative (presence/absence) assessments. It was found that quantitative source tracking functioned best when there were equal numbers of different hosts shedding large amounts of highly-specific markers in well-mixed environments (Leach *et al.* 2008). Variation in the distribution and density of faecal contamination in the environment contributed to method error with the highest error associated with sparsely-contaminated and/or unmixed environments. This suggests that temporal variability, a survey of the geographic distribution of host-specific markers, an examination of cross-species marker occurrence and an assessment of environmental decay rates are all important preliminary steps prior to engaging in and contributing to the success of quantitative source attribution projects.

9.5 PRACTICAL UTILITY OF SOURCE ATTRIBUTION STUDIES

Source attribution has been successfully employed in determining the cause of faecal contamination at bathing beaches (Brownell *et al.* 2007, Korajkic *et al.* 2009) and when examining faecal loading as a function of land use (Gourmelon *et al.* 2007, Lee *et al.* 2008). Although many of these studies have focused on human-specific targets, some have included one or more methods for detection of animal faeces (Cole *et al.* 2003, Stewart-Pullaro *et al.* 2006, Gourmelon *et al.* 2007, Stapleton *et al.* 2007, Lee *et al.* 2008, Fremaux *et al.* 2009, Jenkins *et al.* 2009, Stapleton *et al.* 2009, Edge *et al.* 2010). Source tracking is becoming an integral part of the total maximum daily load (TMDL) assessment and implementation strategy in the USA. In Florida, a recent study used markers for human (*Bacteroidales* HF183 and human polyomaviruses), general ruminant, and horse targets to determine the dominant contributors to elevated FIO levels in tributaries of the Hillsborough River (Wapnick *et al.* 2007). The success of this study relied on intensive sampling of the tributaries using low-cost methods (FIO testing by membrane filtration) to determine the most contaminated sites as a precursor to targeted deployment of source tracking methods based on a thorough land use assessment and field survey of probable sources. The use of multiple assessment methods allowed the identification of specific sites and sub-watersheds dominated by ruminant or human pollution as well as those that received mixed inputs. The study results were incorporated into the basin management action plan (BMAP) of Hillsborough County.[3] Of interest, this study found low correlation between marker detection and FIO concentrations;

[3] http://www.dep.state.fl.us/water/watersheds/bmap.htm

the only significant relationship being a weak correlation between human-specific *Bacteroidales* marker by conventional PCR and *E. coli* counts.

A study in a large Oregon coastal watershed used presence/absence data on human and ruminant *Bacteroidales* (HF183, HF134, CF128, CF193) along with *E. coli* counts, water quality measurements and climatic data in order to elucidate temporal and spatial dynamics of the markers and locate specific sources of contamination (Shanks *et al.* 2006). This study demonstrated a strong statistical linkage between ruminant host-specific markers, FIOs and associated FIO loading to specific point and non-point sources of contamination on a per sampling site basis (Bernhard & Field 2000b). A similar study in New Zealand utilized human and ruminant-specific markers to assess pollution sources in a mixed urban and rural watershed (Kirs *et al.* 2008). Although some incomplete specificity of the *Bacteroidales* ruminant marker was noted in the New Zealand study, for example, cross-reactivity with marsupial and horse faeces, the PCR methods proved useful to discriminate human from livestock pollution. An important observation made in several field studies is that source tracking data are more reliable on high-flow samples, and/or when faecal contamination is high (Gourmelon *et al.* 2007, Stapleton *et al.* 2009). In addition, correlation of source tracking markers with FIO density is much stronger under these conditions. This conclusion was supported by a modelling study which found that conditions of lower contamination "generated significantly greater variance (17 per cent versus 1.7 per cent for qualitative analyses) and resulted in underestimates for contributions from each host" (Leach *et al.* 2008).

9.6 STUDY DESIGN – INCORPORATING A HOLISTIC APPROACH

Advantages and limitations to various FIOs, alternative or secondary indicators and source attribution techniques have been discussed in this Chapter. The use of site assessment methods in the form of sanitary surveys (USA), beach profiles (EU) or environmental health and safety surveys (Canada) as a means to target monitoring and remediation have also been introduced. An approach not solely based on FIO levels would be able to take into account the source of the contamination which may be identified through the use of sanitary inspections. Another consideration, other than the source of the contamination, is the inherent variability of bacterial density in water environments. Bacterial concentrations may change abruptly both spatially and temporally making microbial assessments based on a single grab sample invariably a snapshot of water quality captured at a single moment in time (Whitman *et al.* 2004b). The

feasibility of a health-based monitoring approach of recreational waters was proposed as the result of an expert consultation sponsored by the WHO and USEPA and resulted in the development of the *Annapolis Protocol* (WHO 1999). The *Annapolis Protocol* suggests a classification scheme for recreational waters (very poor, poor, fair, good, or excellent) based upon health risk. A classification scheme would allow for more flexibility while still measuring microbial indicators of faecal contamination using approved analytical methods since influential factors such as the variable effects of precipitation and wave action can be accounted for within the overall bathing water quality assessment framework. A holistic and systematic integrated approach aimed at providing insight into the sources and mechanisms responsible for degraded water quality, may serve as a model for source determination and remediation in flowing, inland and coastal water bodies worldwide.

The current WHO guidelines for safe recreational water environments base the initial classification of recreational waters on the combined evidence of human faecal contamination sources (sewage, river discharge, and bather contamination) and compliance monitoring using faecal indicator organisms (WHO 2003). Where human inputs are minimal the potential for animal faecal pollution must also be addressed (WHO 2003). The ability to ascribe a level of risk associated with primary water contact could be applied to as an effective public health management action, such as the posting of an advisory notice to discourage recreational water use during incidences of poor water quality. Better management and remediation of contamination sources would allow for the reclassification of bathing beaches previously identified as having poor water quality (WHO 1999).

9.7 CASE STUDIES

In this section two case studies are presented which illustrate the efficacy of source attribution techniques as part of a holistic monitoring approach. In these case studies various assessment schemes are utilized to identify sources of contamination impacting source or surface waters: genotyping of *Cryptosporidium spp* in a drinking-water associated outbreak in the United Kingdom and the use of source attribution techniques as part of monitoring scheme to locate and mitigate pollutant source impacting bathing waters at a beach in the USA. While by no means comprehensive, they provide good examples of how agencies can use the best available science to develop monitoring, public notification and remediation plans for the improvement of source/surface water quality and the reduction of health risks.

Case Study 1 Drinking-water quality: genotyping of *Cryptosporidium* leads to the identification of the source of contamination[4]

Background

Identification of *Cryptosporidium* in treated drinking-water has relied on immunofluorescence microscopic detection of oocysts in water specimens at treatment plants. Positive findings indicate the presence of oocysts but cannot determine the species or subspecies. A dilemma arises when oocysts of *Cryptosporidium* are detected in finished water at the treatment plant but it is not known if those oocysts arise from a species known to infect humans. If the oocysts are potentially infectious for humans swift action should be taken to protect public health and prevent an outbreak. If the oocysts are, however, potentially non-infectious for humans, actions such as a boil-water alert could be expensive, inconvenient and unnecessary. The application of molecular methods to identify species and genotypes, although time consuming and expensive, can prevent unnecessary alarm and enormous costs to the community and the water utility. This application, in conjunction with other observations, provides the evidence to identify organisms with the potential to cause infections in the human population and identify the probable source of those species and genotypes.

Problem

In the area of Northamptonshire, England approximately 250,000 people receive tap water from the Anglian Water Company. In July 2008 an outbreak of cryptosporidiosis was linked to contaminated tap water. On 23–24 June 2008 *Cryptosporidium* spp. oocysts were detected by direct immunofluorescence antibody (DIA) microscopy as part of routine operational monitoring of treated water at a surface water treatment works. *Cryptosporidium* oocysts were also detected by DIA microscopy from the bowel contents of a rabbit carcass removed from a tank at the water treatment works. The positive DIA microscope slides (treated water and rabbit gut) were sent to the UK *Cryptosporidium* Reference Unit (CRU), Swansea, Wales, for molecular typing. On 25 June 2008 a precautionary boil-water notice was implemented and enhanced surveillance for human disease cases was established by the health protection team in the affected area. Enhanced surveillance was initiated on 26 June. Beginning on 27 June and into July, an outbreak involving 23 cases of cryptosporidiosis was detected in the community. The genotype found in these cases matched that of the oocysts found in the water supply and the rabbit carcass.

To reduce the potential for infection within the impacted area Anglian Water vans equipped with loudspeakers were sent out to warn people in about 108,000 homes

[4] Contributed by Ron Fayer, United States Department of Agriculture, and Lihua Xiao, Centres for Disease Control and Prevention, Atlanta, GA, USA.

across 85 communities in Northamptonshire about the problem, a boil-water advisory was announced and local residents had to boil their water for 10 days until the source was found and removed (BBC News, 25 June 2008). Approximately 1000 miles of pipes were cleaned and flushed, bottled water was delivered to 100,000 households, and households were compensated at the cost of £3 million. Twenty schools in the area that relied on tap water for drinking-fountains were temporarily closed.

Improvement initiative

Presumptive and confirmatory testing for *Cryptosporidium* in tap water conducted in a timely manner provided information of immediate public health importance. Acting expeditiously on these data by informing the public through media notification, including boil-water alerts while providing safe drinking-water minimized the number of cases, reducing morbidity and mortally. The *Cryptosporidium* rabbit genotype, which was not previously known to infect humans, was identified as the etiologic agent in an outbreak of diarrhoeal disease and should be considered a human pathogen when found in future tests.

Lessons for the future

Routine monitoring at the water treatment plant enabled the detection of low levels of *Cryptosporidium* at an early stage and allowed the affected part of the treatment works to be swiftly isolated. The system of rapid public notification and the corroboration of positive water samples with the potential source of infectious material and positive specimens from human cases by molecular methods combined with replacement of contaminated drinking-water by bottled water helped to keep the number of outbreak cases relatively low. In addition the discovery of a previously unrecognized human pathogen provided scientific evidence of new pathogen of public health concern (Chalmers *et al.* 2009). The model applied to this outbreak can be adopted by other water treatment companies and public health agencies.

Case Study 2 Using expanded FIO monitoring, sanitary surveys and source attribution techniques to identify pollution sources and guide remediation at a Great Lakes coastal beach[5]

Background

Racine, Wisconsin (WI), USA is a Great Lakes coastal community which derives a portion of its economic livelihood from tourism. In 2000, bathing-water quality

[5] contributed by Julie Kinzelman, City of Racine, Wisconsin, USA

failures at the main public beach reached a high of 66 days, or 62 per cent of the swimming season. While there was public outcry at the loss of the utility, the contamination sources were unidentified and no remediation plans were in place.

Problem

Many coastal communities throughout the Great Lakes region and the USA suffer from frequent water quality advisories at their bathing beaches. These advisories have direct and indirect economic repercussion as well as human health implications. While the problem is recognized, there is no clear direction on how best to navigate through the available monitoring and source attribution techniques in order to gain the most meaningful results at the least expense nor, prior to 2007 (GLRC 2005), was there a standardized site assessment tool which would aid responsible authorities in directing their actions towards the most likely pollution sources. Racine, WI, was one such community where CSO events from neighbouring communities to the North, the local publicly owned treatment works (POTW) and urbanized gull populations were incriminated but not confirmed to be culprits.

A series of research studies was conducted from 2000–2005 to rule in or rule out these incriminated sources and target mitigation measures based on relative contribution to poor surface water quality. Routine monitoring using FIOs was expanded to include multi-depth and spatial distribution studies. Sanitary surveys were employed to determine when and where contamination events were most likely to occur. Results of initial screening and empirical data were confirmed using antibiotic resistance profiles assembled from local source libraries of human sewage, domesticated animals (including urbanized gull populations) and wildlife as well as human- and bovine-specific *Bacteroides* markers. Results of these assessments indicated that water quality was most likely to degrade as a result of direct storm water discharge, surface run-off resulting from rain events greater than 2.5 cm and the exchange between beach sands and near-shore surface water facilitated by wave action. Sources of contamination included sanitary infiltration into the storm sewer system, non-human faecal contamination associated with wet weather events, and urbanized gull populations. In order to improve water quality, each of these sources and their transport mechanisms had to be addressed.

Improvement Initiative

Improvement initiatives were incremental and occurred over a period of nine years, with the most active phase taking place within the first five (Table 9.2).

Pluming studies conducted from 1999–2000 revealed the extent to which a storm drain discharging directly onto beach sands had the ability to adversely impact surface water quality. Two primary underground treatment chambers were installed to retain street debris, grits and oils. From these chambers the first flush storm water was

diverted to a series of nine sand-bottomed infiltration/evaporation cells functioning as a constructed wetland. In summer of 2000, the period of time immediately preceding the re-engineering of the storm drain, the concentration of *E. coli* per 100 ml of storm water exceeded 1000 cfu/100 ml on 36 out of 46 sampling events or 78 per cent of the time. In the summer of 2005 the concentration of *E. coli* per 100 ml of storm water exceeded 1000 MPN/100 ml two out of 14 times or 14 per cent of the time, a reduction of 64 percent (Kinzelman & Hiller 2007). The mean seasonal *E. coli* content in storm water discharging from this catch basin was reduced by two orders of magnitude alleviating many of the bathing water quality failures associated with wet weather events.

Table 9.2 Timeline of improvement initiatives, City of Racine, WI, USA.

Timeline of Improvement Initiatives:	
2000:	Re-engineering of storm water outfall begins; autumn of 2000 (includes both a system for the mechanical removal of solid wastes and a series of infiltration/evaporation basins to retain first-flush storm water)
2001:	Initial beach grooming study conducted by Racine Health Department Laboratory
2002:	Phase II beach grooming study, re-engineering of outfall completed, infiltration beds planted with native wetland plant species
2003:	New beach grooming techniques implemented; provides a 30% reduction in dry weather advisories, signs posted prohibiting feeding of seagulls, city storm drains placarded – "no dumping, drains to lake"
2004:	Additional storm drains placarded and more wetland plants added to storm water outfall site
2005:	Beach slope improved, swales removed, and berm crest enhanced to reduce transport of bacteria from beach sands to near shore waters, additional storm drains placarded, weekly median *E. coli* level from storm water discharge points drops from 3000 MPN/100 ml to 41 MPN/100 ml
2006:	Beach grooming and grading continue according to new best management practices, additional waste receptacles (with liners) added in life-guarded areas, algae & aquatic plant removal occurs as necessary, educational signage installed on Lake Michigan pathway
2007:	Created dunes are used to manage blowing sand, washouts filled in after significant rain events, routine/annual beach sanitary surveys conducted, ongoing predictive modelling and QPCR studies

In order to address dry weather bathing-water quality failures a series of beach sand manipulation measures were executed. Previous beach grooming techniques provided an aesthetically pleasing and debris-free beach, but significantly increased bacterial indicator organism density when beach sands were wet (Kinzelman *et al.* 2003,

Kinzelman, *et al.* 2004). Alterations in conventional techniques envisaged deeper grooming without levelling and compacting beach sands which promoted UV light penetration, more rapid drying of beach sands and a reduction in moisture content (Kinzelman & McLellan 2009). The slope or grade of the beach is important when considering the possibility of transfer of bacterial organisms from foreshore sands to near shore waters, for example, reducing non-point contamination. The removal of deep swales, an improved beach grade, the addition of encouraged dune ridges and a pronounced beach face provided better drainage and limited the encroachment of waves onto the foreshore beach sands.

Another improvement measure included the placement of multiple waste receptacles with rigid liners (which could be emptied on an as-needed basis by the lifeguards) in the backshore and foreshore beach area. This beach management measure improved waste management, an important step in deterring lazing/loafing of urbanized gull populations in near-shore areas. Best practices also include the removal of stranded macrophytes and algae. Preliminary research results indicate water quality degrades over time if mats remain floating or submerged in near-shore waters (Kleinheinz *et al.* 2009). Regulatory measures included enacting an ordinance prohibiting the feeding of shorebirds and disallowing dogs on the beach. Public education initiatives included informational signage, a media campaign and the use of volunteers to stencil storm drains throughout the city.

Lessons for the future

The judicious use of routine and expanded monitoring programmes employing FIOs, source attribution techniques, and sanitary surveys can result in the successful identification of pollutant sources impacting water bodies and the targeting of mitigation measures. The City of Racine, WI, US successfully employed this scheme to reduce water quality advisories from 62 per cent of the bathing season in 2000 to five or less from 2005–2009. Sustainable use of water resources was shown to have positive economic, environmental, and social benefits for coastal communities.(Table 9.3)

Table 9.3 Annual beach advisories/closures posted at North and Zoo Beaches, Racine, WI for the period 2000 to 2009.

Annual Bathing Water Quality Advisories (# days/% season) – Racine, WI			
Year	**North Beach**	**Zoo Beach**	**Beach Season (Total Days)**
2000	62/66%	39/41%	94
2001	17/20%	21/25%	84
2002	27/31%	22/25%	87
2003	31/32%	26/27%	96

(*Continued*)

Table 9.3 (*Continued*).

Annual Bathing Water Quality Advisories (# days/% season) – Racine, WI			
Year	**North Beach**	**Zoo Beach**	**Beach Season (Total Days)**
2004*	22/22%	16/16%	99
2005	5/5%	5/5%	99
2006	3/3%	7/7%	94
2007*	3/3%	7/7%	94
2008*	1/1%	3/3%	87
2009	2/2%	6/6%	93

*Record rainfall recorded for the months of May & June 2004, August 2007, and June 2008. North Beach awarded *Blue Wave* status in **2004**

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10

Comparative risk analysis

Graham McBride, Tom Ross and Al Dufour

10.1 ESSENTIALS OF RISK ASSESSMENT

Risk assessment is a systematic process to estimate the level of risk related to some specific action or activity. It is now commonly applied to a wide variety of human endeavours in which harm to people, the environment or economic interests might occur. In the context of public health, the process attempts to quantify the likelihood and severity of illness to individuals or populations from a specified hazard.

The primary purpose of risk assessment is to provide support for decisions about managing risks associated with those specific actions or activities. This is done by systematically synthesizing information about the factors that contribute to the risk in a coherent framework that enables risk, or relative risk, to be inferred from knowledge of those risk-contributing factors in specific circumstances. Depending on the needs of the risk manager, the risk assessment may attempt to assess the relative effectiveness of proposed risk mitigations, or to estimate the

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magnitude of the risk under different circumstances, or the risk to different populations or sub-groups within the population.

There are many frameworks that describe the interaction between risk assessment[1] and risk management, and a third component known as "risk communication" which involves understanding the interests, concerns and values of "stakeholders", that is, those affected by the risk, so as to guide and optimize risk management decisions. One depiction of the interaction among these aspects of risk analysis is presented in Figure 10.1.

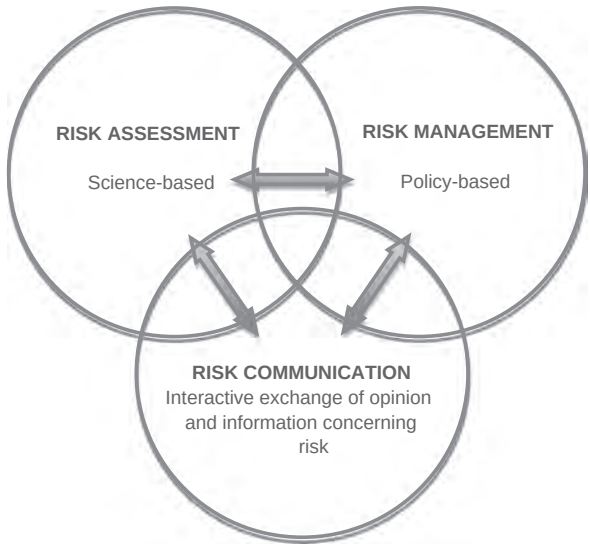


Figure 10.1 A depiction of the interaction between risk assessors, risk managers and those affected by the risk ('stakeholders') within the risk analysis framework developed by the World Health Organization and the Food and Agriculture Organisation of the United Nations. Arrows indicate lines of communication, while separate circles are intended to depict discrete roles of those responsible for risk assessment, those responsible for making decisions about risk, and stakeholders (based on: WHO/FAO 2009).

[1] While some organisations consider risk assessment to encompass risk management, risk communication and risk analysis, many organisations involved in environmental and public health risk assessment consider risk analysis to be the 'umbrella' activity that encompasses risk assessment, management and communication. For example, the Society for Risk Analysis (http://www.sra.org) broadly defines risk analysis "…to include risk assessment, risk characterization, risk communication, risk management, and policy relating to risk." We choose to adopt this convention here.

Risk management involves a balance between the most effective risk mitigation action, based on cost or technical feasibility, and the interests and values of stakeholders. Therefore risk managers will also seek information on costs (compared to the benefits) of risk management options. The efficacy of various approaches and the factors that affect it may be considered in the risk assessment to support the decisions of risk managers. The combination of risk assessment, risk management and risk communication is, in some frameworks, collectively termed 'risk analysis'. Microbiological risk assessment frameworks relevant to waterborne risk are discussed by Haas *et al.* (1999, Chapter 3); WHO (2003); Gale (2001a&b, 2003), Coffey *et al.* (2007) and Goss & Richards (2008).

10.1.1 Key elements

The first step in risk assessment usually is the development of a conceptual model or framework that combines knowledge of risk-affecting factors and how they could interact to cause harm. In many cases this information can be expressed mathematically as a series of equations that, provided sufficient quantitative data and knowledge exist, enable quantification of the risk or, at least, the relative risk. In assessing the public health risk from exposure to microbial pathogens, the "risk assessment" task is often broken down into four discrete components:

- *Hazard Identification* – which involves describing the hazard (pathogens), presenting the evidence that the hazard causes illness and that it can cause illness from the source of exposure being considered, and the type of illness caused.
- *Hazard Characterization* – which presents information about characteristics of the organism that affect its ability to cause illness, such as virulence factors, physiological traits that affect its survival in the environment and, importantly, the severity of disease caused, including consideration of differences in susceptibility of different members of the population exposed and the probability of illness as a function of the dose ingested. In the context of water and sanitation this corresponds to consideration and characterization of elements of "pathogen virulence" and "host susceptibility". This includes consideration of dose-response relationships, which is sometimes considered as a further discrete component (e.g., Haas *et al.* 1999).
- *Exposure Assessment* – which attempts to estimate the exposure of the affected population(s) to the pathogen under scrutiny. In the context of public health risk from recreational/occupational exposure to water this could involve consideration of, for example: sources of contamination,

loads and composition of pathogens in sources of contamination, and factors that would increase or decrease the risk of exposure such as inactivation due to UV irradiation, predation, or human interventions. It should also consider factors that would alter exposure of different members of the population such as age (children may swim more often, or ingest more water when swimming), cultural and gender factors (e.g., women in developing nations may be more likely to be exposed due to their domestic responsibilities), type of water exposure (e.g., full immersion through swimming *cf.* accidental exposure through clothes-washing). In the context of water and sanitation this corresponds to consideration and characterization of elements of load, transport and exposure (see: Chapters 2–7).

- *Risk Characterization* – the systematic and scientific process of synthesizing all the relevant knowledge and information to produce estimates of risk, or relative risk.

10.1.2 Principles of risk assessment

Ideally, the model and the data and knowledge that it is based on will be clearly documented, or 'transparent'. Usually, however, insufficient knowledge and data are available to enable unambiguous assessment of (comparative) risk, or decisions based on that risk, and a number of assumptions will need to be made in the development and application of the risk assessment model. These assumptions, and their potential consequences for the decisions based on them, should also be clearly articulated.

10.1.3 Pathogen selection

Many zoonotic pathogens have been reported in the literature as the causal agents of human infections or outbreaks of disease. The majority of these human infections were transmitted from animals to humans by food products (O'Brien 2005). Several outbreaks have been transmitted by drinking-water and only a small number have been transmitted by recreational water (Craun 2004). Outbreaks may, however, comprise only a minority of the actual cases suffered: "*…probably the vast majority of waterborne disease burden arises outside of detected outbreaks*" (Bartram 2003). The zoonotic bacterial pathogens mentioned most frequently are *E. coli* O157, *Campylobacter*, *Salmonella*, *Leptospira* and *Listeria*. Protozoan pathogens include *Cryptosporidium*, *Giardia* and *Toxoplasma* (Schlundt *et al.* 2004, Pell 1996, Rosen 2000, Bicudo & Goyal 2003). Although most of the zoonotic pathogens have been associated with sporadic and outbreak patterns of disease, a few have not been associated with

waterborne outbreaks or with faecal contamination of water. The following criteria have been adopted whereby pathogens from the list above will be selected for inclusion in our risk comparison process.

- The pathogens are known to be carried by animal or bird species;
- The pathogens are discharged in the faeces of animals or birds;
- The pathogens have been isolated from surface waters;
- The pathogens cause disease in humans.

The zoonotic pathogens that meet these criteria are:

E. coli O157 (more generally, EHEC) which are predominantly carried by ruminants including beef and dairy cattle and sheep (USEPA 2000a, Caprioli *et al.* 2005) and, to a lesser extent, monogastric animals (Chapman 2000, Chapman *et al.* 1997). This organism has been associated with outbreaks of disease that are related to recreational water (e.g. Keene *et al.* 1994) and has been isolated from surface waters. None of the outbreaks have been linked directly to direct contacts with animals. However, some have been associated with food and drinking-water.

Campylobacter species are frequently found in surface waters and this organism is carried by poultry, cattle and sheep (Jones 2001). *Campylobacter* has frequently been linked to outbreaks transmitted by food and water, but its occurrence is predominated by sporadic and endemic patterns, rather than outbreaks. In New Zealand, where campylobacteriosis is a reportable disease, about 300 to 400 cases per hundred thousand population have been reported in recent years (Till & McBride 2004, Till *et al.* 2008).[2] In western and northern Europe, in the late 1990s reported rates per 100,000 varied from ~20 (The Netherlands) to ~100 (England Wales) (Kist, 2002). Annual reported incidence in Australia is ~100/100,000 (NNDSS, 2010) and in Canada seems to be declining but at the time of writing is ~30/100,000 (PHAC, 2010). It is important to note that the actual incidence of this disease in the USA is estimated to be ~40 times higher than the reported rate (Mead *et al.*, 1999), because many cases are not detected by a country's health reporting system.[3]

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[2] This rate has approximately halved in recent years. This has been attributed to management interventions in the poultry industry (French *et al.* 2011, McBride *et al.* 2011).

[3] The many reasons for this state of affairs are often described by the "reporting pyramid" (e.g., Lake *et al.* 2010). Layers in this pyramid depict all the necessary steps that must be taken before it is possible to report incidence. For example, an ill person must visit a doctor who must request that a stool sample be supplied and analysed for the presence of *Campylobacter*, an infected stool must be supplied and analysed correctly, and a positive result must be entered into the reporting system. If any one of these steps is not completed, the case will not be reported.

Salmonella species have been isolated from cattle, sheep, poultry and swine (Davies *et al.* 2004, Li *et al.* 2007). Although *Salmonella* has seldom been linked to outbreaks related to recreational waters, it is one of the most frequently reported foodborne diseases worldwide (Schlundt *et al.* 2004) and has been implicated in illness caused via contaminated drinking-water (Febriani *et al.* 2009). At least two swimming-associated outbreaks of salmonellosis have been reported in the literature (Moore 1954, Anon. 1961).

Cryptosporidium has been isolated from cattle faeces (USEPA 2000a) and from surface waters. Outbreaks of swimming-associated disease caused by *Cryptosporidium* have been reported in the literature (e.g. Hunter & Thompson 2005).

Giardia has been isolated mainly from cattle. In the United States more than 50% of dairy and cattle herds are infected with this organism (USEPA 2000a). It has frequently been isolated from surface waters. Although there is much evidence available that shows infection by the waterborne route is very frequent, little evidence is available about swimming-associated outbreaks. Craun *et al.* (2004) indicated that four outbreaks associated with untreated recreational water in USA lakes and ponds were detected between 1971 and 2000.

These five zoonotic pathogens of primary concern will be the subject of this risk comparison chapter. Notably, the organisms selected also correspond with those non-viral pathogens identified by Coffey *et al.* (2007) as being the greatest causes of waterborne disease outbreaks in USA and Europe during the late 20th century.

10.2 THREE RISK ASSESSMENT PARADIGMS

Microbial risk assessment is often categorised into three main forms: qualitative, semi-quantitative and quantitative (e.g., SA/SNZ 2004, FAO/WHO 2009) although, in practice, there is a spectrum of approaches particularly between the semi-quantitative and quantitative approaches. In the qualitative approach there is no attempt made to quantify risks. Instead, one essentially sets the context of the issues—especially identifying the pathogens of concern—accompanied by narrative statements of risk for different types and locations of sources and exposures, for example, risks are described in subjective, or relative terms, such as risk A is higher than risk B, or risk C is not significantly different to Risk D. In contrast, the semi-quantitative risk assessment approach, often called Comparative Risk Assessment (CRA), does attempt to quantify aspects of risk. It uses the context-setting information that

would be derived in a qualitative approach and uses risk 'scores' to construct a metric for comparing the magnitude of risks from different pathogens, sources and exposure locations. As such it calculates *relative health risks* and attempts to quantify the relative *magnitude* of risks. Typically, this uses the fundamental notion that "risk = likelihood of exposure × consequences". Scores for "consequences" may be broken into sub-metrics for scale, magnitude of exposure and probability of illness, duration and severity of adverse health effects. The outcome can be somewhat dependent on the assignment of scores and the definition of boundaries between them.

Quantitative Microbial Risk Assessment (QMRA, Haas *et al.* 1999) attempts to calculate absolute health risks. Whilst 'deterministic' approaches are sometimes used, QMRA usually estimates risk by considering many possible combinations, via statistical or 'stochastic' modelling of exposures and dose-response. As such it is much more data-intensive than the comparative approach, particularly because it requires data on the variability over time of the degree of pathogen contamination at exposure locations.

In deterministic approaches to risk modelling, risk (or relative risk) is evaluated on the basis of representative values of the risk-affecting variables. The values may be the average, or mode, but to generate conservative estimates of risk, values that are deliberately conservative (e.g., the value that will not be exceeded in 95% of cases) may be used (use of a maximum value is not favoured, because it is always possible that it may be exceeded). This is done so that when the model is calculated to evaluate the risk, the estimate is inherently conservative. Decisions based on that estimate provide a high degree of public health protection, but these estimates may be unrealistically high and lead to poor decisions.[4] Other potential consequences of employing a deterministic approach, compared to stochastic modelling, were exemplified in Nauta (2000) showing the failure to consider variability in risk assessment modelling could lead to erroneous risk management decisions.

For reasons such as those described above, a stochastic simulation modelling approach is often used to be able to better represent and assess the influence of variability in the various elements and circumstances known to influence

[4] A problem with this approach is that in making a series of conservative simplifying assumptions the conservatism compounds and decisions based on the approach represent a level of risk that only occurs in very rare sets of circumstances, rather than being more representative of the risk from the "normal" situation, but with allowance for rare events. This problem was explained, and its implications discussed, by Cassin *et al.* (1996) who coined the term "compounding conservatism" to describe it.

risk. The process involves the construction of a conceptual mathematical model of the way that the risk arises, and then, by systematically varying the inputs of the model and calculating the resultant risk in each of those circumstances, to learn how widely the risk can vary in different circumstances and for different people. By analysing all the results statistically, the risk can be characterised by a most likely value, as well as the extreme outcomes on both ends of the spectrum. Obviously, depending on the complexity of the model and the number of variations that should be investigated, this analysis can be very time-consuming.

Fortunately, the advent of powerful 'user-friendly' software, some of which runs in conjunction with common spreadsheet software, has made stochastic simulation modelling much more readily available. The software runs through the model time after time after time. Each time is called an *iteration*. At each iteration a value is selected from each variable's range, more-or-less at random (according to the probability distribution describing that variable and accounting for any correlation with other variables), and the outcome is evaluated for that set of circumstances. Typically, tens of thousands or hundreds of thousands of iterations are calculated. All of those values are collated by the software and summary of the spread of risk and the most frequent, or most typical, result identified. This kind of software, and the approach of stochastic simulation modelling, implement 'Monte Carlo' methods, indicating their basis in random probability processes such as occur in games of chance.

In a detailed QMRA study of waterborne gastro-intestinal pathogens the variability in risk-affecting factors needs to be obtained from a combination of detailed monitoring and modelling of faecal indicators and pathogens. It should also include uncertainty analysis, especially with regard to dose-response information (Teunis 2009). For the purposes of this text, in its intended application to many types of pathogen sources and environments, that level of environmental data will not be available. Accordingly we present a prototype of a deterministic, comparative risk assessment procedure, based on the notion that risk to public health can be considered as the combination of the likelihood of exposure to a hazard and the severity of the consequences should that exposure occur.

Table 10.1 presents the range of data, in both numeric and narrative form, needed to run the procedure (which is presented as a Microsoft Excel® spreadsheet). The rationale for the entries given in the Table is given in the Appendix.

The model itself is described after first considering four determinants: the diseases selected; the possible sources of their agents; exposure risk factors; and, the population risk from exposure to waterborne microbial hazards.

Table 10.1 Risk affecting characteristics for selected zoonotic pathogens.

Potential risk factors	Pathogen				
	Campylobacter jejuni	***E. coli* (EHEC)**	***Salmonella***	***Giardia lamblia***	***Cryptosporidium (parvum or hominis)***
Pathogen/host					
Infectivity for healthy adults (ID_{50})[a]	897	750	23,600	35	35
Severity of infection	Mild	Severe	Moderate	Mild	Moderate
Higher susceptibility for children?	Yes	Yes	Yes	Yes	Yes
Pathogen in excreta from individual animals (frequency, relative concentrations)[b,g]					
Cattle (10–30 kg/day)[c]	(H, H)	(L, H)[d]	(L, L)	(M, M)	(L, H)
Swine (2.7–4.0 kg/day)[c]	(H, M)	(L, M)	(M, M)	(M, L)	(L, L)
Sheep (0.7–1.5 kg/day)[c]	(H, H)	(L, H)	(L, L)	(L, L)	(L, L)
Poultry (0.1–0.14 kg/day)[c]	(H, H)	(L, L)	(M, M)	(L, L)	(L, L)
Pathogen survival in environment					
Survival, days (faeces, water)[e]	(S, S)	(M, M)	(L, M)	(L, L)[f]	(L, L)[f]

[a] ID_{50} values for these pathogens are reviewed in the Appendix (ID_{50} is the dose for which, on average, half of an exposed population will be infected).

[b] L, M, H = Low, Medium, High. These judgements have been made in the light of the data summarized in the Appendix.

[c] Typical faecal load from each animal group.

[d] Marked seasonal effect; highest in summer.

[e] S, M, L = Short, Medium, Long. The metric for "Survival" is the T_{90}: the time for 90% of the original population of pathogens to be inactivated.

[f] Marked seasonal effect, longer survival times in cooler conditions.

[g] The model also includes the category "supershedders". The values for this are the same as for *E. coli* in cattle but with the relative concentration of enterohaemorrhagic *E. coli* given an extremely high value, that is, 1000 times greater than the "average" concentration.

10.2.1 The diseases

The severity of consequences of exposure depend on a number of factors related to the pathogen and the host including the infectiousness of the pathogen, the dose ingested, and the severity of disease that is the usual outcome. In turn, the severity of the disease depends on the susceptibility of the host to infection by the pathogen. As discussed above, there is variability in each of these risk-affecting factors but in the risk assessment tool developed here we adopt average values to characterise the relative risk. As noted above[4], if conservative or worst-case values are taken for each variable, the resulting risk estimate is characterized by an extremely improbable event. It should also be noted that point estimates based on measures of central tendencies, for example, average, or mode, will not necessarily lead to an answer that represents the most likely outcome and can lead to large errors (Cassin *et al.* 1996). Nonetheless, we have included different categories in the risk ranking tool where appropriate to be able to distinguish situations when risks are systematically higher or systematically lower. For example, children are often more susceptible to infection than adults and for this reason we have included options in the tool that can differentiate this risk, or if there is some correlation between sporadic contamination and the likelihood that people will be exposed to the recreational water. (Differential susceptibility is discussed in greater detail below.) In the case of differential exposure due to age, the population exposed can be selected from "general", "children" only or "adults" only. More sub-categories could easily be included in the tool if there were data that showed that specific populations were physiologically more likely to become infected. Note, also, that some populations are more likely to be exposed due to custom, location, and so on. but this aspect is addressed in a separate question concerning frequency of exposure to the recreational water resource being assessed.

10.2.2 Assessing infectivity

The infectiousness of a pathogen is sometimes characterised as "the infectious dose". This is inappropriate because there is variability in the number of pathogens required to cause infection or illness (depending on the pathogen itself and the susceptibility of the consumer). Recognising this, infectiousness is often characterised by the ID_{50}: the number of cells of the pathogen that results in 50% of the exposed population becoming infected. The relationship between probability of infection and dose ingested is described as the dose-response curve and can be described by a variety of mathematical equations. A detailed review of dose-response relationships for infection processes, both from a biological and mathematical perspective, is presented in FAO/WHO (2003).

For many diseases evaluation of ID_{50} has come from clinical trials using healthy adult volunteers. It therefore ignores any elevated health risks that may be faced by children, by immuno-compromised people, and by the elderly (USEPA 2000*b*, Nwachuku & Gerba 2004, Wade 2008). The pattern for children may be of considerable importance for developing countries where it is known that children can exhibit campylobacteriosis rates many times higher than those for adults (Blaser 1997, Rao *et al.* 2001, Teunis *et al.* 2005). In developed countries such as New Zealand the reported illness rates for all five of the selected diseases exhibit higher rates among children (see www.nzpho.org.nz, Lake *et al.* 2011) and similar findings have been made for Scotland (Strachan *et al.* 2009) and in USA (Denno *et al.* 2009). Accordingly, differential rates between children and adults need careful attention. A good example of differential sensitivity of identifiable sub-populations has been demonstrated for listeriosis: the susceptibility to infection from this food-borne pathogen ranges over 1000-fold between the healthy, young adult, population and those who are immuno-compromised due to underlying illness (e.g. AIDS) or medical treatment such as organ transplant recipients (Marchetti 1996).

10.2.3 Assessing severity

The dose-response relationship also does not consider the severity of the illness, For example, *Salmonella* infections are usually self-limiting and of relatively short duration, while infections from enterohaemorrhagic *E. coli* often lead to life threatening illness which is clearly more severe. Similarly, reliance only on clinical trial data frequently ignores any sequelae that may arise. For example, Guillain-Barré Syndrome may affect about 0.03% of people who have contracted campylobacteriosis (McCarthy & Giesecke 2001). Infections with *Salmonella* or *Campylobacter* have been found to increase the short term risk of death and long term mortality (Helms *et al.* 2003).

One way of comparing disease severity is to use the metric of the "disability adjusted life years" (DALY) concept, originally developed by Murray and Lopez (1996) and adopted by the World Health Organization to inform global health planning (AIHW 2000, Kemmeren *et al.* 2006). The DALY is a measure of the years of "healthy" life lost due to illness or injury, that is, time lived in states of 'less-than-full' health. DALYs are calculated as the sum of years of life lost due to premature death (YLL) and the equivalent years of "healthy" life lost due to poor health or disability (YLD). The YLD considers the extent of the disability that is endured, that is, YLD is weighted according to the severity of the disability. The origin and application of the DALY concept, particularly in

relation to the establishment of disability estimates, and their relevance to undeveloped nations, was reviewed King & Bertino (2008).

10.2.4 The sources

The sources considered here will be confined to four major groups of domestic animals in the world: cattle, swine, sheep and poultry. The world-wide animal census developed by the Food and Agriculture Organization of the United Nations (http://faostat.fao.org) in 2009 lists cattle as the largest animal population at 1.34 billion. Sheep are the next most numerous at 1.09 billion (note that sheep tend to shed similar amounts of annual faecal material per unit area, compared with cattle—Wilcock 2006) and hogs follow at 0.92 billion population. Poultry outnumber all of the above three large animal populations by 5.3 to 1 with a world population of 17.86 billion. These high population numbers are mainly due to the great commercial value associated with these domestic birds and animals. They are the major groups related to food production around the world. This particular group of domestic livestock is also of special significance because in many countries they are held in Concentrated Animal Feeding Operations (CAFO) where many thousands of animals are confined in very small areas. Faecal wastes from CAFO's are usually treated in septic lagoon systems before discharge to receiving waters. The risks of illness associated with exposure to the discharged animal wastes is, however, largely unknown (see: Chapter 11).

The world population of other birds and animals, such as geese, ducks, horses and goats, are very small relative to the above four species. Although urbanized geese and gulls are well recognized as major polluters of bathing beaches, they will not be considered because of their relatively small populations. Wild animals and wild birds are likewise not considered, even though their population densities in the world might be quite high and their faecal contribution to recreational waters is well recognized. Furthermore, good estimates of feral bird and animal populations in the world are not available and the linkage of human enteric illness attributable to zoonotic pathogens from feral animals and birds is not very strong.

10.3 THE EXPOSURES AND RISK FACTORS

We consider risk of human infection from recreational water contact by ingestion only, excluding any risk from inhalation. We do not consider the "knock-on" effects whereby food gets contaminated *via* water (e.g., irrigation, or processing

water), that is, whereas an illness was foodborne, the source was contaminated water.

10.3.1 Ingestion rates

The ingestion of water during swimming activities can be a significant factor affecting risk. There is a dearth of empirically collected data on ingestion of water by swimmers. In a study of divers wearing masks, estimates based on self-reported volumes of water that the divers believed they had swallowed were mainly in the range of 30 mL or less, but with some reporting much larger volumes (Schijven & de Roda Husman 2006). These self-assessed volumes of ingested water were not dissimilar from the amounts of water swallowed by recreational swimmers in a pilot study conducted in a swimming pool (Dufour *et al.* 2006). In that study, ingestion of water was estimated by the amount of cyanuric acid measured in a 24 hour urine sample collected after a one hour swimming activity in a pool disinfected with chlorine isocyanurate.[5] The average amount of water swallowed by 53 participants was about 30 mL. Swimmers less than 16 years old swallowed about 37 mL of water, which was more than twice that swallowed by adults (average 16 mL). These systematic differences should be taken into account in risk assessments because they directly affect exposure.

10.3.2 Climate change

Risk of illness associated with exposure to non-human faecal pollution may be significantly affected by global warming and climate changes. Events similar to those which might occur under global warming conditions have been observed in recent years (Epstein 2005, Patz *et al.* 2005). Weather extremes related to atmospheric and ocean warming have resulted in heat waves and extensive flooding, and these phenomena have given a preview of what may be expected under full-scale global warming. In North America, weather extremes have resulted in drought and very high temperatures, and in unusually heavy rainstorms that have caused extensive flooding. Curriero *et al.* (2000) have documented an association between extreme rainfall and waterborne disease outbreaks in the United States. They showed that over 50% of the drinking-water outbreaks of disease in the United States were associated with rain events above the 90th percentile value of total monthly precipitation. Similarly, Thomas *et al.* (2006) have shown that in Canada "high impact" weather events are associated with waterborne disease outbreaks. The association between outbreaks of disease and extreme rainfall events described in these studies may be a harbinger of

[5] Similar results were obtained in a follow-up study (Evans *et al.* 2006).

increased risk of disease related to global warming and climate change. Conversely, high temperatures may also have the effect of decreasing risk associated with animal or faecal pollution in some regions. Higher temperatures and lack of rainfall may cause desiccation of faecal material in open areas and thereby enhance the die-off of pathogens that might otherwise survive and contaminate water resources (Sinton *et al.* 2007a, Meays *et al.* 2005). Risk modifying events of the type described above will have to be anticipated in future assessments of the relationship between animal and bird faecal pollution, and human health.

10.4 THE COMPARATIVE RISK MODEL

10.4.1 Background and objectives

Microbial risk assessment modelling is gaining importance in relation to water quality and protection. According to Coffey *et al.* (2007) risk assessment models are critical to protect human health from contaminated water sources.

A number of models that are intended for, or could be adapted to, microbial waterborne risk assessment do currently exist and were reviewed by Coffey *et al.* (2007). Some are qualitative while others are quantitative and complex in terms of data needs and computational structure. While qualitative approaches can provide an effective means of assessing risks with minimum resources and limited data, they lack the precision and predictive ability of fully quantitative approaches. Conversely, the quantitative models are complex and require large amounts of data, are variable in their accuracy and, in the evaluation of Coffey *et al.* (2007), no one model could account for all hydrological and geological factors of relevance and also model the physical transport of bacteria in surface run-off. The best performing models were of medium to high complexity in terms of user expertise and the quantity of data required for their implementation.

Coffey *et al.* (2007) observed that the most common bacterial model used to estimate bacterial loadings was HSPF (Hydrological Simulation Programme—Fortran), but that it was complex, requires large quantities of monitoring data, needs extensive calibration, and had a limited capacity to accurately represent diverse watershed topography and land uses. They further noted that some models were not full, qualitative models, but can give a good initial estimate of risk from pathogens and highlight requirements for a full quantitative assessment.

Ross & Sumner (2002) presented a simple, spreadsheet-based, comparative risk assessment model for microbial food safety risk assessment. That model, or slight variations on it, has found utility among a range of users (e.g., FAO 2004, AECL 2005, Pointon *et al.* 2006, Rasco & Bledsoe 2007, Mataragas *et al.* 2008, NSWFA 2009, Perni *et al.* 2009, Tian & Liu 2009). Due to its acceptance for

some uses in food safety risk assessment, in this Chapter we seek to translate that model into a format appropriate for use in microbial water quality risk assessment and present it for evaluation.

It is not intended nor inferred that the model presented can provide accurate estimates of recreational waterborne risks under all circumstances and scenarios. Rather, it can provide quick screening of relative risk, and the effects of multiple factors in combination on overall risk. It is also intended to illustrate an approach that could make risk assessments for recreational waters more accessible and also has great utility in teaching the principles and philosophy of quantitative risk assessment.

However, the model clearly has limitations. For example, while the model is superficially simple to use, it relies on a relatively high level of knowledge of the watershed being considered to be able to answer the questions appropriately. If answers to the questions posed are inappropriate, the relative risk estimates from the model will be unreliable (in other words: "garbage in – garbage out"). While the logic inherent in the model is essentially correct, the weightings used for responses to the answers may not be appropriate in all situations and this could lead to unrealistic or illogical conclusions in some circumstances. Furthermore, the model only considers one source of faecal contamination at a time when, in many circumstances, there will be multiple sources of contamination. Nonetheless, the model could be used to estimate which source represents the greatest risk by assessing each source separately, or assessing the combined risk from multiple sources.

Users should be aware of the uses and limitations of the model. Such limitations and caveats were discussed in detail by Ross and Sumner (2002) in relation to the food safety risk assessment model and most apply equally to the model presented here.

10.4.2 Model structure and interface

Evaluation of the health risk from a water source requires knowledge of the strength ("load") of the hazard, and an understanding of the modification of pathogen numbers together with the characteristics of the "transport" (Goss & Richards, 2008) and routes of exposure. The structure of the decision tool corresponds to that generally accepted paradigm (i.e. load, transport, exposure) and can be considered as three banks of questions corresponding to those three aspects of risk.

The model attempts to consider the collective contribution of many factors to the overall risk to the public exposed to bodies of water, whether due to

recreation, domestic needs (e.g., clothes washing in developing nations; food gathering) or employment (e.g. irrigation farmers, fishermen).

The model is implemented in spreadsheet software and uses an approach similar to the microbial food safety risk model of Ross & Sumner (2002). The benefits of the use of spreadsheet software are that it:

- Allows automation of the calculations required to estimate the risk, facilitating a quick exploration of the effect of different assumptions by the user;
- Is widely available and used, that is, users do not need special training nor to have access to specialized software.

Users are presented with a series of sixteen questions and asked to select from a list of possible answers to those questions. Figure 10.2 shows the 'user interface' of the tool. Question 1 relates to the animals that are the source of the contamination to estimate the severity of the microbial hazard. Questions 2 to 5 are used to estimate the pathogen "load". Questions 6 to 11 relate to mobilisation and transport of the pathogens to the recreational water body being assessed. Questions 12 to 16 relate to exposure to the water body being assessed.

The model requires users to provide answers based on 'average' situations, not extreme or unusual circumstances, for the purposes of estimating relative risk. However, users could potentially use the model to estimate the relative risk increase, or decrease, due to unusual circumstances that may be of interest or relevance for water safety management.[6]

In its current form the model is limited to consideration of faecal contamination of recreational water by farmed cattle, sheep, pigs or poultry.

10.4.3 Model logic

The overall principal of the tool is that the answers that are chosen by the user for each of the qualitative Questions 1 to 16 are assigned numeric values. The numeric values are assumptions about the relative risk contribution of the alternative answers offered for each question. Those values can then be used in calculations to generate estimates of relative risk.

[6] Note, however, that in the model if only a single value is changed, the predicted change in risk will in nearly all cases simply reflect the difference in "weight" applied to the subjective answers offered to the user. The weights are a very simplified measure of relative risk contribution from each factor and changing the answer to one question will not usually generate a reliable estimate of the increased relative risk, because the weights used are, for most questions, arbitrary. The benefit of the model is to assess the influence on relative risk of simultaneous changes in multiple risk-affecting factors.

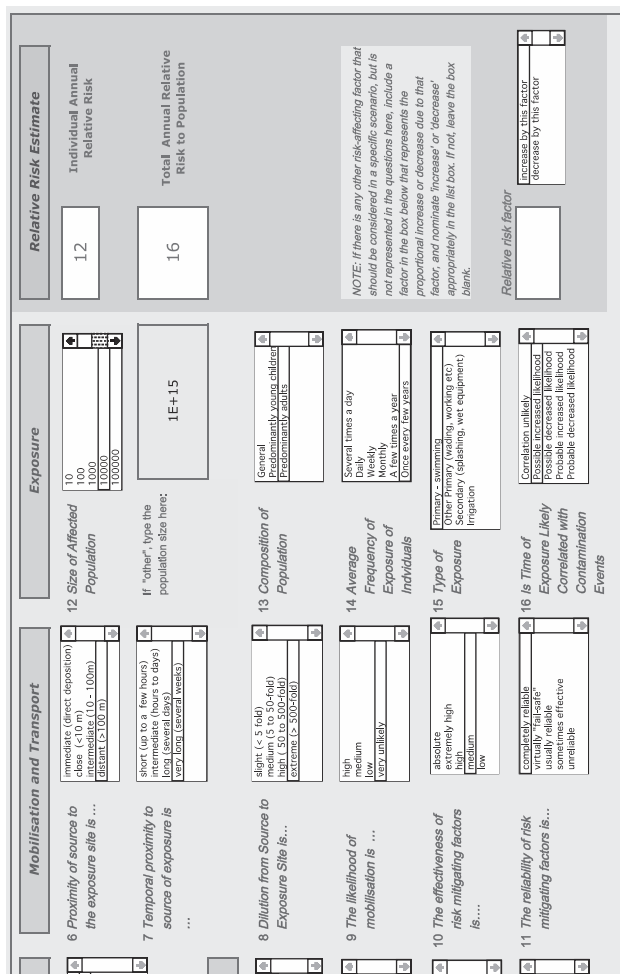


Figure 10.2 Image of the 'user-interface' of the recreational waterborne microbial risk assessment model, showing the questions, alternative answers and risk outputs. Users select answers from the 'pull down' menus, which are translated into numerical values used to calculate the risk indices.

In some cases the values ascribed are similar to relevant absolute values (e.g., the ID_{50} values used in Question 1; in Question 15 weekly exposure is weighted four times as heavily as monthly exposure, etc). In other cases the values are relative to the most extreme value. For example, for Question 5: "continuous contamination" has a weight of '1', and other frequencies of input/contamination are weighted relative to that value, for example, "frequent" contamination has arbitrarily been assigned a value of 0.3, "rare" has been assigned a value 0.001.

The relative risk of different scenarios is calculated, essentially, as the simple product of the relative risk from each of the risk-contributing factors explicitly addressed in Questions 1 to 16. There are three exceptions, however, where a logical test is also applied. The first relates to assessment of the efficacy estimates of actions taken to reduce contamination of the water resource being considered, and aims to jointly assess the ability of the action to reduce contamination as well as the reliability of the process. The logic involved is discussed in detail later, in the sections describing those questions.

The second case relates to the possibility that human exposure is, in some way, correlated with contamination events. Question 16 asks the user to comment on whether correlations, either positive or negative, between contamination events and human exposure are possible and to rank these as "possible" or "probable". If, however, contamination is "continuous" or "frequent" or if exposure is "daily" or more frequent, it is assumed any such correlation is irrelevant because exposure frequency and likelihood of contamination are such that there is near certainty of exposure to contaminated water and that the relative risk cannot be increased nor decreased. However when exposure to the water is low and contamination is rare, the risk will be under-predicted if there is a correlation between exposure to the recreational water and contamination, for example, if contamination, while rare, were more likely in summer, when people are more likely to swim. Conversely, if contamination events are rare but are detected in sufficient time to alert swimmers prior to the contamination reaching the recreational water, there would be a negative correlation between contamination events and exposure.

The third use of a logical test, as explained in the next section, is to determine the greatest hazard potential from pathogens in different animal sources of contamination.

10.4.4 The questions

The following section provides advice on interpretation of the sixteen questions as well as describing the relative risk weights applied to each of the possible responses.

10.4.4.1 Identifying the source

Question 1 asks the user to select the type of farmed animal population that contributes most to the source of contamination of the recreational/working water body. Doing so determines both the pathogen considered to present the greatest risk from that animal species and also the relative risk, based on information presented in Table 10.1, as described below. The overall hazard presented by the pathogen is considered to arise from:

- its relative prevalence among herds/flocks of the animal selected;
- the concentration of the pathogen in faeces of the species selected;
- the relative survival of the pathogen in the environment (expressed as T_{90});
- the relative severity of the disease caused by the pathogen;
- the infectiousness of the pathogen as expressed by the ID_{50}.

For each animal group a simplified index of "hazardousness" for each of the five pathogens considered is calculated by the following formula:

$$\begin{aligned} \textit{Relative pathogen risk} = (&\text{relative prevalence} \times \text{relative concentration} \\ &\times \text{relative survival (or } T_{90}) \\ &\times \text{relative disease severity})/ID_{50} \end{aligned}$$

The maximum of the values generated for each pathogen for the animal species selected is the relative risk value assigned as the answer to Question 1 and is used in further calculation of relative risk. It should be noted that this approach is based on several subjective decisions and assumptions. The most apparent is the translation of qualitative assessment of pathogen prevalence and concentration (*see* Table 10.1) into relative quantities. Table 10.2 indicates the values, or relative weights, that were applied. The weightings are based on factors of ten for simplicity but could be altered if reliable, representative, quantitative data were available.

To allow the estimation of the consequences of the effects of more extreme hazards, for example, a "supershedder", or an epidemic level of pathogen excretion within a herd/flock, an additional choice reflecting a higher level of pathogen excretion is added to the range of responses to Question 1. Currently, selection of this option only has the effect of increasing the modelled concentration of *E. coli* in the faeces of cattle by 1000-fold, but other options could be included.

In practice, the combination of the above weights and ID_{50} values results in *Cryptosporidium* representing the greatest level of hazard when cattle or poultry are selected, *Giardia* and *Cryptosporidium* when pigs are selected, and EHEC

for sheep except when the "supershedder" option is selected. In that case, enteropathogenic *E. coli* is evaluated to be the greatest source of risk overall. Modification of the weightings (see: Table 10.2) could change the relative risk estimates and predicted relative importance of each pathogen in each animal species. Epidemiologically, *Cryptosporidium* scores the highest as cause of detected waterborne infections (Coffey *et al.* 2007). The relative severity of illness might also be made less subjective by deriving estimates of DALYs lost for each pathogen but was not undertaken at this time. The qualitative descriptions of severity for the five pathogens applied in this example are in accordance with those presented by Goss and Richards (2008).

Table 10.2 Weighting factors applied when assessing hazard relative importance.

Hazard Characteristic	Qualitative Description	Numerical weight assigned
Severity of infection	Severe	1
	Moderate	0.1
	Mild	0.01
Survival (in faeces, water)	Long (L)	1
	Medium (M)	0.1
	Short (S)	0.01
Prevalence of pathogen in faeces	High (H)	1
	Medium (M)	0.1
	Low (L)	0.01
Concentration of pathogen in faeces	High (H)	1
	Medium (M)	0.1
	Low (L)	0.01

The estimation of risk also relies on consideration of the dose ingested and the likelihood that the dose will lead to a symptomatic infection. The relative risk calculations in the model assume that there is a direct proportionality between the dose ingested and the probability of illness. This is generally in accord with the predictions of the dose response models considered herein (single-parameter simple exponential or two-parameter beta-Poisson, as used in the discussion of ID_{50} values in the appendix to this chapter). If the dose is rather lower than the ID_{50}, the risk can be considered to be directly proportional to the dose (Haas 1996, Gale 2001a&b, 2003), because the dose-response relationship is linear at low doses. The assumption of proportionality is incorrect, however, if the dose in significantly greater than the ID_{50} for the pathogen of interest. This is because the dose-response curve for probability of infection is asymptotic and non-linear

at higher doses, with the asymptote being approached at doses that are at least an order-of-magnitude higher than the ID_{50}.[7] However, due to the effects of dilution, inactivation and the volume of water ingested, it is assumed that, in most practical situations, the dose ingested will be below the ID_{50} for most pathogens. In cases where this assumption is not valid (e.g., direct contamination adjacent to a point where people are exposed) the consequence will be an underestimation of the relative risk of other situations compared to that most extreme situation.

The selection of the animal source of contamination in Question 1 is also is used to assign a relative weight of faeces produced per animal type, which will also affect the load. The following relative quantities of faeces per animal are assumed and used in the relative risk calculations:

cattle	relative quantity (= relative risk) = 1
pigs	relative quantity (= relative risk) = 0.17
sheep	relative quantity (= relative risk) = 0.06
poultry	relative quantity (= relative risk) = 0.006

10.4.4.2 Estimating load

Questions 2 to 5 relate to the load of pathogens expected to arise from the herd or flock considered as the source of contamination. The questions, and alternative answers provided, are relatively self-explanatory from the discussion presented earlier, but the weightings applied are presented below for transparency.[8] They aim to estimate the load of pathogen entering the water body by estimating the level of pathogens based on the rate and scale of faecal contamination entering the water body on the basis of animal type, herd size, herd density and frequency of contamination, and the manner in which contamination of the water occurs. More detailed discussions of factors affecting load, and approaches to minimising load in animal faeces, are presented in Chapters 3 and 4.

Question 2: Density of Animal Population. The question is phrased as a statement to be completed, that is, "*The density of the animal population causing contamination is …"* with four possible responses offered. Those responses, and the relative risk assigned to them are:

high (>1000 per hectare)	relative risk = 1
medium (100 to 1000 per hectare)	relative risk = 0.1

[7] The assumption will also be incorrect if the pathogens act co-operatively to cause infection and disease, requiring more complex dose-response models (FAO/WHO, 2003).

[8] These weightings greatly simplify the complex set of "pathogen delivery processes" operating in the environment, such as modeled by Collins & Rutherford (2004) for *E. coli*.

low (10 to 100 per hectare)	relative risk = 0.01
very low (<10 hectare)	relative risk = 0.001

Question 3: Size of Herd or Flock. The question is phrased as a statement to be completed, that is, "*The size of the herd or flock causing contamination is …"* with four possible responses offered. Those responses, and the relative risk assigned to them are:

large (1000s of animals)	relative risk = 1
medium (100s of animals)	relative risk = 0.1
small (scores of animals)	relative risk = 0.01
very small (a few animals)	relative risk = 0.001

Question 4: Mode of Contamination. The question is phrased as a statement to be completed, that is, "*The mode of contamination is …"* with five possible responses offered. Those responses, and the relative risk assigned to them are:

direct – untreated	relative risk = 1
diffuse – for example, agricultural run-off grazing	relative risk = 0.01
diffuse – for example, manure spreading occurs	relative risk = 0.5
direct – primary treated effluent	relative risk = 0.1
direct – secondary treated effluent	relative risk = 0.001

Other factors affecting the risk from the mode of contamination are considered in Questions 6–11, relating to mobilisation.

Question 5: Frequency of contamination. The question is phrased as a statement to be completed, that is, *"The frequency of contamination is …"* with five possible responses offered. Those responses, and the relative risk assigned to them are:

rare	relative risk = 0.001
sporadic	relative risk = 0.01
intermittent	relative risk = 0.05
frequent	relative risk = 0.3
continuous	relative risk = 1

10.4.4.3 Estimating contamination of the recreational water

Pathogen concentrations in the water body to which people will be exposed will affect the probability of illness, that is, the greater the contamination level and the dose ingested, the greater the probability of illness. Questions 6 to 11 relate to mobilisation and transport of pathogens from the source to the water body of concern to estimate the reduction in pathogen load due:

- Die-off in the environment due to time and distance;

- Dilution, and
- The effectiveness and reliability of implementation of actions taken to minimize contamination of the water from the source considered.

More detailed discussion of mobilisation and transport, and means of assessing and minimising this, are presented in Chapters 6, 7 and 8. Growth of pathogens in the environment is assumed not to occur under scenarios relevant to this comparative risk model.

Question 6: Proximity of Source to Exposure Site. In addition to the effect of time on the extent of pathogen inactivation (*see* Question 7, below), the likelihood that the pathogen will reach the water body is reduced due to absorption onto soil particles, predation, and so on. Accordingly, risk to recreational water users will be reduced the further the contamination source (i.e., the animals that are the source of the faeces) is from the water body, or water supplying the water body. The question is phrased as a statement to be completed, that is, *"Proximity of source to the exposure site is …"* with four possible responses offered. Those responses, and the relative risk assigned to them are:

immediate (direct deposition)	relative risk = 1
close (<10 m)	relative risk = 0.1
intermediate (10–100 m)	relative risk = 0.01
distant	relative risk = 0.001

Question 7: Temporal proximity to source of exposure. Pathogen die-off in the environment will be greater, under a given set of inimical conditions, the longer they are exposed to those conditions. As such, greater time between the point of contamination and the moment of exposure will reduce the level of pathogen in the water and, consequently, the risk of illness. Question 7 is phrased as a statement to be completed, that is, *"Temporal proximity of source to the exposure site is …"* with four possible responses offered. Those responses, and the relative risk assigned to them are:

short (up to a few hours)	relative risk = 1
intermediate (hours to days)	relative risk = 0.1
long (several days)	relative risk = 0.05
very long (several weeks)	relative risk = 0.005

The weighting factors used reflect that microbial inactivation is usually characterised as a log-linear decline over time.

Question 8: Dilution from Source to Exposure Site. As noted above, dilution of pathogens would be expected to decrease risk because the dose ingested from a

given mode of exposure (see Question 15) will be less, leading to decreased probability of infection. Question 8 is phrased as a statement to be completed, that is, *"Dilution from Source to Exposure Site is…"* with four possible responses offered. Those responses, and the relative risk assigned to them are:

slight (<5-fold)	relative risk = 0.4
medium (5 to 50-fold)	relative risk = 0.04
high (50 to 500-fold)	relative risk = 0.004
extreme (> 500-fold)	relative risk = 0.002

The weights used are proportional to the means of the ranges of dilution specified.

Question 9: Likelihood of mobilisation. Increased mobilisation of contaminants will increase the load reaching the water body of concern. Mobilisation can be affected by agricultural practices, for example, tile-drain systems are the most frequently reported route by which liquid manure can contaminate surface water courses, but a range of other factors and practices affect mobilisation, for example, the slope and direction of land and how it affects run-off, irrigation practices, vegetation and so on. as discussed by Goss & Richards (2008). Question 9 is framed as a phrase to be completed: "*The likelihood of mobilisation is …*" with four possible responses offered. Those responses, and the relative risk arbitrarily assigned to them, are:

high	relative risk = 1
medium	relative risk = 0.1
low	relative risk = 0.01
very unlikely	relative risk = 0.001

Questions 10 and 11: Effectiveness, and reliability of risk mitigating actions are included in recognition that sufficient knowledge exists to be able to reduce risk of contamination of surface water by various practices and actions, but only if they are reliably implemented. Such actions include "streambank retirement", fences near waterways to prevent animal ingress, bridges over water courses to allow animals to cross without entering the water. The answers to both questions are based on subjective assessments. Question 10 is framed as a statement to be completed: "*The effectiveness of risk mitigating factors is …* " with five possible responses, as follows:

absolute	relative risk = 0
extremely high	relative risk = 0.0001
high	relative risk = 0.01
medium	relative risk = 0.1
low	relative risk = 1

Question 11 is also framed as a statement to be completed: "*The reliability of risk mitigating factors is …*" with five possible responses, as follows:

completely reliable	relative risk = 0
virtually "fail-safe"	relative risk = 0.01
usually reliable	relative risk = 0.1
sometimes effective	relative risk = 0.5
unreliable	relative risk = 0.9

The first response to both questions are unusual compared to all other responses in the risk model because both have the potential to reduce the risk estimate to zero, that is, no risk. However, a process that completely eliminates pathogens, but is unreliable still has a risk associated with it. Thus, the degree to which a process is unreliable reduces the effective risk reduction. Conversely, a completely unreliable process that has little effect on pathogen numbers cannot increase the relative risk and the relative risk score must remain as "1". To model these logical considerations of the interplay between process efficacy and process operational reliability the two scores are combined and a logical test applied. Thus, the relative risk due to both of these factors in combination is taken as the sum of the two relative risk scores. However, to prevent the model from predicting an increase in risk from a low efficacy process operated unreliably, the "MIN" function in Microsoft Excel is used so that if the combined relative risk score is greater than "1", the model substitutes a value of "1". The net effect of this on the relative risk score for mitigations is shown in Table 10.3, below.

It is noted that the answers to the above questions may be subjective and can have a profound affect on the risk estimate. Accordingly, it is advised that users make careful considerations and seek guidance as needed, when assessing reliability.

10.4.4.4 Exposure

The risk to people from contaminated recreational waters depends not only on the level of contamination but also the magnitude of the exposure to that contaminated water. This depends on how frequently people are exposed and the manner in which exposure occurs. For the sake of this example of the approach to relative risk estimation we have limited the scope to the risk due to ingestion of contaminated water.

Risk can be expressed as risk to an individual or risk to an entire population, and can be affected by the susceptibility of individuals or sub-groups within the population, for example, young children may be more susceptible to pathogens because they have not yet experienced the organism nor developed immunity.

Table 10.3 Combined relative risk scores from responses to Questions 10 and 11.

Question 10 response	**Question 11 response**	**Completely reliable**	**Virtually "fail-safe"**	**Usually reliable**	**Sometimes reliable**	**Unreliable**
	relative risk score	**0**	**0.01**	**0.1**	**0.5**	**0.9**
Absolute	0	0	0.01	0.1	0.5	0.9
Extremely high	0.0001	0.0001	0.0101	0.1001	0.5001	0.9001
High	0.01	0.01	0.02	0.11	0.51	0.91
Medium	0.1	0.1	0.11	0.2	0.6	1
Low	1	1	1	1	1	1

The elderly can be more susceptible to infectious disease because immune function begins to diminish with age. The magnitude of risk is also affected by the time period considered, that is, a greater time period usually increases the risk of exposure. The questions in this section enable estimation and discrimination of risk on the basis of these factors.

Question 12: Size of Affected Population. This question is included to enable overall public health risk to be estimated, in addition to risk to individuals. In risk assessment, risk encompasses elements of probability and severity of outcomes. Severity can be considered to include both the severity and magnitude of the conseqences of exposure to the hazard, that is, the number of people affected by the hazard. Question 12 simply requests the user to indicate the size of the population exposed to the microbiological hazard in the recreational water being considered. An option is included for the user to nominate the size of the population exposed, rather than to use one of the options presented. The same approach could have been used with other question as well, that is, to allow the user to specify their own estimate of relative risk for any other factor specifically considered in the model but was not implemented in this example to maintain simplicity for the sake of demonstrating the approach.

Question 13: Composition of Population. As noted elsewhere, susceptibility to infection varies with medical condition, age and other factors. In this question, susceptibility on the basis of age only is considered. The choices presented, and the relative risk rating assigned to those sub-population, is shown below, where the highest relative risk is assumed to apply to young children.

General	relative risk = 0.7
Predominantly young children	relative risk = 1
Predominantly adults	relative risk = 0.1

Question 14: Frequency of Exposure. Frequency of exposure is self-evidently a factor that contributes to the risk from hazard in a contaminated body of water. The response choices are based on common units of time and the weights applied based on the actual relationships of those times, as shown below:

Several times a day	relative risk = 1
Daily	relative risk = 0.365
Weekly	relative risk = 0.052
Monthly	relative risk = 0.013
A few times a year	relative risk = 0.003
Once every few years	relative risk = 0.0003

Note that the risk is ranked relative to a person who is exposed to the potentially contaminated water body several times a day.

Question 15: Type of Exposure. There are various ways in which people can be exposed to pathogens in contaminated recreational waters. We extend the scope here slightly to include occupational exposures as well, for example people harvesting food from such waters or washing clothes, or involved in religious or other custom. Exposure to contaminated irrigation waters, for example, from applying water or exposure to spray from overhead irrigations, is also considered. The options presented and relative risk weightings are:

Swimming	relative risk = 1
Other primary (wading, working, etc.)	relative risk = 0.3
Secondary (splashing, wet equipment)	relative risk = 0.1
Irrigation	relative risk = 0.1

Question 16: Is Time of Exposure Likely Correlated with Contamination Events. As discussed earlier, there may be situations in which contamination events and exposure are correlated. Where correlations may significantly alter risk, the following relative risk factors are applied:

Correlation unlikely	relative risk = 1
Possible increased likelihood	relative risk = 10
Possible decreased likelihood	relative risk = 0.1
Probable increased likelihood	relative risk = 100
Probable decreased likelihood	relative risk = 0.01

These factors are only included in the risk calculations where both the exposure frequency is "weekly" or less *and* the contamination frequency is "intermittent" or less. An additional question is included to enable users to model the effect of scenarios and factors not easily assessed *via* the other questions in the model. For this question, the user enters a value to represent how much better, or worse, the risk to human health from exposure to that recreational water would be with the additional factor considered. Thus, if the situation, due to some other factor is ten times worse, then the user should enter "10" in the space provided and select "increase by this factor" in the list provided. If the situation is only half as bad due to some intervention or other factor not specifically included in the model, the user would enter "2" in the space provided and select "decrease by this factor" in the list provided. If there is no effect the user can enter "1", or "0", or leave the box empty.

10.4.4.4 Relative risk calculations

The answers to the above questions are translated into the relative risk values shown above. These values are then used in calculations to establish how the relative risk from each factor affects the relative risk overall. In general the

answer is simply calculated as the product of the relative risk factors. Thus, the "Individual Annual Relative Risk" is based on the following calculation:

= source relative risk (based on the calculation described under "Question 1", above)

× relative amount of excrement produced per animal of the species selected in Question 1 (as described above under "Question 1", *above*);
× relative risk due to density of animal population (Question 2);
× relative risk due to size of herd or flock causing contamination (Question 3);
× relative risk due to mode of contamination (Question 4);
× relative frequency of contamination (Question 5);
× relative risk due to proximity of faecal contamination to recreational water (Question 6);
× dilution between source and recreational water (Question 7);
× relative risk reduction due to time between source and recreational water (Question 8);
× the likelihood of mobilisation (Question 9);
× relative risk reduction due to reliability and efficacy of mitigation actions (combination of Questions 10 and 11 as described in Table 10.3, *above*);
× relative risk due to composition of affected population (Question 13);
× relative frequency of exposure (Question 14);
× relative risk due to type of exposure (Question 15);
× relative risk adjustment for possible correlation between infrequent contamination and infrequent exposure (Question 16) and, if included by the user;
× relative risk adjustment due to other factors not explicitly considered in the model.

The above calculation leads to a number, on an arbitrary scale, based on risk over a year of potential exposure for an individual. The higher the number, the greater the relative risk.

Assuming the most extreme relative risk (i.e., '1') for each factor, and combining this with the relative risk estimate based on the animal species considered to represent the greatest hazard, generates a maximum score of 5.33×10^{-4}. Conversely, assuming the lowest relative risk for each factor, leads to a prediction of "No Risk". The next lowest predicted relative risk is 2.25×10^{-40}, obtained if all answers are selected to represent the lowest relative risk, but with Questions 10 and 11 answered as "Very High" and "Completely Reliable" respectively, or as "Absolute" and "Virtually fail-safe" respectively. These extremes set the scale of relative risk for the model presented. To make the scale more 'natural' to users, the logarithm of the above calculation is taken

and 41 added to avoid generating negative values under some other scenarios. Similarly, the calculated value is rounded to the nearest integer. This results in a scale of relative risk from 1 to 38. (Note that the upper value can be increased if the effect of other risk-increasing factors is included using the additional question.) Because the scale is logarithmic, every unit increase in the relative risk score corresponds to a ten-fold increase in risk, that is, due to the combined effect of probability of infection and the expected severity of infection.

To calculate the relative population health risk, the individual risk is multiplied by the population size (Question 12). To set the risk calculation on a similar scale, the logarithm of the population size is added to the individual risk index.

The model is available for download from: http://www.foodsafetycentre.com.au/risk-assessment.php

10.5 CONCLUSIONS

Comparative risk assessment is an approach for evaluating and quantifying risks without resorting to the complex, time-intensive quantitative microbial risk assessment process. It also provides a relative quantitative aspect not available in the qualitative risk assessment process, relying instead on some narrative to describe risk when dealing with different types or sources of exposure (e.g. low, medium, high for animal excretion rates, as in Table 10.1). The comparative risk model in this chapter makes use of an interactive spreadsheet programme that can be applied in a form that is readily understood and easy to use.

While the model presented here has been developed for very specific zoonotic pathogens, it might have other applications which may be very attractive for evaluating risk under various situations. For instance, risk differences between local, regional or larger areas can be evaluated using the comparative risk model, thereby providing water resource managers with a means to prioritize where they should apply the greatest risk reduction efforts and in what order. The model could also provide risk managers with a means to determine the most effective treatment or management options regarding public health risks associated with recreational activities. Furthermore, it may provide a tool for risk managers, wherein various scenarios might be developed and evaluated to determine which approach provides the greatest public health protection. Lastly, the spreadsheet approach for applying the model may be very useful as a training tool for those not entirely familiar with the risk assessment process.

Although the comparative risk model sacrifices some of the detailed aspects of the quantitative microbial risk assessment paradigm, the relative nature of this approach is valuable for examining many of the issues associated with risk assessment. The model presented here should be considered a prototype for determining risk posed

only by specific waterborne zoonotic pathogens. It has however proved to be effective in the foods area where it has been used to evaluate risk associated with meat and fish products. The true value of the model for estimating risks to recreationists posed by waterborne zoonotic pathogens, however, will be known only after it has been evaluated under actual conditions in the field.

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APPENDIX: BASIS OF VALUES PRESENTED IN TABLE 10.1

This appendix provides an explanation and reference to published literature for values presented in Table A10.1, which describes characteristics of the selected microbial hazards relevant to the risk they pose to people exposed to recreational waters contaminated by them.

ID_{50} VALUES

ID_{50} values are taken from best-estimate dose-response relationships as reported in the literature. No explicit account is taken of uncertainty, though that is often desirable in particular quantitative risk assessments (Teunis 2009).

CAMPYLOBACTERIOSIS

The two parameters for the beta-Poisson dose-response curve were derived by Medema *et al.* (1996), using data for healthy urban adult volunteers reported by Black *et al.* (1988). This curve, for probability of infection given an average dose, is given generally by $Pr_{infection} = 1–(1 + d/\beta)^{-\alpha}$, where d is the average dose given to each group of volunteers, α is a shape parameter and β is a scale parameter. They obtained the parameter values as $\alpha = 0.145$, $\beta = 7.589$, from which ID_{50} (for infection) $= \beta(2^{1/\alpha}–1) \approx 897$ (see also Teunis & Havelaar 2000). Teunis *et al.* (2005) later analysed campylobacteriosis rates among two sets of children at school camps, which indicated an ID_{50} (for illness) < 10. In other words, even in developed countries children exhibit markedly higher rates of campylobacteriosis than is the case for adults (see also Rao *et al.* 2001).

E. COLI O157:H7 INFECTION

Teunis *et al.* (2008) analysed several outbreaks for illness using the two-parameter beta-Poisson dose-response model and obtained prediction parameters for the heterogeneous case ($\alpha = 0.248$, $\beta = 48.80$), which results in ID_{50} (for illness) ≈ 750.

SALMONELLOSIS

Haas *et al.* (1999) analysed infectivity of *Salmonella* (non-typhoid strains) in human volunteers in studies reported by McCullough and Eisele (1951a&b), obtaining ID_{50} = 23,600. A more recent study (Bollaerts *et al.* 2008) has analysed a larger set of data which generally suggests lower ID_{50} values, particularly for the "susceptible" component of a population (see also Blaser & Newman 1982 and Rose & Gerba 1991).

GIARDIASIS

Rose *et al.* (1991) fitted the "simple exponential model" to infections exhibited by volunteers in studies reported by Rendtorff (1954) and Rendtorff & Holt (1954), using the exponential dose response model in which $\Pr_{\text{infection}} = 1 - e^{-rd}$ where d is again the average dose given to each group of volunteers and r is the probability that a single *Giardia* cyst could cause infection. They obtained $r = 0.01982$. Therefore the $ID_{50} = -\ln(½)/r = 0.693/r \approx 35$.

CRYPTOSPORIDIOSIS

Clinical trials for infectivity of oocysts of *Cryptosporidium parvum* were done as part of a set of three studies in the Medical School of the University of Texas.[9] Individual analyses for each set have generally indicated that the appropriate dose-response curve is the single-parameter "simple exponential model". But a meta-analysis has identified different infectivity levels when fitting a number of candidate curves to each trial's dataset, such that the differences depend on the particular isolate used and on the method of "passaging" the *Cryptosporidium* in the laboratory (Teunis *et al.* 2002a, 2002b). Having regard to all these studies USEPA (2003), in developing its "Long Term 2 Enhanced Surface Water Treatment Rule" for drinking water, concluded that the dose-response function (for infection, *cf.* illness) should indeed be of "simple exponential" form, with a particular value of its single parameter ($r = 0.09$). This gives rise to $ID_{50} \approx 8$. However, two further studies have since been reported.[10] Teunis (2009) has interpreted all five studies together, together with a sixth,[11] and this (omitting the infectious TU502 *Crypto. hominis* data, because it has a human source) leads to a conclusion that on average the ID_{50} for *Cryptosporidium* can be taken as approximately the same as is inferred for *Giardia* (i.e., about 35).

PATHOGENS IN ANIMAL EXCRETA

The following material has been particularly guided by information presented by Soller *et al.* (2010) and USEPA (2010), along with some other published literature. Chapter 3 of this text gives further detailed information.

Tables A10.1–A10.5 present summaries of studies of prevalence and concentration of the five pathogens considered in this chapter, each including the four animal groups

[9] These studies were conducted for the TAMU, Iowa and ICP isolates (Okhuysen *et al.* 1999).

[10] The Moredun *Crypto. parvum* isolate (Okhuysen *et al.* 2002) and the TU502 *Crypto. hominis* isolate (Chappell *et al.* 2006).

[11] The 16W (*Crypto. parvum*) isolate.

Table A10.1 *Campylobacter*.

Reference	*Prevalence (%)*	*Concentration*	*Notes*
Cattle			
Berry *et al.* (2007)	2.2–14.9	–	Beef cattle feedlots
Besser *et al.* (2005)	1.6–62.2	–	Beef cattle feedlots
Brown *et al.* (2004)	36	–	Rural Cheshire, UK
Devane *et al.* (2005)	97.8	–	New Zealand dairy cattle (all positive for *C. jejuni*)
Hakkinen & Hänninen (2009)	49.7	–	Substantial differences between herds
Hutchison *et al.* (2004/5)	12.8	320 cfu/g (g.m.)	Fresh composite farm manure, UK. max. = 1.5×10^5 cfu/g
Kwan *et al.* (2008)	35.9	–	Five NW England farms, prevalence range = 26.4% (winter) to 50.8% (summer)
McAllister *et al.* (2005)	30–47	–	Cows (Ontario, Canada)
	41.7	–	Calves (British Columbia, Canada)
McAllister *et al.* (2005)	41.7	–	Canada
Moriarty *et al.* (2008)	–	430 cfu/g (med.)	New Zealand: Concentration range 15–1.8×10^7 cfu/g
Stanley *et al.* (1998a)	–	610 MPN/g (ave.)	UK beef cattle at slaughter
	–	69.9 MPN/g (ave.)	UK cows
	–	33,000 MPN/g (ave.)	UK calves
Swine			
Dorner *et al.* (2004)	45.9, 79.7	–	Canadian sows and gilts (females, not yet mated)
Hutchison *et al.* (2004/5)	13.5	310 cfu/g (g.m.)	Fresh composite farm manure, UK. max. = 1.5×10^4 cfu/g

(*Continued*)

Table A10.1 (*Continued*)

Reference	*Prevalence (%)*	*Concentration*	*Notes*
Weijtens *et al.* (1997)	–	$10^{3.6}$–10^{5} cfu/g	Five samples. Shedding dominated by *C. coli*, less infectious to humans *cf. C. jejuni*
Sheep			
Açik & Cetinkay (2006)	49.5	–	Intestinal contents, gall bladders and faeces from 610 healthy sheep
Brown *et al.* (2004)	25	–	Rural Cheshire, UK, for *C. jejuni*; 21% positive for *C. coli*
Devane *et al.* (2005)	59.8	–	New Zealand dairy cattle (52/66 positive for *C. jejuni*)
Hutchison *et al.* (2004/5)	20.8	390 cfu/g (g.m.)	Fresh composite farm manure, UK. Max. = 2100 cfu/g
Rotariu *et al.* (2009)	22	2.7×10^{4} cfu/g	Cattle vs. sheep. No statistically significant difference in prevalence or average concentrations for cattle or sheep between hosts or regions in Scotland.
	25	2.0×10^{5} cfu/g	
Stanley *et al.* (1998b)	91.7	10^{4}–10^{7} MPN/g	Thermophilic *Campylobacter in* lambs. See also Skelly & Weinstein (2003)
	29.3	–	Adult sheep
Poultry			
Cox *et al.* (2002)	–	$10^{2.8}$–$10^{3.9}$cfu/g	Breeders: composite samples from 35 farms
	–	$10^{3.5}$–$10^{6.5}$cfu/g	Broilers: composite samples from 35 farms

(*Continued*)

Table A10.1 (*Continued*)

Reference	*Prevalence (%)*	*Concentration*	*Notes*
El-Shibiny *et al.* (2005)	–	10^6–10^9 cfu/g	
Hutchison *et al.* (2004/5)	19.4	260 cfu/g (g.m.)	Fresh composite farm manure, UK. max. = 2.9×10^4 cfu/g
Stanley & Jones (2003)	Up to 100%	–	Varies between flocks

"g.m." = geometric mean, "ave." = arithmetic mean, "max." = maximum

Table A10.2 Pathogenic *E. coli*.

Reference	*Prevalence (%)*	*Concentration*	*Notes*
Cattle/cows/calves			
Besser *et al.* (2001)	–	≤30 to ≥10^7/g	Calves, Washington State
Chase-Topping *et al.* (2006/7)	–	–	Demonstrated presence of "super-shedders" within herds
Donkersgoed *et al.* (1999)	19.7, 0.7	–	Summer, winter
Duffy (2003)	0.1–62	–	Irish stock (review of 26 studies)
Fegen *et al.* (2003)	–	<3 to 2.4×10^4	Australia
Hancock *et al.* (1997)	1.8	–	Cattle feedlots
Hutchison *et al.* (2004/5)	13.2	1200 cfu/g (g.m.)	Fresh composite farm manure, UK. max. = 2.6×10^8 cfu/g
Meyer-Broseta *et al.* (2001)	0–100	–	France
Reinstein *et al.* (2009)	9.3	–	Kansas, organically fed: range = 0–24%.
	7.2	–	Kansas, naturally raised: range = 0–20.3%
Robinson *et al.* (2004)	–	up to 10^6 cfu/g	
Swine			
Chapman *et al.* (1997)	0.4	–	
Hutchison *et al.* (2004/5)	11.9	3900 cfu/g (g.m.)	Fresh composite farm manure, UK. max. = 7.5×10^5 cfu/g
Sheep			
Hutchison *et al.* (2004/5)	20.8	780 cfu/g (g.m.)	Fresh composite farm manure, UK. max. = 4.9×10^4 cfu/g
Kudva *et al.* (1998)	<10^2–10^6 cfu/g	–	
Ogden *et al.* (2005)	6.5	<10^2 to >10^6 cfu/g	United Kingdom
Poultry	0	–	Negligible presence/excretion: Chapman *et al.* (1997), Hutchison *et al.* (2004/5)

"g.m." = geometric mean, "ave." = arithmetic mean, "max." = maximum

Table A10.3 *Salmonella.*

Reference	*Prevalence (%)*	*Concentration*	*Notes*
Cattle/cows/calves			
Hutchison *et al.* (2004/5)	7.7	2100 cfu/g (g.m.)	Fresh composite farm manure, UK. max. = 5.8×10^5 cfu/g
Rodriguez *et al.* (2006)	0.4	–	From five USA states; rectal swabs
Swine			
Davies (1998)	4, 60	–	In two North Caroline herds
Hutchison *et al.* (2004/5)	7.9	600 cfu/g (g.m.)	Fresh composite farm manure, UK. max. = 7.8×10^4 cfu/g
Sanchez *et al.* (2007)	17	–	Literature survey on subclinical infections in swine
Rodriguez *et al.* (2006)	6.0	–	From five USA states; rectal swabs
Sheep			
Hutchison *et al.* (2004/5)	8.3	710 cfu/g (g.m.)	Fresh composite farm manure, UK. max. = 2.0×10^3 cfu/g
Poultry			
Li *et al.* (2007)	30.8	16.2 /g	North Carolina; average over all bird ages
Hutchison *et al.* (2004/5)	17.9	220 cfu/g (g.m.)	Fresh composite farm manure, UK. max. = 2.2×10^4 cfu/g
Rodriguez *et al.* (2006)	0.2	–	From five USA states; rectal swabs

"g.m." = geometric mean, "ave." = arithmetic mean, "max." = maximum

Table A10.4 *Giardia.*

Reference	*Prevalence (%)*	*Concentration*	*Notes*
Cattle/cows/calves			
Heitman *et al.* (2002)	<10–60	5800 cysts/g (ave.)	North Saskatchewan River Basin, Alberta
Hutchison *et al.* (2004)	3.6	10 /g	Fresh composite farm manure, UK. max. = 5.0×10^3 cfu/g
McAllister *et al.* (2005)	8.7	85.9 cysts/g (ave.)	Cows (Ontario, Canada)
	36	–	Calves (British Columbia, Canada), max. = 113,000 cysts/g
Nydam *et al.* (2001)	–	–	An infected calf could produce 3.8×10^7 oocysts in 6 days
Swine			
Heitman *et al.* (2002)	16.1	–	North Saskatchewan River Basin, Alberta
Hutchison *et al.* (2004)	2.4	68 /g (g.m.)	Fresh composite farm manure, UK. max. = 2.95×10^4 cfu/g
Sheep			
Hutchison *et al.* (2004)	20.8	3.8×10^2 /g (g.m.)	Fresh composite farm manure, UK. max. = 1.6×10^5 cfu/g
Ryan *et al.* (2005)	8.7	–	Australia
Poultry			Not detected (Hutchison *et al.* 2004)

"g.m." = geometric mean, "ave." = arithmetic mean, "max." = maximum

Table A10.5 *Cryptosporidium.*

Reference	*Prevalence (%)*	*Concentration*	*Notes*
Cattle/cows/calves			A wide range of prevalence is reported in various studies, tending to be higher amongst youngstock (Atwill *et al.* 1999a&b, 2006). See also chapter 3 of this text.
Davies *et al.* (2005a)	–	331 / gdw, (ave.)	Adult cattle; represents about 10^7 oocysts per animal per day
Heitman *et al.* (2002)	0–12	249 oocysts / g (ave.)	
Hoar *et al.* (2001)	1.1	–	Canadian adult beef cattle
Hutchison *et al.* (2004/5)	5.4	19 cfu/g (g.m.)	*C. parvum* in fresh composite farm manure, UK. max. = 3.5×10^3 cfu/g
McAllister *et al.* (2005)	18.4	–	Cows (Ontario, Canada)
	13	–	Calves (British Columbia, Canada), max. = 132,000 oocysts/g
McAllister *et al.* (2005)	1.1	12,323 / g (max.)	California
Nydam *et al.* (2001)	–	–	An infected calf could produce 3.89×10^{10} oocysts in 6 days
Santín *et al.* (2008)	8.7, 36	–	Cows, calves
Starkey *et al.* (2005)	–	1.3×10^5 / g	Calves
Swine			
Ferguson *et al.* (2009)	–	–	Australia. Prevalence highly variable; average shedding rates from prior studies = 14.3 oocysts/g for adults and 472 oocysts/g for juveniles (6–8 weeks old)
Heitman *et al.* (2002)	0	–	Canada

(*Continued*)

Table A10.5 (*Continued*)

Reference	*Prevalence (%)*	*Concentration*	*Notes*
Hutchison *et al.* (2004/5)	13.5	58 cfu/g (g.m.)	*C. parvum* in fresh composite farm manure, UK. max. = 3.6×10^3 cfu/g
Sheep			
Hutchison *et al.* (2004/5)	29.2	10 cfu/g (g.m.)	*C. parvum* in fresh composite farm manure, UK. max. = 2.5×10^2 cfu/g
Ryan *et al.* (2005)	2.6	–	Australia
Poultry			
Ferguson *et al.* (2009)	–	–	Two studies: (i) prevalence in US 6% (broilers) and 27% (layer chickens); (ii) prevalence = 27% (16 samples) in The Netherlands. Average shedding rate = 2100 oocysts/g faeces for Netherlands layers.
Hutchison *et al.* (2004/5)	0	–	*C. parvum* in fresh composite farm manure, UK.

"g.m." = geometric mean, "ave." = arithmetic mean, "max." = maximum

(full descriptions of these studies are of course given in the cited references in these tables). Note that these studies typically analyse composite samples, rather than faecal material from individual animals. Therefore the variation between individual animals is smoothed somewhat. This is appropriate for risk studies, because faecal contamination of environmental water typically arises from groups of animals, not from an individual animal.

PATHOGEN SURVIVAL (T_{90}) AND EFFECT OF SUNLIGHT

Campylobacter, *E. coli* and *Salmonella*

Sinton *et al.* (2007a) reported inactivation rates of *Campy. jejuni*, *E. coli*, and *Salm. enterica* (*inter alia*) in bovine faeces on pasture. In the first one to three weeks, there were increases (up to 1.5 orders of magnitude) in the counts of *E. coli* (three seasons) and *Salm. enterica* (two seasons), but there was none for *Campy. jejuni*. Thereafter, the counts decreased, giving an average ranking of the times necessary for 90% inactivation of *Campy. jejuni* (6.2 days from deposition) < *S. enterica* (38 days) < *E. coli* (48 days). Sinton *et al.* (2007b) studied the inactivation of the same microorganisms in river water and seawater. All sunlight inactivation rates, as a function of were far higher than the corresponding dark rates. All the T_{90} values were higher in winter than in summer. Seasonal values for *Salm. enterica* and *E. coli* were similar and both were considerably larger than those for *Campy. jejuni*. The rapid inactivation of *Campy. jejuni* was attributed to a high susceptibility to photo-oxidative damage.

Giardia and *Cryptosporidium*

Olson *et al.* (1999) reported that *Giardia* cysts were noninfective in water, faeces, and soil following one week of freezing to −4°C and within two weeks at 25°C. At 4°C *Giardia* cysts were infective for 11 weeks in water, seven weeks in soil, and one week in cattle faeces. *Cryptosporidium* oocysts were more environmentally resistant. At −4°C and 4°C, the oocysts could survive in water and soil for >12 weeks but degradation was accelerated at 25°C. *Cryptosporidium* oocysts also were degraded more rapidly in faeces and in soil containing natural microorganisms. Davies *et al.* (2005b) reported T_{90} for closed non-irradiated soil microcosms ranging from 13–24 days at 35°C to 45–66 days at 20°C depending on soil type. In laboratory studies of *Crypto. parvum*, Ives *et al.* (2007) reported first-order $\log_{10}$ inactivation coefficients ranging from $k = 0.0088$ per day at 5°C to 0.20 per day at 30°C. These correspond to T_{90} values of approximately 114 and 5 days respectively [using the first-order

relationship $T_{90} = -\log_{10}(0.1)/k = 1/k$]. Similar studies for *Giardia* have not been cited, but it seems plausible to assume that a similar situation applies.

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11

Epidemiological studies on swimmer health effects associated with potential exposure to zoonotic pathogens in bathing beach water – a review

Al Dufour, Timothy J. Wade and David Kay

11.1 INTRODUCTION

Humans, animals and birds discharge billions of tons of faecal material into the environment every year. Much of this faecal material reaches water bodies either indirectly through discharge after treatment or directly by being washed off the surface by rainfall or through defecation directly into water bodies. This faecal material can carry pathogenic microbes that may pose a risk to humans exposed to contaminated surface water.

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Treated wastewater has been shown to be related to swimming-associated gastrointestinal illness in individuals in contact with contaminated water for recreation. Studies conducted in both marine and freshwater environments have shown that as the level of faecal contamination increases the frequency of gastrointestinal illness in swimmers also increases (Wade *et al.* 2003, Pruss 1998, Zmirou *et al.* 2003). Incidence is directly related to the densities of faecal indicator bacteria in the water. This relationship has provided regulators and water resource managers with a means to protect the health of swimmers. Faecal indicators have been used for many years to measure water quality and to maintain the safety of water for swimmers.

One of the shortcomings of faecal indicator bacteria is, however, that they are carried by all warm-blooded animals including birds. Thus, contamination of water by animal and bird faeces cannot be distinguished from contamination by human faeces when using these faecal indicator bacteria. Since the extent to which risk associated with the former differs from risk associated with the latter is unknown, it has been the practice of regulators and water resource managers to consider the risk as being the same for all waters regardless of the source. Current knowledge about the occurrence of pathogens in faeces leads us to believe that the risks related to human and animal faecal sources are not equivalent. A large proportion of the swimming-associated gastrointestinal illnesses (in unchlorinated water) are thought to be caused by viruses (Hendrikson *et al.* 2001). It is well-known that viruses commonly responsible for gastrointestinal illness (e.g. norovirus, rotavirus) tend to be species-specific. This relationship holds up quite well among the viruses that cause gastrointestinal infections, but less well for respiratory infections, where the species barrier is frequently crossed. The species barrier would imply that the risk associated with exposure to water contaminated by animals and birds would have to be lower than the risk associated with exposure to water contaminated by human faeces; however, this risk difference has not been well defined for individuals who swim in recreational waters.

The main challenge in defining the risk of infection associated with exposure to zoonotic pathogens that may be present in water contaminated with animal/bird faeces is the lack of a technique to determine the source of the faeces and to make quantitative attributions to different sources. Currently an extensive research effort is on-going to develop methods to identify sources of faecal contamination in water (USEPA 2005, Rochelle & De Leon 2006, Santo Domingo *et al.* 2007). Most of the efforts involve detecting and quantifying highly specific sections of DNA from the genome of bacteria that have established an ecological niche in the gastrointestinal tract of human and non-human species. None of the methods developed to date have the specificity

and sensitivity to be useful for characterizing the source of faecal contamination in recreational and other surface waters. In the absence of effective source identification methods, risk assessment remains confined to studies at sites where the dominant or only source of faecal contamination is known. This approach has been successful in studies of health effects in swimmers exposed to point sources of pollution, such as treated wastewater effluents.

Few studies have addressed the health effects in swimmers exposed to bathing beach waters contaminated by animal and bird faecal wastes. Only two studies in the literature were specifically designed to answer the question whether an excess of swimming-associated health effects can be related to exposure to waters affected by non-point source faecal wastes (Calderon *et al.* 1991, Colford *et al.* 2007). In a third study, which was designed to look at health effects associated with polluted waters, two of nine beaches were contaminated with animal faecal wastes and swimmer exposure at these beaches may be helpful in answering our question (Cheung *et al.* 1990). One other study was designed to determine if health effects were different in populations exposed to rural run-off conditions as opposed to exposure to beach waters affected by human wastewaters treated with oxidation pond processes (McBride *et al.* 1998). These studies will be described in some detail to determine if a general conclusion can be drawn from their results with regard to health effects associated with exposure to animal and bird faecal contamination of bathing waters.

11.2 HONG KONG STUDY

In 1990, Cheung *et al.* reported the results of epidemiological studies undertaken in 1986 and 1987 in Hong Kong. Although these studies were not conducted to determine the effect that animal faeces-contaminated beach waters might have on exposed swimmers, the data generated by these studies may have a bearing on the issue. The group studied water quality and swimmer health effects at nine beaches. Six of the beaches were contaminated by human sewage discharged through sea water outfalls or storm water drains. Two of the beaches ("Old Cafeteria" and "New Cafeteria") were contaminated by livestock waste (mainly pig excreta) discharged from the mouth of a river. One of the nine beaches had mixed human and animal faecal wastes as the source of contamination of the beach.

Water samples were obtained from beaches on weekend days every two hours between 9 a.m. and 5 p.m. The water samples were tested for nine water quality indicators, including *E. coli* and enterococci.

The study population included beachgoers recruited at the beach. Follow-up telephone interviews were conducted the next day to collect demographic and swimmer activity information. A second telephone interview was conducted

seven to ten days later to determine if any episode of illness had occurred since the swimming experience.

Swimmers were defined as individuals having significant exposure of upper body orifices to beach water. Non-swimmers were beachgoers who did not immerse their heads in beach water. The health endpoint, highly credible gastrointestinal illness (HCGI), was defined as any one of the following: vomiting; diarrhoea with fever or a disability condition; and nausea or stomachache accompanied by a fever.

We re-analyzed the data reported in Cheung *et al.* (1990) with respect to source and swimmer illness. The data for both beach water quality and health status from the two animal waste-affected sites were combined. This was also done with the data from six of the seven remaining beach sites that were contaminated by faecal wastes from sewer outfalls or stormwater drains. The seventh site was not included in the analysis because the faecal sources were described as being from a sewage outfall and a river, a mix of both human and animal faecal contamination. The data extracted from Table 11.3 of the Cheung study are shown in Table 11.1. The average *E. coli* density at the animal faeces-contaminated beach sites was 978 per 100 ml, with a range of 243 to 1714 *E. coli* per 100 ml. At the human sewage contaminated beach sites the average *E. coli* density was 187 per 100 ml, with a range of 69 to 269 per 100 ml.

Table 11.1 Analysis of data from Cheung *et al.* (1990) for swimmer-associated gastroenteritis among beaches grouped by source of faecal contamination.

Faecal source	E. coli density (range, per 100 ml)	Category	Total number	Number ill	p Value[1]
Animal	243–1714	Swimmers	960	2	0.5246
		Non-swimmers	366	0	
Human	69–269	Swimmers	11,748	25	0.0418
		Non-swimmers	3368	2	

[1]p-value for the difference between swimmer/non-swimmer illness rates based on Fisher's exact test.

The total number of individuals who swam at the animal faeces-contaminated beach waters was 960; 366 non-swimmers at these beaches participated in a study. In these two groups HCGI illness was observed in two of the swimmers while no HCGI illness was observed among the non-swimmers. At the beaches with bathing waters contaminated by human sewage 11,748 swimmers

participated in the study and 3,368 individuals participated as non-swimmers. Twenty-five of the swimmers and two of the non-swimmers suffered HCGI illness. Fisher's Exact test was used to determine if it was reasonable to assume that illness rates in swimmers and non-swimmers were identical.

The results of this statistical analysis indicate that the illness rate in swimmers was different from that of non-swimmers only at beaches that were contaminated by human sewage. At beaches contaminated by animal faecal wastes no significant difference in illness rate was observed between swimmers and non-swimmers despite much higher levels of faecal contamination at the animal impacted sites. The mean *E. coli* densities in the beach waters contaminated with animal faeces were about five times greater than those in the beach waters contaminated with human sewage. Enterococci densities in the animal contaminated waters were about twice the mean density observed in the waters contaminated with human sewage. Although the resolution to detect a difference was low, since very few respondents reported episodes of gastroenteritis, one interpretation of these analyses is that exposure to animal faeces carries less risk than exposure to human sewage.

11.3 CONNECTICUT USA STUDY

In 1991 Calderon *et al.* published a prospective epidemiological study at a beach on a small freshwater pond located in central Connecticut, USA. The pond, an impoundment with a surface of about three acres, was formed by damming a river. The pond was fed by two small streams. One side of the pond had a sandy beach about 200 yards in length. There were no human sources of faecal contamination in the surrounding watershed contributing to the streams that fed the pond. There were no direct sources of human faecal contamination of the pond either, such as leaking septic tanks. The main source of the faecal contamination of the pond was associated with bird or animal faeces from the surrounding forestland.

The study participants consisted of families from the local community who had exclusive use of the beach area. Community members frequently used the pond for swimming. Attendance at the beach was recorded daily by study participants. The health status of beach attendees was recorded in a daily diary kept by each family member. Gastrointestinal illness was defined as having experienced any of the following symptoms: vomiting, diarrhoea, stomachache or nausea. If symptomatic illness occurred one, two or three days after a swimming experience it was designated as swimming-associated. Exposure was defined as complete immersion of the head and body beneath the surface of the water. Individuals who swam at other locations were excluded from the study.

Multiple indicators of water quality were measured daily over the course of the bathing season. Water samples were collected daily at two sites at the beach at 10 a.m., 2 p.m. and 5 p.m. Water samples were analyzed for *E. coli*, enterococci, faecal coliforms, *Staphylococcus* and *Pseudomonas aeruginosa*. Rainfall also was measured daily.

The results of the study indicated that there was significant swimming-associated gastrointestinal illness, but that it was not related to the level of faecal indicator bacteria in the water. A strong relationship between illness and swimmer density or *Staphylococcus* density led the authors to conclude that the excess gastrointestinal illness in swimmers was probably caused by swimmer-to-swimmer transmission and not by exposure to faecal contamination of animal or bird origin.

11.4 NEW ZEALAND STUDY

In 1998 McBride *et al*. reported the results of epidemiological studies conducted at beaches contaminated by human faecal contamination from oxidation ponds (three beaches) and beaches contaminated by run-off from rural areas contaminated by animal faeces (two beaches). Two control beaches with minimum faecal contamination impact were also included in the study.

Water samples were collected twice daily at 11 a.m. and 3 p.m. on weekends and holidays at three locations along each beach. Each sample was tested for *E. coli*, faecal coliforms and enterococci using membrane filter techniques.

Multiple health endpoints were used, including highly credible gastrointestinal illnesses (HCGI). The symptoms associated with HCGI in this study included vomiting; loose bowels with fever; loose bowels with disability (one or more days away because of illness or days unable to do normal activity, medical advice sought or hospitalization); nausea with fever; or, indigestion with fever.

Three categories of exposure were defined: swimmers, paddlers, and non-exposed individuals. Exposure was defined as the act of entering the water. Swimmers were individuals who immersed their head beneath the surface of the water. Paddlers were individuals who entered the water but did not immerse their head beneath the surface.

Potential study participants were recruited at the beach. The initial contact was used to gather information about swimming exposure and demographic information. At this time questions were also asked about the types of food recently eaten and details were collected for a follow-up contact.

The follow-up questionnaire was designed to determine types of swimming activity, symptoms that were evident since a swimming experience or food types ingested since the swimming event. Questions were also asked to determine if

other family members had become ill or if the participants had had contact with animals.

None of the faecal indicator bacteria occurred at very high densities. The median values for enterococci at all beaches were less than 10 MPN per 100 ml. The median values for *E. coli* at the control and rural beaches were approximately 10 per 100 ml or less. *E. coli* median values in beach waters with possible human faecal contamination ranged from less than 10 to about 30 per 100 ml. All of the median faecal coliform densities were less than 30 per 100 ml.

The relationship between health effects associated with swimming and *E. coli* or faecal coliforms did not show any increase in illness risk through increasing quartiles. Similarly, the risk for exposed groups (those entering the water or entering the water and immersing their heads beneath the surface of the water) did not show a statistically significant association with increasing quartiles of enterococci concentrations.

These findings were associated with swimmers from all of the beaches regardless of the source of the faecal contamination. The combination of data from beaches where swimmers were exposed to faecal contamination from different sources made it difficult to determine if exposure to one source or the other posed a greater risk to swimmers. The authors indicate that, "No evidence has been found to suggest any merit in separating beachgoers illness risk on the basis of the types of faecal material present (i.e., from rural areas versus from oxidation ponds treating human wastes)." They also concluded, "Illness risks at control beaches were significantly lower than at beaches believed to be impacted by oxidation pond effluent and by rural runoff." The authors indicated that because of very low densities of faecal indicator bacteria in the waters at the beaches, exposure may not have been great enough to elicit an observable health effect in swimmers.

11.5 SAN DIEGO, CALIFORNIA STUDY

In January 2007, Colford *et al.* published a report describing epidemiological studies expressly conducted to examine health effects experienced by swimmers and the relationship of these effects to water quality indicators in water predominantly contaminated by non-human faecal sources. Their study was conducted on Mission Bay in San Diego, a 2,287 acre man-made estuary. The Bay has 27 miles of shoreline, 19 of which are sandy beaches. Multiple beach sites were used for the epidemiology study. This study was unique, in that the year before the epidemiology study was initiated, a source identification project (Gruber *et al.* 2005) had been conducted to determine the sources of faecal contamination of the Bay. Faecal sources were identified with two separate source identification methods, one a library method (ribotyping) and the other a

non-library method (Polymerase Chain Reaction). The results of this bacterial source identification study showed that the major source of faecal contamination to the Bay were birds and that less than 9% of the faecal contamination was from human sources.

In this prospective cohort study participants were recruited each sampling day and their current health and degree of exposure to the water were recorded. Six beaches in the Mission Bay were used for the study, which was conducted on weekends and holidays.

Water quality samples were collected at the six selected beaches at eighteen sites, with the number of sites per beach ranging from two to five depending on the beach length and anticipated swimming activity. The quality of beach waters was measured using three traditional faecal indicator bacteria, enterococci, faecal coliforms and total coliforms.

Study participants recruited at the beach had to meet certain criteria including (1) they could not have participated previously in the study; (2) at least one family member had to be 18 years old or older; (3) they had to have a home address in the United states, Canada or Mexico; and (4) they had no history of swimming in the previous seven days. Participants were asked to complete a questionnaire about possible exposures at the beach and illnesses experienced in the previous two to three days, prior to departure from the beach. Follow-up telephone interviews were conducted about 14 days after the beach visit. Participants were interviewed about demographic information; swimming and other exposures since the beach day; pre-existing health problems and health problems experienced since the beach visit.

Health outcomes included gastrointestinal (GI) illness, respiratory symptoms, dermatologic symptoms and other non-specific symptoms. GI symptoms included nausea, vomiting, diarrhoea and stomach cramps. Grouped symptoms were defined as HCGI-1 (vomiting, diarrhoea and fever or cramps and fever) and HCGI-2 (vomiting plus fever).

Eight thousand seven hundred and ninety-seven of the enrolled participants completed the follow-up telephone interview. The results of the study showed that there was a significant excess of diarrhoea among swimmers. However, there was no correlation between traditional water quality indicators (*Enterococcus*, faecal coliforms or total coliforms) and the risk of illness. Although swimmers experienced more diarrhoea than non-swimmers and the incidence of symptoms increased with increased exposures, an increased risk was not observed for the more severe symptoms, such as fever, vomiting or HCGI-1 or HCGI-2. The authors concluded, “Our findings do not agree with earlier studies reporting association between bacterial indicators of water quality and illness. We believe these results are due to a lack of human sources of

traditional faecal indicator bacteria, supported by our lack of virus detection and an independent microbial tracking survey."

11.6 OTHER STUDIES

Outbreaks of human infection with zoonotic pathogens have often been linked to bathing or incidental contact with untreated surface water (Kramer *et al.* 1998, Ackman *et al.* 1997). Most, however, have identified other bathers rather than animals as the likely source of contamination. A large outbreak of *E. coli* 0157: H7 in Swaziland (Effler *et al.* 2001) and several cases of *E. coli* 0157:H7 (Ihekweazu *et al.* 2006) were associated with contact with untreated surface waters where cattle were implicated as the likely source, but definitive linkages to cattle faeces were not established. Outbreaks in drinking-water systems, such as the outbreak in Ontario (PPHB 2000, Hrudey 2003) of *E. coli* 0157: H7 and *Campylobacter* spp., also provide indirect evidence of the transmission of zoonotic illnesses through contact with untreated water. The municipal water supply was contaminated by nearby livestock demonstrated to be infected with genetically identical strains of the responsible pathogens. While the Walkerton example is not directly applicable since, for obvious reasons, drinking-water exposures would be considerably greater than bathing water exposures, it does support the general plausibility of similar situations involving contamination of untreated surface waters and recreational or other incidental contact.

Case-control studies of the five zoonotic pathogens addressed in this book: *Campylobacter* spp. (Denno 2009, Schoneburg-Norio 2004), *Cryptosporidium parvum* (Pintar 2009, Roy 2004), *E. coli* 0157:H7 (Denno 2009, Slutsker 1998, Werber 2007), *Salmonella* spp. (Denno 2009) and *Giardia* spp. (Stuart 2003) have identified exposures to untreated surface waters as a significant risk factor for infection and illness. In the northwestern United States, a recent study attributed 10 per cent of sporadic *Campylobacter* and 21 per cent of sporadic *Salmonella* infections to swimming in or contact with a natural source of water (Denno 2009). None of these studies, however, made an attempt to characterize the sources of contamination affecting the water bodies. Since case-control studies investigate many different exposures, detailed investigations of all exposures are usually not feasible.

11.7 CONCLUSIONS

The epidemiological studies reviewed in this chapter do not provide evidence for associations between swimming-associated gastrointestinal illness and exposures to bathing waters contaminated with faeces from animals or birds. Other studies,

such as outbreak investigations and case-control studies, have provided logical linkages to human infections with zoonotic pathogens and recreational or occupational exposures to water, but they have not established a definitive link between water contamination and specific animal sources.

Three of the prospective studies were conducted in marine or estuarine waters and one was conducted at a freshwater beach. Two of these studies were specifically designed to answer the question "Do bathing waters contaminated by non-human faeces pose a risk to swimmers?" In both studies efforts were made to ensure that animals and birds were the dominant source of the faecal contamination. This was done in one case by surveying the watershed and determining human wastes were not being discharged into streams that fed into the bathing water area (Calderon 1991). In the second case (Colford 2007) an extensive pre-study was performed using microbial source identification methods to determine if human faeces were contaminating the beaches. In the third case (Cheun 1990) the dominant source of non-human faecal contamination was identified as being related to wastes that were discharged to a river that contaminated the water at two of the beaches that were studied. In the fourth case (McBride1998) the beaches studied were characterized as receiving discharges from oxidation ponds, rural runoff or not receiving known sources of faecal contamination. The sources of faecal contamination were not specifically identified.

The levels of faecal contamination at the study locations, as measured by faecal indicator bacteria, were not unusual. Enterococci and *E. coli* were measured at three of the study locations while only enterococci was measured at the San Diego location. The enterococci geometric mean densities per 100 ml at all four locations ranged from 17 to 144. The geometric mean *E. coli* densities per 100 ml at the Hong Kong, Connecticut and New Zealand locations ranged from 25 to 1,705. In similar epidemiological studies conducted at beaches contaminated by human sources (sewage effluents) with indicator densities at these same levels, swimming-associated gastroenteritis has been frequently observed. In the study where the beaches were characterized as receiving rural run-off, the densities of faecal indicator bacteria were very low, averaging eight per 100 ml at 1 beach and 30 per 100 milliliters at a second beach. This unusually low level of faecal contamination is likely to have hindered the ability to draw clear-cut conclusions about whether rural run-off is related to swimming-associated gastroenteritis.

All of the studies used a health endpoint similar to or identical to the Highly Credible Gastrointestinal (HCGI) symptomatology first described by Cabelli (1983), in which combinations of the symptoms (vomiting, diarrhoea, nausea and fever) were used to confirm a case of gastroenteritis.

After observing the results of these studies the question can be asked, can the lack of excess faecal-associated illness in swimmers be taken as evidence that animal

contaminated waters do not pose a health risk to swimmers? One possible explanation for the observations is that the conditions under which these studies were conducted prevented finding a health effect in swimmers. These studies were conducted at sites where water quality standards were based on data gathered from human epidemiological studies at sites impacted by human sewage. This is important because human sewage is usually treated and disinfected before being discharged into surface waters. Treatment and disinfection significantly re-arrange the relationship between faecal indicator organisms (FIO) and potentially present pathogens. The ratio of FIO to pathogens is changed from a very high ratio to a very low ratio after disinfection and it is the latter ratio which is used to develop bathing beach standards. On the other hand, faeces from animals are not treated and the high ratio of FIO to pathogens that are observed at the animal source may be maintained for long periods. This could result in levels of FIO that would not be allowed at a beach, or if distances were involved, dilution or die-off of pathogens would take place before faecal wastes reached a beach. In light of this scenario, the conclusions drawn above do not completely answer the question whether exposure to animal-contaminated waters poses a health risk to swimmers. The exposure to zoonotic pathogens is unlikely to have occurred at beaches meeting local beach water quality standards.

The real question remains whether epidemiological studies to determine if swimming associated gastroenteritis is related or not to exposure to waters contaminated by animal or bird faeces can be properly conducted under conditions where animals or birds are the source of recreational water contamination. There are many impediments to carrying out the necessary prospective cohort types of studies.

First, because of the putative low frequency of illness associated with zoonotic pathogens, large populations would need to be recruited. Such large study populations might not be available at rural beaches where animal exposures might take place.

Second, faecal indicator bacteria may occur in very high numbers, relative to the numbers of zoonotic pathogens. Unlike the wastewater treatment plant systems used for eliminating faecal indicator bacteria and pathogens from human faecal wastes, the wastes from animals are normally not treated, except in the case of confined animal feedlot operations. This means that a waiver from local regulations would have to be obtained to allow high indicator counts at animal-impacted beaches.

Third, some zoonotic pathogens can cause serious disease, especially in children. For example, *E. coli* 0157:H7 not only causes severe gastrointestinal disease, but also haemolytic uremic syndrome which frequently results in the death of children. Therefore, a study at a site which is contaminated enough to

elicit health effects may pose too great a health risk for severe illness and infection and thus raise ethical issues related to conducting an epidemiological study.

A fourth challenge is that traditionally designed epidemiology studies may be poorly suited to quantifying and understanding the sporadic and episodic risks associated with animal wastes. Furthermore, a single study cannot encompass risks from all types of animal waste (cattle, birds, pigs, etc.) and no study to date has addressed the risks associated specifically with cattle waste. While the studies considered here had no obvious major flaws in design, the impact at the specific sites may not have been enough to allow for a risk to be detected, or a risk may only have been present following a heavy rain or specific sporadic contamination events, which also in turn, could affect swimming behaviour and decrease the likelihood of exposure.

In the presence of all these formidable barriers and confounding factors, which severely hinder our ability to quantify the relationship between water quality and swimming-associated disease caused by zoonotic pathogens, some other means will have to be used to measure the risk posed by the presence of zoonotic pathogens in bathing beach waters. Alternate approaches to traditional prospective (observational or randomized/intentional exposure) epidemiology studies should be explored to better quantify and understand health risks from waters contaminated with animal faeces. Approaches could include case-control studies targeting specific zoonotic pathogens combined with detailed source characterizations, or studies targeted at highly exposed populations. Incorporation of human-specific indicators into studies can help better define exposure. Future epidemiological studies designed to address this issue must carefully consider site selection, sample size, exposure and measurement of appropriate indicators.

Quantitative microbial risk assessment has been proposed as another alternative approach to defining risks associated with zoonotic pathogens. However, it is generally agreed that this is not an ideal substitute for epidemiological evidence. Until the time when information is available to regulators for developing water quality criteria for waters contaminated by non-human faecal wastes, they may be left with no other choice than regulating animal-polluted bathing water as if it poses the same risk as human-contaminated bathing water.

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12

Economic evaluation

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12.1 INTRODUCTION

This chapter considers the economic evaluation of interventions concerned with the contamination of recreational and other waters by microbial pollution from livestock waste. The objective of such interventions is to reduce adverse impacts on water quality and public health. Because resources are scarce, regulatory choices imply trade-offs between the resources needed to manage the problem of water contamination, and other potentially competing uses of those resources. It is important that resources are used as efficiently as possible in the sense that society should make the most of the resources available by comparing what is gained from using those resources with the gain from alternative uses – the so-called opportunity costs. Economic evaluation is thus about determining whether an intervention is an efficient use of society's resources and can be defined as the comparative analysis of alternative courses of action in terms of both their costs and consequences (Drummond *et al.* 1987). The tasks included

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in an economic evaluation include the identification, measurement, valuation and comparison of the costs and consequences of the alternatives being considered.

In the context of recreational water, as an example of the more general case to be made for water quality, this chapter looks at the economic evaluation of interventions addressing water contamination by livestock waste. It provides a summary of the concepts underpinning economic evaluation, including economic value, cost-benefit analysis, cost-effectiveness analysis, as well as the methods employed to value costs and benefits. The chapter also includes a brief review of some empirical studies that have sought to estimate the economic value of interventions to improve contaminated recreational bathing waters. This is then followed by a more detailed case study outlining the economic cost-benefit approach, as applied to the bacteriological contamination of recreational bathing waters in the Netherlands.

12.2 ECONOMICS, ECONOMIC VALUES AND ECONOMIC EVALUATION OF INTERVENTIONS

Economics is defined as "the science which studies human behaviour as a relationship between ends and scarce means which have alternative uses" (Robbins 1935). The objective of economics is to maximise human welfare or utility. Thus, it is important that the allocation of resources in society is done as efficiently as possible. In economic terms, an efficient allocation of resources is defined as one that takes advantage of every opportunity to ensure that some individuals will be better off while not making anyone else worse off. Any intervention that sets out to reduce the adverse impacts on water quality and public health of microbial contamination of recreational and other waters by livestock waste will necessitate a reallocation of society's resources. Economists argue that in looking at whether an intervention represents an efficient use of resources, rational management decisions should be based on an informed assessment of the costs of reducing the adverse impacts on water quality and public health, as well as the benefits of reducing them. This requires that we place "economic" values on these elements.

The economic definition of *value* is a rigorously defined theoretical concept, but one which is also grounded in empirically observed phenomena. Nevertheless, despite its familiarity and role in people's everyday lives, much confusion continues to surround the concept and much abuse is heaped upon it. Economic value is used in welfare economics to assess the efficiency of a proposed change from the point of view of society's welfare. Because human welfare is an intangible concept that cannot be directly measured, economists use a transformation of welfare into a more general single-scale composite indicator,

referred to as a numeraire (Pearce 1986). It is then possible to define economic value very narrowly in terms of economic behaviour in the context of supply and demand. Put simply, it is the maximum amount of goods or services – or equivalent money income – that an individual is willing to forego (willingness to pay – WTP) in order to obtain some outcome that increases his/her welfare.[1] These sums of money are demonstrated or implied by the choices people make, and thus reflect individuals' preferences for the change in question (Pearce 1986).

In promoting economic evaluation, there is concern that there is too much emphasis on assigning monetary values to aspects of health and the environment that are difficult—if not impossible—to quantify. There is also concern that decisions about health and environmental protection interventions might be made strictly on the basis of whether their quantifiable benefits outweigh their monetized, quantifiable costs.

It should be noted though, that decision-makers will often find it hard to interpret and decide upon health or environmental endpoints that are the subject of interventions. They will generally find it easier to interpret monetary values for the purposes of making decisions about an intervention. Considering the incremental costs and benefits associated with alternative interventions (including: no intervention) can help to clarify the tradeoffs and implications associated with those interventions and help to set priorities.

Economic evaluations can be undertaken at the individual level (e.g. individual farmer), sectoral level (e.g. farming sector) or societal level (e.g. single country or the EU). The perspective taken determines which potential costs and benefits are included in an economic evaluation. Policy decisions related to public health and water are mostly taken from the perspective of society, since such interventions will have an impact on society as a whole. Nevertheless, further boundaries may have to be defined. For example, if an intervention in one country has cross-border impacts that benefit individuals in another country, then the geographical scope of the analysis will determine whether the benefit derived by the latter individuals is included in the economic analysis. It is important to note that in addition to looking at whether an intervention represents an efficient allocation of resources, it may be equally important for the welfare of people to consider how those resources are distributed in society. Costs and benefits may not be borne equally across society. As discussed later,

[1] If the outcome reduces welfare then this utility loss is measured by the minimum amount of money that the individual would require in compensation (willingness to accept – WTA) in order to offset the outcome.

distributional concerns can be accommodated in economic evaluation in a number of ways.

12.2.1 Cost/Benefit analysis

Cost/Benefit Analysis (CBA) provides a systematic assessment of the costs and benefits associated with an intervention. The benefits of an action are contrasted with the associated (opportunity) costs within a common analytical framework. Economic theory defines a benefit as a change that increases human well-being, whilst a cost is defined as a change that decreases human well-being. As described above, and for the purpose of comparison, these increases and decreases in well-being are measured using money as the common denominator. The net benefit of a change is given by the difference between the costs and benefits. Delayed benefits and costs are converted to their present day equivalents through a process called discounting. The change is said to be economically efficient if the present value of net benefits (NPV) is positive, or the ratio of total benefits to total costs (B/C ratio) is greater than one.

In general, the following steps are included in a CBA:

Step 1: Define the objective of the intervention.
Step 2: Define the baseline, that is, what would happen if no action is taken.
Step 3: Define the alternative options to achieve the objective.
Step 4: Quantify the investment costs of each option compared to the baseline.
Step 5: Identify and quantify the positive and negative welfare effects of each alternative option compared to the baseline.
Step 6: Value the welfare effects in monetary terms, using market prices and economic valuation methods.
Step 7: Calculate the present value of costs and benefits occurring at different points in time using an appropriate discount rate.
Step 8: Calculate the Net Present Value (NPV) or Benefit/Cost (B/C) ratio of each alternative option.
Step 9: Perform a sensitivity analysis.
Step 10: Select the most efficient intervention option.

Carrying out a CBA is a multi-disciplinary process, involving expertise from different fields and the input from policy and decision-makers. While economists are involved in all steps, environmental expertise of many kinds is also needed, especially in steps 2, 3 and 5.

Preferably all costs and benefits included in a CBA are quantified in monetary terms. In cases where there is no market for the resource under consideration and hence no market price to reflect its economic value, or where there are non-priced environmental or health effects to be considered, several economic valuation methods are available that allow placing a value on such non-marketed costs and benefits. These are discussed further below. It will hardly ever be possible, however, to monetise all impacts all of the time: those impacts that cannot be monetised are often left out of the analysis. Non-monetised impacts, if considered relevant, can nonetheless be included in a qualitative discussion accompanying the CBA results, as *pro memoriam* items in the balance sheet or provide the basis for a multi-criteria analysis (Brouwer & van Ek 2004).

With regards to the distributional issue of who gains and who loses, CBA accommodates such concerns in two main ways: either by portraying the distributional incidence of costs and benefits along with the size of those costs and benefits; or by attaching 'weights' to the benefits and costs accruing to particular groups. The weights implicit in a conventional CBA are those determined by the existing distribution of income and this means that the preferences of the rich may be weighted more heavily than the preferences of the poor. This can be corrected by modifying the implicit weights.

12.2.2 Cost-effectiveness analysis

Cost-effectiveness analysis – CEA (sometimes also known as least cost analysis) is used to identify the most cost-effective option for achieving a pre-set objective or criterion that is not measurable in monetary terms (e.g., some health outcomes). The relevant objective is set, options for achieving it are identified and the most cost-effective option is identified as that with the lowest present value of costs. Where the costs are related to an effect that differs in magnitude between alternative interventions, then the results can be stated in terms of net cost per unit of effect.

Cost-effectiveness analysis is suitable for use in situations where valid and reliable estimation of the benefits of alternative options is not feasible. Instead of attempting to identify and value the benefits, the most cost-effective means of achieving a desired objective is identified. Cost-effectiveness analysis is suited, for example, to situations where clear and defensible health goals exist which can be measured in terms of appropriate units. For example, health goals relating to mortality and morbidity effects of interventions are sometimes combined into single units such as QALYs (Quality Adjusted Live Years), DALYs (Disability Adjusted Live Years), HYEs (Health Years Equivalent), and other health indices. CEA can also be used to identify the most effective option

for a fixed amount of funding that has been allocated to achieve a policy objective. The drawback of cost-effectiveness analysis is that it does not identify the benefits of actions or the willingness of society to pay for improvements. For these reasons, CBA is, if practicable, the evaluation approach of choice.

The various steps distinguished in a CEA are described below:

Step 1: Define the objective involved.
Step 2: Determine the extent to which the objective is met.
Step 3: Identify sources of pollution and impacts now and in the future over the appropriate time horizon.
Step 4: Identify measures to bridge the gap between the reference (baseline) and target situation.
Step 5: Assess the effectiveness of these measures in reaching the objective.
Step 6: Assess the costs of these measures.
Step 7: Rank measures in terms of increasing unit costs.
Step 8: Assess the least cost way to reach the objective.

Once again, carrying out a CEA is a multi-disciplinary exercise. A number of approaches are used in practice at varying levels of complexity, scale, comprehensiveness and completeness for carrying out a CEA. These are discussed, for example, in Zhang & Folmer (1998). A distinction is made between bottom-up and top-down approaches. The bottom-up approach focuses on technological details of measures and their impact on individual enterprises (micro level), whereas top-down approaches usually consider the wider economic impacts of pollution abatement measures and strategies, often without detailed technical specification of the proposed measures (macro level).

Economic evaluation using CBA or CEA involves multiple assumptions and often produces uncertain results. Estimates of the costs and benefits associated with alternative interventions rely on data to the extent that they are available, relevant, and accurate, but also rely on judgments, values, assumptions, and extrapolations. When undertaking economic evaluation, the sources of uncertainty should be identified, characterized, and communicated clearly, for example by conducting a sensitivity analysis. In this respect, reporting on the uncertainties and conducting sensitivity analyses are important components of any evaluation and the presentation of benefits and costs of an intervention should not be expressed as though they are precise measures of actual economic costs and benefits.

It may also be necessary to consider intervention options that differ in the temporal pattern of benefits and costs, or that differ in their duration. It is then

necessary to adjust the streams of benefits and costs, using a rate of time preference (discount rate) to yield discounted present values.

We now consider in more detail the methods employed to estimate the value of the costs and benefits associated with changes in water quality and public health.

12.3 VALUING THE BENEFITS OF INTERVENTIONS

Regulatory interventions regarding waters contaminated by livestock waste set out to reduce the adverse impacts on water quality and public health. As discussed in Chapter 10, the adverse impacts of contaminated recreational waters consist mainly of human health concerns. This section uses the case of recreational water quality and public health as the context to determine the benefits of interventions aimed at improving the quality of water microbially contaminated by livestock waste.

12.3.1 Human health benefits

The benefits of reducing the human health consequences of degraded water quality as a result of contamination with livestock waste can be calculated in a step-by-step fashion (called a "damage function" approach), where the levels of contamination are associated with health effects and monetary values are associated with reducing risks of these health effects. Quantification of benefits thus requires the identification of well-defined, economically meaningful health effects associated with the contaminant; the change in health effect expected to result from the intervention that reduces exposure to the contaminant; as well as the change in incidence of the health effect in the exposed population. Finally, it is necessary to estimate the economic value of adverse health effects avoided, and multiply this unit value by their reduced incidence in the population to derive the monetised benefits.

The economic consequences of a case of the adverse health effects averted will include:

(1) health-care and medical costs – such as out-of-pocket medical expenses of the affected individual (or family), the opportunity costs of time spent in obtaining treatment, plus, for example, costs paid by the insurance. The individual may be unable to undertake some or all normal chores and thus require additional special care-giving and services not reflected in basic medical costs;
(2) work loss – this includes lost personal income, plus lost productivity (irrespective of whether the individual is compensated or not. Whilst some individuals may receive sick pay and hence not perceive any

income loss, this is nevertheless a cost to society and in this respect reflects lost productivity); and,

(3) other social and economic costs – these include lost opportunities to enjoy leisure activities, discomfort or inconvenience (pain and suffering), anxiety, concern and inconvenience to family members and others. In addition, individuals may engage in defensive and averting expenditures and activities associated with attempts to prevent the health impacts.

The health care costs, plus work loss (consequences 1 and 2), constitute the measure of welfare known as the Cost-of-Illness approach. This seeks to identify the real costs of illness in the form of lost productivity and output and the increase in resources devoted to health care (and hence measures the *ex-post* or realised damages rather than the *ex-ante* valuation of WTP at the moment choices are made). Its theoretical legitimacy rests on the assumption that national income is a valid measure of welfare. However, the COI approach can be misleading in that it fails to capture the variety of behavioural responses to illness and to the threat of illness. Furthermore, when evaluating health hazards that strike with some degree of randomness, so that no-one could predict exactly who will actually suffer from the associated risk or benefit from its prevention (as in the case of recreational water health hazards addressed through publicly financed health programmes), then the approach is not appropriate for assessing *ex-ante* policy decisions. More generally, since the COI approach does not include other social and economic costs it will not reflect the total welfare impact of a cleanup or management intervention.

Leaving aside for a moment the issue of how it is measured in practice, the maximum WTP to reduce all the adverse impacts on human health is a comprehensive measure of welfare. It reflects all the reasons for which an individual might want to avoid an adverse effect, including financial and non-financial concerns.

A few things should be noted in considering the basic model of valuation regarding human health. First, the welfare of an individual must be related to the health effect, either in terms of actual or perceived health, otherwise no economic benefit is derived. Second, the health endpoints associated with the intervention have to be weighted appropriately in terms of their incidence or probability in a given population, as well as in terms of a factor that reflects the impact or severity of the illness on the welfare of the individual and society. Whether this latter factor reflects the health related quality of life or monetary impact will determine whether the weighting is in terms of disease burden or economic value, respectively.

It may also be necessary to consider issues such as the role of time lags between exposure and changes in health status, the duration of illness, or multiple changes

in health status that occur concurrently or in a specific sequence. All of these issues can be of importance when undertaking economic benefits valuation.

12.3.2 Other benefits

In addition to dealing with public health concerns, regulatory interventions addressing the contamination of recreational and other waters by microbial pollution from livestock waste may have other benefits with an impact on societal well-being and hence have an economic value. These additional benefits may include, for example, increases in tourism and employment, farm production increases, ecological impacts, and other aesthetic, recreation, amenity, (shell)fish harvesting, drinking-water and non-use improvements.

Tourism expenditures by beach visitors (e.g. food, accommodation, shopping) and employment related to tourism are sometimes perceived as benefits since they may be important for the development of regional coastal economies. However, from a national perspective, they are likely to be transfers, that is, the activities would have taken place elsewhere in the country, and hence there is no net increase in economic activity across the country. Although they can legitimately be added to an economic impact analysis, they should not be included in a cost-benefit analysis unless they represent net economic gains (Loomis & Helfland 2001).

Interventions to reduce the adverse impacts on water quality and public health from contaminated livestock waste frequently have the potential to result in additional costs for farmers, but these may be countered by reductions in production costs related to animal health. For example, control of pathogens that, apart from a public health impact, also have an impact on animal health, can result in improvements in livestock growth and reproduction abilities.

Other benefits related to marine and wildlife ecology, aesthetics, recreation, amenity and non-use improvements can all be considered legitimate components of the Total Economic Value (TEV) of recreational and other water quality changes and hence should be included in assessments of the benefits of interventions targeting contamination from livestock waste.

12.3.3 Benefit valuation steps

The steps in the economic valuation of benefits are described below:

Step 1: Identification of the goods and services (including environment- or health-related aspects) provided by recreational and other waters amenable to robust valuation.

Step 2: Assessment of their provision (target) level, including quality attributes, compared to the baseline (reference) level of provision.
Step 3: Identification of the groups of people in society (users and non-users) who benefit from the goods and services involved or who suffer a loss when they are degraded.
Step 4: Identification of the possible values (use and non-use values) attributed to the goods and services involved by these groups in society.
Step 5: Selection of the appropriate economic valuation method(s).
Step 6: Estimation of the economic value of the change in provision level of the goods and services involved, accounting for substitution and income effects and other contextual factors.
Step7: Quantification of the "market size", that is, the total population of beneficiaries over which the economic value is aggregated, accounting for possible distance-decay effects (people living further away may attach less value to the goods and services involved).
Step 8: Estimation of the total economic value (TEV).

12.3.4 Economic valuation methods

The question remains how credible estimates of economic value, as represented by WTP, are derived in the context of goods such as recreational water quality, where there are either no apparent markets or very imperfect markets.

In such situations, various techniques can be used to estimate WTP measures of value. These can be grouped into two basic approaches.[2] "Stated preference" methods rely on data from structured survey questionnaires in which preferences are conveyed via individuals' responses to questions regarding hypothetical markets or choices. "Revealed preference" approaches infer values from individuals' market choices regarding goods which are related to the one being investigated, for example, by looking at expenditure on holidays as a reflection of preferences for higher quality recreational waters. Both revealed preference and stated preference approaches to valuation have been used to investigate changes in recreational water quality in practice.

The principal method under the "revealed preference" approach used to value recreational water quality changes has been the "travel cost" method (Bockstael & McConnell 2006). In this method, the costs incurred in reaching a recreational site are used as a proxy for the value of recreation. Expenses differ

[2] Further details of the underlying theory and application of the approaches and techniques is provided in texts including, Braden & Kolstad (1991), Freeman (2003), Bateman *et al.* (2002), Mitchell & Carson (1989), Champ, Boyle & Brown (2003), Kanninen (2007), Bockstael & McConnell (2006).

between sites (or for the same site over time) depending on the site characteristics, including water quality. Different approaches exist, with the zonal travel cost model as the most important. This approach is based on aggregate information of travel behaviour of people living in different zones and individual travel cost and random utility models based on individual household travel behaviour. The Travel Cost method can be data intensive, especially when it requires information about individual household travel behaviour and associated travel costs to the site of interest. It is furthermore important to point out that the method derives economic values for recreational water quality based on the assumption that water quality is an important determinant of individual travel behaviour. Advanced models use data about both travel behaviour and associated costs to different sites and their characteristics to predict changes in travel behaviour based on changes in site characteristics.

In the "stated preference" approach two main methods have been used: Contingent Valuation and Choice Modelling. The Contingent Valuation method (Mitchell & Carson 1989; Bateman *et al.* 2002) uses a questionnaire survey to ask individuals how much they would be willing to pay to have a single, specified change occur. So long as people are able to understand clearly the change being offered, and answer truthfully, this approach is ideal. It measures precisely what the analyst wants to know: the individual's strength of preference for the proposed change expressed through his/her financial commitment. Several practical difficulties arise with this approach, however, and a central issue is whether the intentions people indicate *ex ante* accurately describe their behaviour *ex post*, when people face no penalty or cost associated with a discrepancy between the two.

Choice modelling methods are most appropriate when a project or policy affects individual aspects of a resource (Bennett & Blamey 2001). Data collection is again via a survey consisting of a series of questions; for each, respondents have to make a choice between two or more options. By varying the options in these choices, analysts can see how respondents value the different characteristics (referred to as attributes) that define differing recreational water qualities and beach experiences. This permits valuation of marginal changes in those attributes (e.g. water quality, beach facilities).

Sometimes it is not necessary to initiate a new original valuation study. Existing valuation estimates from previous studies can be used to undertake so-called "benefits transfer". This approach transposes monetary values estimated at one site (study site) to another (policy site). The study site refers to the site where the original study took place, while the policy site is a new site where information is needed about the monetary value of similar benefits. The benefits transferred from the study site could have been measured using any

of the stated preference or revealed preference valuation approaches outlined above.

Benefits transfer is still in its infancy, in part because for many environmental policy issues only a limited number of high quality valuation studies have been completed. It has, however, the potential to become a significant and useful estimation approach. The most important reason for using previous research results in new policy contexts is that it saves a lot of time and resources. Applying previous research findings to similar decision situations is an attractive alternative to expensive and time-consuming original research to inform decision-making. Some previous valuation studies relating to recreational water quality that may be used for benefits transfer are discussed below.

12.3.5 Recreational water quality valuation studies

A considerable body of literature of an applied nature has evolved over the past two decades, relevant to the valuation of the benefits of interventions concerning recreational water quality. Most of the studies obtain value measures by utilising some form of travel cost/random utility model, contingent ranking exercise or some form of contingent valuation method survey. Table 12.1 summarises various studies that have been undertaken internationally on valuing recreational water quality improvements. Derived estimates have been converted to common values in pounds sterling (UK£) at 2006 prices. It is immediately clear that, although few of the studies relate directly to animal waste, the application of the various "revealed preference" and "stated preference" studies to recreational water quality more generally is well established.

Although all estimates are converted into common terms, the range of mean WTP estimates found is quite wide. This can be partly explained by differences in the valuation methods used as these yield theoretically different estimates (Bateman & Jones 2003), but is also a reflection of the variety of scales or "scope" of water quality changes being considered in different studies. Furthermore, there may also be differences across the studies in the range of benefits examined. For example, while some studies only consider health benefits, others also consider ecological, aesthetic, recreational and amenity improvements. While these differences in study design and remit naturally yield a range of value estimates, some consistent findings emerge, including, most clearly, that individuals hold significant and positive values for improvements in recreational water quality. These values imply substantial aggregate benefits across populations as a whole.

Table 12.1 Recreational water quality valuation studies and benefit estimates (UK£ 2006).

Study	Approach	Location	Stressor	Quality change	Value per visit	Value/year
Barton (1998)		Costa Rica	Sewage, FC	To swimmable	–	101.34–121.60
Bockstael *et al.* (1987)	RUM/TCM	Boston, USA	Oil, faecal coliforms, COD	10% improvement	0.52	10.47
Bocksteal *et al.* (1987)	RUM/TCM	Boston, USA	Turbidity, FC, COD	30%	1.37	33.10
Bocksteal *et al.* (1989)	CVM	Chesapeake Bay, USA	–	Unacceptable to acceptable	–	134.27
Brouwer & Bronda (2004)	CVM	Netherlands	Sewage, FC	Revised EUReduction in illness risk level from 10/100 to 5/100 swimmers	–	24.44
Day *et al.* (2001)	CVM	Ayr and Irvine, Scotland	Sewage	Current EU Mandatory – AyrCurrent EU Mandatory – Irvine	–	10.43–13.735.98–8.62
Choe *et al.* (1996)	CVM	Philippines	Sewage	To swimmable	–	10.13–20.27
Feenberg & Mills (1980)	RUM/TCM	Boston, USA	Oil, total bacteria, colour	10% improvement	–	3.91
Georgiou *et al.* (1998)[1]	CVM	East Anglia, UK		Current EU Mandatory – AchieveCurrent EU Mandatory – Maintain	–	16.2018.35

(*Continued*)

Table 12.1 (*Continued*).

Study	Approach	Location	Stressor	Quality change	Value per visit	Value/year
Georgiou *et al.* (2000)[1]	CVM	East Anglia, UK	Sewage	Current EU Mandatory → Revised EU	–	35.14
Hanley, Bell & Alvarez (2003)	CVM	South-West Scotland	Sewage	Current EU Mandatory	0.55	9.03
Le Goffe (1995)	CVM	France	Eutrophication, sewage	To swimmable/ shellfishable	–	36.90
Machado & Mourato (1999)	CVM/CR	Lisbon, Portugal	Sewage, FC, FS	Current EU mandatoryCurrent EU guideline	8.1514.05	40.90104.19
Mourato *et al.* (2003)	CE	United Kingdom	Sewage, FS	Revised EUGI Illness risk level ↓ by 1/100 swimmersAdvisory Note System (During Poor WQ Events)	—	1.226.21
McConnel & Ducci (1989)	CVM	Barbados	Sewage	–	–	11.82–159.88
Mantymaa (1999)	CVM	Finland	Sewage, nutrients	From swimmable to drinkable	–	33.78
McConnel & Ducci (1989)	CVM	Uruguay	Sewage	To swimmable level	–	15.63
Niklittschek & Leon (1996)	CVM	Chile	Sewage	To swimmable	–	89.51

Niklittschek & Leon (1996)	TCM	Chile	Sewage	To swimmable	–	118.22
Sandstrom (1997)	RUM/TCM	Sweden	Nutrients	50% improvement	16.89-38.84	–
Zylicz *et al.* (1995)	CVM	Poland	Eutrophication	To swimmable	–	15.20–59.96

Revealed Preference: RUM – Random Utility Model; TCM – Travel Cost Method;
Stated Preference: CVM – Contingent Valuation Method; CR – Contingent Ranking; CE = Choice Experiment
Original value estimates converted to £STG using annual average exchange rates (Bank of England figures) and adjusted by GDP deflators (UK Treasury figures) to give 2006 prices.
[1] Estimates based on revised figures for these studies reported in Georgiou (2003).

12.4 VALUING THE COST OF INTERVENTIONS

Turning to the opportunity costs of interventions to improve the quality of waters contaminated by livestock waste, these include the value of the goods and services lost by society resulting from the use of resources to comply with and implement such interventions and from associated reductions in output. These costs generally fall under five headings that must be included in social cost analyses (EPA, 2010):

(1) Real-resource compliance costs: these are the direct costs associated with purchasing, installing and operating new pollution control equipment; changing relevant production processes by using different inputs or different mixtures of inputs; and, capturing the polluting wastes and selling or re-using them.
(2) Government regulatory costs: these include the monitoring, administrative and enforcement costs associated with regulation.
(3) Social welfare losses: these are the losses in welfare associated with the rise in the price (or decreases in output) of goods and services that occur as a result of policy.
(4) Transitional costs: these include the value of resources that are displaced because of regulation-induced reductions in production and the private real resource costs of re-allocating those resources.
(5) Indirect costs: these other costs include the adverse effects policies may have on product quality, productivity, innovation and changes in markets indirectly affected by the policy.

The challenge in developing an estimate of the social costs of recreational water quality improvements is to consider the markets being affected by the policy, assess the available data and analytical methods and adopt an analytical approach that will yield an estimate suitable for use in CBA.

12.4.1 Measuring costs

There are three general approaches to measuring the costs of interventions, namely an engineering analysis approach, a cost survey approach and econometric estimations of costs (Fearne *et al.* 2004).

Under the engineering analysis approach the costs of an intervention are estimated for each step of the process involved in implementing the intervention. The approach may, for example, make use of technical details on livestock production in order to estimate the potential changes in the production system or management. If some new additional technical piece of equipment is required to reduce contamination by livestock waste, then the estimated

annuities for long term investments of this new equipment, as well as any additional annual variable costs, would be estimated. Engineering cost estimations have the advantage that they are transparent and usually easy to understand. However, the method does require that clearly defined interventions are planned for implementation.

The cost survey approach seeks to measure the costs of interventions through surveys of relevant stakeholders, such as companies or farmers. Although the approach considers how intervention measures have been implemented in practice, it nevertheless suffers from the fact that the quality of any analysis is only as good as the quality of the survey and its responses. In most developed countries, statistics are available for cost prices of, for example, farm inputs, as well as farm-to-gate prices for livestock products. However, data on potential changes of inputs and costs further up or down the livestock food chain may be scarce or not available at all. In addition, the approach can be time-consuming and not applicable where new intervention measures are being proposed, or where other information that is unavailable to survey respondents is required. Nevertheless, expert consultations are often the only way to get information on the potential costs related to interventions.

Finally, econometric estimates can be undertaken, being applied at either individual, sectoral, national or international level. This approach allows for the control of other important variables, as well as effects on trade and spill-overs to other sectors and markets. The approach is data-intensive and can be time-consuming, depending on the level of sophistication of the model. Another problem is that such datasets may not necessarily collect the specific efforts, costs and outcomes related to the particular improvements that are the subject of the intervention. This then requires that other sources of data or proxies be used to measure such variables, thus reducing their level of validity.

As was the case in organising and presenting measures of the benefits of recreational water quality improvements, it is also necessary when considering social costs to take account of the issues of discounting and uncertainty.

Case Study: The benefits and costs of recreational bathing water quality improvements in the Netherlands

The following case study concerns recreational water quality improvements in the Netherlands. In order to support policy and decision-making regarding the revision of the 1976 EU Bathing Water Directive (BWD) in 2003 and to establish new standards for bacteriological water contamination, the extent and cause of the recreational water quality problem was investigated and measures identified in order

to resolve expected future problems. The costs and effectiveness of these measures were estimated and the least cost way to achieve the new standards established. Given the uncertainties involved, these were accounted for explicitly in relation to the sources of bacteriological recreational water contamination, and the costs and effectiveness of identified measures. The socio-economic benefits of the new standards were assessed in a large-scale household contingent valuation survey commissioned by the Dutch National Water Authority under the Ministry of Traffic and Water. A subsequent cost/benefit analysis assessed the economic net benefits of the newly proposed standards. Full details can be found in Brouwer and Bronda (2005) and Brouwer and Deblois (2008).

Recreational bathing water quality generally consists of the following three main aspects: hygiene, transparency and toxicity as a result of algal blooms. The EU BWD focuses mainly on hygiene, that is, faecal contamination of bathing water originating from humans and animals. The 1976 Directive established nineteen parameters against the then prevailing background of knowledge and experience with water quality problems. In 2002, the European Commission (EC) proposed a reduction in the number of parameters from nineteen to two key microbiological parameters in the new Directive, complemented by visual inspection (algal bloom, oil) and pH measurement in fresh waters. Under the 1976 Directive, three microbiological parameters were monitored: Total Coliforms (TC), Faecal Coliforms (FC) and Faecal Streptococci (FS). The first two parameters (TC and FC) are in the same family of bacteria and have a legally non-binding 'guide value' and legally binding 'threshold value'. The third parameter (FS) only has a 'guide value' for recreational bathing water quality and is therefore measured only incidentally in the Netherlands.

The two indicators retained in the revised Directive are Intestinal Enterococci (IE) and *Escherichia coli* (EC). An assessment of monitoring results and trends led to the conclusion that microbiological faecal pollution is, in the vast majority of cases, the limiting factor for achieving good recreational bathing water quality. An essential prerequisite of the proposed parameter values is that the level of protection of European citizens is maintained. However, establishing scientifically sound and generally acceptable relationships between concentration levels of polluting substances in water and their impacts on humans, plants and animals is surrounded by many uncertainties as a result of a lack of fundamental scientific knowledge and empirical evidence. The proposed threshold values for the two microbiological indicators IE and EC in the revised BWD were based on available scientific evidence provided by only two epidemiological studies of the relationship between faecal pollution and health impacts in recreational waters. One had been conducted in the United Kingdom and the other in Germany. Based on these two studies the EC proposed a legally binding 'Good Quality' value and 'Excellent Quality' guide value for IE and EC concentrations in recreational bathing waters. The proposed standards were equivalent to a risk of 5 per cent (good quality) and 3 per cent

(excellent quality) for contracting gastro-enteritis (GE) and 2.5 per cent (good quality) and 1 per cent (excellent quality) for contracting acute febrile respiratory illness (AFRI). The 1976 BWD guideline values carry a recreational bathing risk of 5 per cent for GE and the obligatory standards a risk of about 12 to 15 per cent.

The proposed new recreational bathing water quality standards for EC and IE were expected to result in a substantial increase in the number of non-complying recreational bathing sites. Under the 1976 Directive, non-compliance was limited to less than 5 per cent of all six hundred officially monitored recreational bathing sites in the Netherlands. The revised standards were expected to result in non-compliance at more than 30 per cent of all recreational bathing sites. Most of these non-complying sites (95%) are inland waters, only a few are coastal recreational bathing locations. At all these sites, measures would have to be taken in order to comply with the new recreational bathing water quality standards. However, before identifying possible measures, the underlying sources of the observed bacteriological contamination at these sites had to be identified first.

In view of the limited time and financial resources available, it was impossible to investigate all non-complying recreational bathing sites in detail. Therefore, a stratified sample of 30 sites was selected from the expected non-complying 170 recreational bathing sites: 27 non-complying freshwater inland locations and 3 non-complying coastal recreational bathing sites. At each of these 30 sites, the potential sources of pollution were identified with the help of a previously developed geographic information system (GIS), which included geo-referenced information about the location of potential pollution sources and relevant pressures such as storm water overflow, marinas, effluent from WWTP and the direct discharge of manure into surface water. Furthermore, a questionnaire was sent to the water managers responsible for the water quality at the 30 recreational bathing locations, asking them to confirm which of the sources identified with the help of the GIS-model they considered responsible for recreational bathing water contamination. The results from this survey were compared with the findings from the GIS model. In those cases where the results did not correspond, follow-up telephone interviews were held with the responsible water managers to find out what really might be causing the problem at a specific site. In some cases, the outcome of these interviews was that a source, which had not been identified before, was added to the list based on the information provided by the water manager. In other cases, the assessment of sources of pollution by the water manager could be dismissed based on available factual data and information about the presence of potential sources.

The sources of bacteriological contamination identified at these 30 sites are presented in Table 12.2. Given the scientific uncertainties in establishing quantified causal relationships between pollution sources and water quality at the selected recreational bathing sites, an important starting point in the assessment is that each potential source or pathway is considered a factor of influence unless it can be proven not to be. From Table 12.2 the following six sources were identified at more

than 30 per cent of the investigated sites: wastewater from combined stormwater overflow (CSO), wastewater discharge from boats and marinas, bathers and pets at beaches, and bird colonies near recreational bathing locations. The last column in Table 12.2 shows the number of recreational bathing locations where specific sources of pollution can not be excluded as a determining factor, because it is not possible to prove that they are non-existent. For instance, untreated sewerage can be shown to be a determining factor at one third of all the locations investigated, and cannot be excluded as a determining factor at two-thirds of the locations.

Table 12.2 Results from the assessment of sources of pollution of bacteriological contamination at a random sample of bathing sites in the Netherlands.

Source of pollution	**# bathing locations where the source was proven to be a factor of influence**	**# bathing locations where the source could not be excluded as a factor of influence**
Discharge of untreated sewerage	1	2
CSO without measures	7	4
CSO with measures	0	3
Insufficiently treated discharge from WWTP	1	2
Manure from farm	0	1
Manure from agricultural land	0	2
Manure from cattle drinking at water side	0	2
Wastewater discharge at marinas	6	5
Wastewater discharge from recreational boats	9	3
Wastewater discharge from commercial ships	3	2
Pets in water or at bathing water location	6	8
Bathers	12	7
Bird colonies at or near bathing location	9	7
Large international rivers	1	0

Source: Brouwer & Bronda (2005).

Three potential sources identified with the help of the GIS model (discharges from food processing industries, slaughterhouses and non-functioning sanitary facilities at recreational bathing locations) could not be identified at any location. Illegal discharges of animal waste were also suspected but difficult to prove and therefore not mentioned by any of the water managers. They may nevertheless play an important role explaining why sites are contaminated, but the extent to which illegal discharges play a role is unknown.

In order to get an indication of the relative weighted contribution of the different sources to the overall recreational bathing water quality problem the identified sources of pollution were linked to the recreational bathing sites' percentile values with which they exceed the new BWQ standard. A source which contributes ten percent to a 95th percentile value twenty times higher than the standard is, for instance, considered relatively more important than a source which contributes 100 per cent to a 95th percentile value twice as a high as the standard. The weighted contribution of the various sources is presented in Figure 12.1.

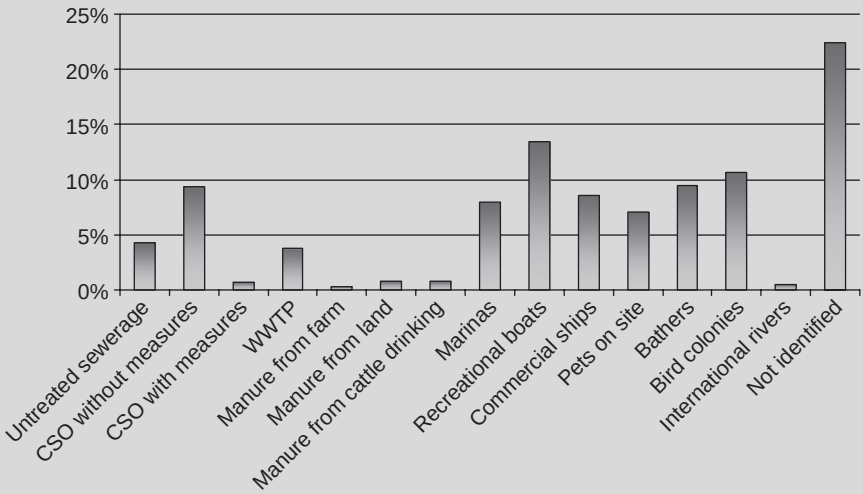


Figure 12.1 Estimated relative contribution of pollution sources to the overall problem of bacteriological bathing water contamination.
Source: Brouwer & Bronda (2005).

Important observations from Figure 12.1 are, first of all, the high percentage (22%) of non-identified sources of pollution. These include a mix of different pollution sources at or near large flowing surface waters, the exact origin of which is difficult or impossible to determine. Secondly, the large contribution of diffuse sources is remarkable, that is, wastewater discharge from recreational boats (13%), bathers

(10%) and bird colonies (11%). This makes it hard if not impossible to identify the exact causes underlying the observed recreational bathing water quality problem as the basis for the subsequent identification of remedial measures.

On the basis of the inventory of potential sources of pollution and their pathways, four different types of measures were identified:

- measures targeted at point sources;
- measures aimed at the elimination or relocation of discharges;
- measures targeted at the pathway of bacteriological contamination;
- instruments aimed at changing human behaviour.

Examples of the first type of measures are the upgrading of treatment of effluents from wastewater treatment plants (WWTP), such as enlargement of the existing treatment capacity and/or modifying disinfection techniques, and increasing overflow capacity or the use of individual wastewater treatment systems. Examples of the second type of measures include the connection of unconnected households and plants to the sewer system, the relocation of a WWTP or marina outside the perimeter of a recreational bathing site or the designation of non-grazing buffer zones for cattle along rivers. The third type of measures consists of, for example, hydrological isolation of recreational bathing water through the construction of a dam or refreshment of recreational bathing water at isolated sites with stagnant water. Examples of the fourth type of measures include information and education programmes or signs aimed at changing the behaviour of bathers, recreational boaters and people who walk their dogs near recreational bathing sites, or regulation such as the prohibition of the presence of pets and horses at beaches.

Compared to technical measures, the effectiveness of the latter type of social and economic instruments (e.g. awareness programmes) is less certain. Social instruments are aimed at changing often unpredictable or inconsistent human behaviour. Even with prohibition signs in place, people may still ignore them. Also the use of economic instruments such as fines or penalties faces uncertainty as to what exactly their effect will be as this depends upon public perception of the degree of enforcement, that is, the perceived probability of getting caught, and public attitudes and behaviour towards compliance with rules and laws.

Sets of measures were identified per recreational bathing location and the costs and effectiveness of these measures were estimated on the basis of an existing database. The cost and effect estimations were supplemented with expert judgement and in a few cases additional field research. Refreshment of recreational bathing water (circulation or suppletion[3]) and disinfection (UV and chloride) of suppleted water are the most frequently proposed measures at one third of all sites investigated,

[3] A process in which freshwater is added to reduce pollution concentration levels.

followed by the prohibition of pets and horses (proposed at one quarter of all recreational bathing locations), and the construction of wastewater disposal tanks for recreational boating at marinas. Putting up nets in order to create buffer zones around agricultural land situated along watercourses to prevent cows and sheep to get too close to the waterside (and hence to prevent that their excrements directly enter water courses) is the cheapest measure. The proposed measures mainly refer to recreational bathing sites with standing waters. Coming up with effective measures for sites with flowing waters is difficult as these measures often have to deal with a mix of diffuse sources of pollution. The potential for control at these sites is therefore much lower.

In those cases where sets of measures could be identified (75 per cent of the inland sites and 33 per cent of the coastal sites), the total investment costs amounted to two million euros (€) to reduce pollution for the inland recreational bathing water sites and €360,000 for the coastal recreational bathing water locations.[4] The corresponding annual operating costs were about €360,000 and €23,000, respectively.

The costs to reduce by one colony forming unit (cfu) per litre of water range from €0.8 to €8.4 per year for standing freshwaters and €0.2 to €8.8 per year for flowing freshwaters.[5] The unit costs for a single coastal location are €3.2 per year. Relating the investment and operating costs to the maximum number of bathers counted at the sites during the recreational bathing season in 2001, the annual costs per bather were also calculated. These costs vary from €1 to almost €4.000 per bather per year. On average, these costs are €221 per bather per year for standing recreational bathing water and €1051 per bather per year for flowing freshwater. In the case of coastal recreational bathing water, the estimated costs are €10 per bather per year.

In a final step, the estimated costs were scaled up to national level. This was done by multiplying the estimated costs for the inland freshwater recreational bathing locations with a factor 7, assuming that the 24 randomly selected inland recreational bathing sites are representative for all 167 non-complying recreational bathing sites in the Netherlands.[6] In the case of the coastal recreational bathing water sites, all non-complying sites were included in the analysis, even though no set of measures

[4] It was only possible to determine measures for one coastal bathing water location due to all the uncertainties involved regarding pollution sources. Although all the sites required measures, it was not possible to identify pollution sources (and hence to design adequate measures) for all the sites.

[5] Investment costs were translated into annual capital costs.

[6] The extent to which the selected sample sites are representative for the whole population of expected non-complying bathing water locations was also examined insofar as possible on the basis of available information. Important criteria were (1) the nature of the source(s) causing bathing water quality deterioration, (2) the number of bathers visiting the locations, (3) the physical characteristics of the bathing water location (i.e. current or standing water systems) and (4) the geographical location of the sample sites.

could be identified for two of the three sites and hence also no costs could be estimated. This results in a total estimated investment sum of €14.5 million and annual operating costs of €2.5 million.

Improving recreational bathing water quality is expected to have significant and substantial recreational benefits. The estimated number of people swimming at non-complying sites on a hot summer day is about 125,000. Most importantly, the health risks of recreational bathing in open waters are expected to be reduced by 50 per cent. In 2002, one in every ten bathers ran the risk of getting one or more of the following health symptoms when recreational bathing water quality standards were not met: infections to eyes, ears and throat and stomach upset (gastroenteritis) such as diarrhoea. Meeting the proposed new recreational bathing water quality standards means that the health risks of recreational bathing would be reduced to one in every twenty bathers. The above mentioned health risks are especially high when swimming, for example, during a hot day directly after heavy rainfall causing storm water overflow at or near recreational bathing locations (i.e. discharge of excess rainwater together with untreated sewerage) or when swimming in standing waters with increased algal blooms during hot weather periods.

Public perception and valuation of improved recreational bathing water based on the new proposed BWQ standards was assessed based on a large scale contingent valuation survey. In December 2002 a questionnaire was sent to 5,000 randomly selected households in the Netherlands. In the questionnaire, households were asked about:

- their recreational bathing behaviour (how often, where);
- their perception of recreational bathing water quality in the Netherlands (distinguishing between freshwater and coastal waters);
- whether they ever get ill after swimming in open water and whether they saw a doctor for this;
- whether they are aware of and informed about existing recreational bathing water quality standards;
- how they feel about being unable to swim in open water during the recreational bathing season;
- how urgent and important they believe improving recreational bathing water quality is;
- to what extent they are able to relate the information provided in the questionnaire about the new proposed recreational bathing water quality standards and the reduced health risks to themselves;
- whether they are willing to pay additional taxation in order to improve recreational bathing water quality in the Netherlands and hence reduce the health risks involved;
- their demographic and socio-economic background;
- their ability to answer the willingness to pay question based on the information provided.

More than 1,500 questionnaires were returned (response rate of 31 per cent). Based on the information provided about respondent demographic and socio-economic background (age, household size, education, income), it was concluded that the sample was representative for the whole of the Netherlands. Sixty percent of all respondents indicated that they swim in open waters in the Netherlands. A quarter of those who said they never swim in open waters gave water quality as their main reason. Fifteen percent do not like swimming or cannot swim, almost ten per cent only swim in public swimming pools, and another ten per cent said he or she is either too old or claims the water is too cold. More than half (55 per cent) of those who said they swim in open waters mentioned coastal locations as their most important recreational bathing site. On average over the past five years, respondents swim eight days per year. During the 2002 recreational bathing season, respondents indicated to have swum between six and ten days. Thirteen per cent of all respondents indicated to have suffered from symptoms of poor recreational bathing water quality such as eye, ear and throat infections and diarrhoea. Forty per cent of them went to see a doctor with these symptoms.

A remarkable finding is that people perceive coastal water quality and inland freshwater quality as being significantly different. The quality of coastal recreational bathing water is perceived higher than the quality of inland freshwater. The same applies when asking respondents how dangerous they believe swimming in coastal and freshwaters is for their health (i.e. health risks as a result of water quality, not drowning risks as a result of for instance currents or collisions with surfers or boats). Coastal waters are judged safer than inland freshwaters. A third of all respondents feel that they are being insufficiently informed about recreational bathing water quality. Half of all respondents feel they are sufficiently informed. Eighty-five per cent of all respondents said that they know that there exist standards for recreational bathing water quality in the Netherlands. A majority of sixty per cent of all respondents is willing to pay extra to improve recreational bathing water quality in the Netherlands and hence reduce the health risks involved. A quarter of all respondents would not mind if they are unable to swim and is also not willing to pay extra to improve recreational bathing water quality. Fifteen per cent have no opinion or are unsure whether they are willing to pay for improved BWQ. The most common reason why people were not willing to pay was that the polluter should pay, followed by reasons like ‘I never swim in open water’, ‘the current situation is good enough’ and ‘I don’t believe that the money will be spent on improving recreational bathing water quality’. The latter reasoning (mistrust that the money will be spent on what it is intended for) are indicative of what are usually called ‘protest bidders’ in the literature. A large amount of protest to the WTP question can seriously invalidate the research. Thorough pre-testing is an essential prerequisite to produce valid research results in this type of studies. In this study, a total of 138 protest bidders were detected, that is, 8 per cent of the total response. This is considered a reasonable result. Combined with the fact that a majority of 62

per cent indicates that they have no problem answering the WTP question and 75 per cent of all respondents claims that the information provided in the questionnaire is sufficient to answer the WTP question, this supports the validity of the survey.

Those who replied positively to the willingness to pay (WTP) question were subsequently asked whether they are willing to pay every year a specific amount of extra money in general taxation in order to improve recreational bathing water quality and hence reduce the health risks involved. Twelve different money amounts (referred to as 'bid levels' in the literature) were used in a dichotomous choice referendum format. These bid levels, ranging from €1 to €200 per year, were based on extensive pre-testing of the questionnaire and randomly allocated to the randomly selected households. It was furthermore emphasised in the questionnaire that this amount of money will be used exclusively to fund the additional costs of measures to improve recreational bathing water quality and reduce the health risks involved. As expected, the probability of saying 'yes' to a specific bid amount decreases as the bid level increases.

The estimated mean WTP is €35 per household per year (Table 12.3). A distinction can be made between mean WTP for people who bathe in open water in the Netherlands and people who do not bathe in open water, usually referred to as users and non-users. As expected, non-users are willing to pay, on average, less than users, but are still willing to pay a substantial amount of money (just over €20 per household per year).

Table 12.3 Mean WTP values for users and non-users (price level 2002).

Category	**Mean WTP (€/household/year)**
Whole sample population	35 (3.6)
Users (bathers)	41 (3.8)
Non-users (non-bathers)	22 (6.6)

Note: (standard error between brackets).

Aggregating the overall WTP estimate across the whole population that benefits from improved recreational bathing water quality (6.9 million households), this results in a total economic value of €242 million per year. Aggregating the user value (€41/household/year) across those in the population who actually bathe in open water in the Netherlands (users) (60% of the 6.9 million households in the Netherlands), we get a total economic value of €170 million per year.

If we compare the estimated least costs to achieve the new recreational bathing water standards (€3.3 million per year) with the estimated benefits in terms of public WTP for improved recreational bathing water quality and hence reduced health risks (€170 million per year), it is clear that the annual benefits exceed the estimated annual costs. Discounting the estimated costs over a period of twenty

years at the prescribed four per cent discount rate results in a total cost of approximately €50 million. Discounting the estimated benefits over the same time period at four per cent yields a total benefit of €2.4 billion, which is almost fifty times higher than the estimated costs. It was furthermore estimated that approximately 125,000 bathers are protected on a hot summer day at sites that cannot be expected to comply with the new proposed recreational bathing water standards. Based on these findings the conclusion is that it is economically efficient to improve recreational bathing water quality and reduce the health risks involved.

However, the pre-feasibility cost-benefit analysis carried out here is surrounded by a number of important uncertainties, requiring careful interpretation of the results. Perhaps the most important source of uncertainty is the reliability of the existing monitoring results and the extent to which non-complying recreational bathing sites face structural or incidental problems of bacteriological contamination. The monitoring data used as the basis for the assessment of future non-compliance of sites is based on two-weekly measurements at the more than 600 sites in the Netherlands. At each site one sample is taken every two weeks. Important factors, which may have caused non-compliance with the new standards, including weather conditions, are not taken into account. It is therefore impossible to assess the nature of non-compliance, that is, structural or incidental, as a result of, for instance, heavy rainfall and storm water overflow the night before the sample was taken.

Another important source of uncertainty is the complex diffuse nature of bacteriological contamination of recreational bathing water, especially flowing waters. The estimated least costs to achieve the new proposed recreational bathing water standards only refer to cost-effective measures that can be taken at about two-thirds of all the non-complying sites (mainly isolated standing waters). In a third of all cases, mainly flowing water systems, no effective set of measures could be identified due to (1) the diffuse nature of the sources of bacteriological contamination (either no source could be identified at all or a mix of diffuse sources were expected to be responsible for non-compliance) and (2) sources which are located outside the sphere of influence of the responsible water manager, such as bacteriological contamination from abroad. More in-depth research is needed to identify which sources exactly are underlying recreational bathing water quality problems and to what extent the problem is a structural and not merely an incidental one, in order to be able to identify adequate measures. Moreover, also the effect of algae and viruses on recreational bathing water quality was not considered in the study, nor was the cost-effectiveness of closing non-complying recreational bathing sites.

With respect to the option of bathing site closure, more research is needed regarding its effect on the number of swimmers visiting these sites (and the possibilities they have to visit other sites nearby) and the economic revenues lost in the associated recreation sector. In a non-published study conducted in 2002, it was estimated that the annual loss of income in retail and catering business and marinas at coastal recreational bathing sites in the Netherlands could add up to between €5 and €8 million if recreational bathing water standards would not be reached.

12.5 CONCLUSIONS

This purpose of this chapter has been to consider the economic evaluation of interventions concerned with the contamination of recreational and other waters by microbial pollution from livestock waste. Policy makers and regulators face a number of dilemmas in the regulation of livestock waste contamination of recreational and other waters. Regulators and governments have to balance the public desire for better environmental quality with the opportunity costs of any actions as well as considering the economic impact of policy changes on both those who have to pay the costs of interventions and those responsible for maintaining water quality.

Although the economics of reducing animal derived pathogens and faecal indicator organism loads has not been adequately researched and quantified directly, a parallel exists for recreational waters impacted by a mix of human and animal loads. This chapter has outlined some of the concepts and issues relevant to undertaking a systematic evaluation of costs and benefits, as well as reviewing some of the literature on the application of economic valuation methods to the issue of recreational bathing waters. Given the relative scarcity of resources any decision regarding allocation of resources to the management of livestock wastes implies foregoing something else. Economic analysis and evaluation is an important tool in helping policy makers understand the balance between society's desire for better environmental quality and human health with the costs of actions necessary to achieve this objective.

While health clearly is a 'right', economics establishes the incontrovertible fact that such rights come at a cost. Costs mean sacrificing scarce resources and this in turn implies that something else of benefit could have been secured. What matters is that the decisions about levels of expenditure on preventing and mitigating the effects of livestock contamination of water quality are optimally informed.

Economic analysis can support this process by indicating and providing insight into: the costs and benefits of an intervention; the potential for finding more cost-effective ways of securing a given objective; eliciting the ways in which society values risks so that risk, cost and benefit can be more transparently analysed.

The case study example demonstrates the use of such economic analysis to the case of bacteriological water contamination of recreational bathing waters in the Netherlands. The case study showed how costs and benefits can be estimated in order to help policy makers and regulators decide on whether expenditures on reducing the contamination of recreational waters represent effective and efficient use of resources. Although based on a number of assumptions with a significant degree of uncertainty attached to them, it shows that it is

economically efficient to improve recreational bathing water quality and reduce the health risks involved.

Summing up then, economic evaluation should be carried out whenever an intervention is being proposed in order to help policy-makers and stakeholders make an informed decision. Economists and health/environmental scientists need to work together in order to provide more complete economic evaluations. Economists require information on economically meaningful environmental and health effects in order to undertake their evaluations and hence must clearly convey their needs.

At the same time, economists and health/environmental scientists need to be aware of the limitations surrounding their disciplines. Economics merely supports the decision-making process, it does not substitute the decision-making process. The reason economics is relevant is that no society has the resources to pursue any social goal in absolute terms – whether it is health care, environment, crime prevention or education. All such goals entail the use of limited resources. Hence, of necessity, there must be 'trade-offs'. And, reduced to its bare essentials, economics is about the analysis of these trade-offs.

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