Waterborne Zoonosis –

Potential transmission of Diseases to
Livestock and Humans from a rural water supply

Report prepared as part of research collaboration between the University of Melbourne and Goulburn-Murray Rural Water Authority on Microbial Risk Assessment

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ABS	*Australian Bureau of Statistics*
CFU	*Colony-forming unit*
DHS	*Department of Human Services*
G-MW	*Goulburn-Murray Rural Water Authority*
HAV	*Hepatitis A virus*
HEV	*Hepatitis E virus*
PFU	*Plaque forming units*
spp	*Species*
UK	*United Kingdom*
USA	*United States of America*
WHO	*World Health Organization*

1. Executive Summary

This document reviews the environmental route of transmission of zoonotic disease (diseases that can be transmitted between (or are shared by) animals and humans) and the links connecting transmission of waterborne pathogens between wildlife, livestock, domestic animals and the human population. There is an emphasis on how contaminated water supplies in Australia may act as a link in waterborne zoonosis.

Zoonotic microbes (bacteria, viruses and parasites) that may be transmitted via a waterborne route are presented in Tables 1, 2 and 3. Table 5 presents data on microorganisms or their toxins that should not be excluded when considering water borne disease.

Bacterial zoonosis are discussed in Section 3.2 (page 10). This section covers the association of Aeromonas, Campylobacter, Escherichia, Salmonella, Shigella, Enterobacter, Klebsiella, Yersinia, Vibrio, Mycobacterium and Legionella in waterborne disease. Data is presented to show that while some of these bacteria are recognized major pathogens responsible for major disease outbreaks others have a less well recognized role in human disease.

Causes of viral illness are presented in section 3.3. *Norovirus* infection in adults and *Rotavirus* infection children are leading causes of gastroenteritis worldwide. Both can be transmitted through contaminated water. Astroviruses, Adenviruses and Enteroviruses can persist in the environment for extended periods and have all been detected in faecally contaminated water.

Waterborne parasites (section 3.4) include a diverse array of organisms with complex lifecycles. *Giardia* and *Cryptosporidium* have both been associated with major waterborne disease outbreaks and are commonly found in Australia.

2. Background

This literature review was prepared to fulfill Task 2 of the research agreement between Goulburn-Murray Rural Water Authority and The University of Melbourne.

3. Literature Review

3.1 Introduction

Water is required for many agricultural operations, from livestock watering and dairy shed cleaning, to crop irrigation, and even as an ingredient in food processing. Poor quality water may lead to a number of negative impacts including nuisance factors such as plugging of distribution pipes and reduced efficiency in cleaning operations, to reduced farm profits from animal deaths or transmission of plant pathogens.

Outbreaks of diseases caused by infectious and toxigenic bacteria, parasites and viruses can be directly linked to contact of agricultural products with contaminated water. Sources of pre-harvest contamination of produce include; manure, manure compost, sewage sludge, irrigation water, runoff water from livestock operations, and wild and domestic animals. Microorganisms are easily dispersed and have diverse characteristics that allow them to not only tolerate but survive in a variety of extreme environments.

The presence of waterborne enteric pathogens (bacteria, viruses, and protozoa) in rural and domestic water supplies represents a potentially significant human health risk. Even though major outbreaks of waterborne disease are comparatively rare, there is substantial evidence that human enteric pathogens, that are frequently present in rural and domestic water supplies, are responsible for low-level incidence of waterborne microbial disease. Although these diseases are often mild in healthy adults, enteric pathogens can cause severe illness, even death, for young children, the elderly, or those with compromised immune systems such as people taking steroids or undergoing cancer treatment. There is a growing global public health concern about new and reemerging infectious diseases particularly those associated with food and water. Some new microbial pathogens have recently emerged, while others no longer thought a problem have re-emerged.

Access to safe water can also be jeopardized by a natural disaster. Diarrheal disease outbreaks can occur after drinking water has been contaminated and have been reported after flooding. An outbreak of diarrheal disease after flooding in Bangladesh in 2004 involved more than 17,000 cases; *Vibrio cholerae* and enterotoxigenic *Escherichia coli* among the pathogens isolated. A large cholera epidemic in West Bengal in 1998 was attributed to preceding

floods, and floods in Mozambique in January–March 2000 led to an increase in the incidence of diarrhea.

3.1.1 Zoonotic disease

Zoonotic diseases are described as those diseases that can be transmitted between (or are shared by) animals and humans. The World Health Organization (WHO) defines Zoonoses (Zoonosis, sing.) as "Those diseases and infections which are naturally transmitted between vertebrate animals and man" (Anonymous, 2006). The impact of zoonoses can be considerable with illness, monetary loss, adverse effect on morale of personnel, unfavorable publicity and legal implications, all possible outcomes of zoonotic disease.

World wide data indicates that about 75% of emerging infectious disease is caused by zoonotic pathogens and some research studies report that about 80% of zoonotic disease could be waterborne (Bolin *et al.*, 2004; Reilly and Browning, 2004).

Zoonotic disease is generally spread via faeces, urine, saliva, blood and milk by means of:

- an incidental host - not required for the perpetuation of the organism,
- link host - bridges the gap between the maintenance host and man, or
- amplifier host - increases the number of the infective agents (viruses and bacteria) to which man may be exposed.

Water can act as a host for disease in a number of ways and disease can be considered to spread through following mechanisms, (also see Appendix 3).

1. Disease spreads because water acts as a carrier for a pathogen e.g. cholera.
2. Disease is spread by vectors that live in or close to water e.g. malaria.
3. Disease is caused by contact with infectious agent that have an essential part of their lifecycle in water e.g. schistosomiasis.

3.1.2 Waterborne disease

While 100% of the population in Australia is considered to be provided with some form of improved water supply, the WHO estimates that world-wide this figure is only 82% (4.9 billion) (WHO, 2000). The usage of water with poor microbiological quality increases the risk of human illness and plays a significant role in the transmission of infectious disease. Consumption of contaminated water is considered to contribute significantly to death from infection and the WHO estimates over 2 million people die each year from infectious disease caused by drinking contaminated water.

A wide variety of microorganisms are known to cause waterborne disease, however the likelihood of any organism to cause disease is dependant upon a number of factors (also see Appendix 4). These include the ability to stay viable for extended periods in water, the total number of cells required to cause infection (in parasites this is often only one cell but bacteria may

require 10-1000 cells to establish an infection) and the ability to find a susceptible host (Leclerc *et al.*, 2002).

The different classes of pathogens do not equally share all of these factors. Viruses for example cannot grow in receiving waters but the infective dose is low. Bacteria have strict requirements for nutrients and generally prefer a warm temperature but given optimum conditions they can proliferate rapidly in stagnant water. Many bacteria also require a specific host to establish infection. Parasites like *Giardia* and *Cryptosporidium* are highly resistant in the aquatic environments and like viruses they cannot multiply outside a host. However, only a few cells can cause disease and it is for this reason that they are most commonly associated with major waterborne disease outbreaks.

3.1.3 Zoonoses and water related illness

Indirect zoonoses require passage of an infectious agent through a vector or vehicle such as contaminated water. In Figure 1, taken from Slifko (2000), a simplified version of the intricate nature of the connection between water, food and human health is demonstrated but probably underplays the role of animal faeces on human health. Both animal and human faeces act as a significant part of the vehicle for transmitting disease.

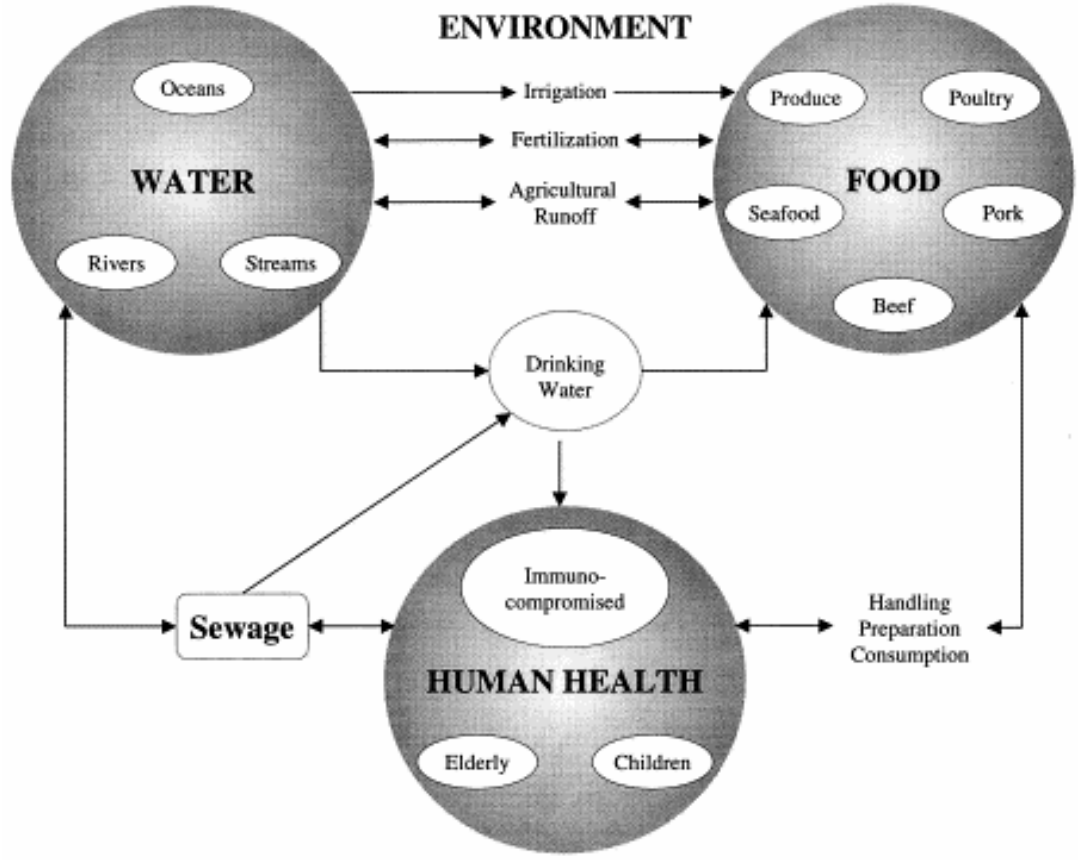

Figure 1. Food-water connection between human health and the environment (Slifko et al., 2000)

3.1.4 Food processing practices produce handling.

Consumer awareness of fresh fruits and vegetables as a healthy choice in a balanced diet has meant that the fresh fruit and vegetable industry seek to supply consumers with a wide range of high quality produce year-round. However, changes in agricultural practices, including harvesting, distribution and processing have contributed in recent times to outbreaks of human illness associated with the consumption of raw vegetables and fruit and products made from them that have not had a heat treatment step.

Vegetables and fruits are generally colonized by a wide variety of microorganisms, such as bacteria, yeasts and fungi that cause spoilage. While human pathogenic bacteria were thought to be primarily associated with animal products, it is increasingly recognized that human pathogens, may be associated with fresh produce. Contamination of produce can occur in the field or orchard, during harvesting, post harvest handling, processing, shipping, or in the home. Contact with mammals, birds, and insects offer yet another avenue through which pathogens can contaminate produce. Pathogens such as *Listeria, Clostridium* and *Bacillus* are naturally present in soils and water and their presence on fresh produce is expected. *Salmonella* sp., *E. coli*, *Campylobacter* sp., parasites and viruses are more likely to contaminate fresh produce through contamination by raw or improperly composted manure, irrigation water or contaminated wash water.

3.2 Waterborne pathogens and causes of zoonosis - Bacterial

Microorganisms play an important role in the biosphere, such as cycling of carbon and other elements. They have an essential role in fixation of atmospheric nitrogen, denitrification, sulfate reduction, methane production, and degradation of vegetable matter. Bacteria are the most diverse living beings on earth and only a fraction of them has been identified. In surface water, the prevalent residents are autotrophic bacteria, those that use carbon dioxide as the principal source of carbon and heterotrophic bacteria, that is, those that use pre-formed molecules as the principal carbon source.

Assessment of water quality currently relies heavily on routine bacteriological testing (Wohlsen *et al.*, 2006). Routine tests often measure total coliforms, faecal coliforms and *E. coli*. (Coliforms are a broad class of bacteria found in our environment and in particular the faeces of mammals.) *Escherichia coli* and other groups of coliforms may be present where there has been faecal contamination originating from warm-blooded animals (Chao, 2006). Bacterial genera in the coliform group include *Escherichia, Enterobacter, Klebsiella,* and *Citrobacter*. The presence of coliform bacteria in drinking water does not necessarily prove the presence of harmful, disease-causing organisms so specifics tests for *E. coli* help indicate if the coliforms might be from a faecal source. However, the absence of these bacteria in water does not necessarily guarantee the absence of pathogens (Krewski *et al.*, 2004). For example an analysis of several reservoirs and creeks in Australia has demonstrated no relationship between indicator bacteria and the detection of Cryptosporidium and/or Giardia (Thurman *et al.*, 1998).

Many bacterial pathogens, e.g. *Samonella*, *Shigella* and *Campylobacter*, are considered to be of faecal origin, hence the current reliance on 'indicator tests' to indicate if a water supply is contaminated with human waste. However other bacterial pathogens are endemic in water supplies. These include *Aeromonas*, *Vibrio* and *Legionella*.

Bacterial pathogens considered most likely to be associated with disease when water is the vector are discussed in sections 3.2.1 and those considered less likely but possible pathogens are considered in section 3.2.2. All bacteria known to be transmitted by a waterborne route are shown in Table 1.

3.2.1 Principal bacterial causes of infection transmissible by a waterborne route in the Australian environment.

3.2.1.1 Campylobacteraceae

Campylobacter is the most frequently identified cause of bacterial diarrhea in industrialized countries. Campylobacters are classified under *Campylobacteraceae*, a bacterial family comprised of genera *Campylobacter*, *Arcobacter* and *Sulfurospirillum* (Vandamme, 2000).

Among the 16 species and six subspecies of *Campylobacter*, two are most commonly isolated from stool samples of human gastroenteritis (Vandamme, 2000). They are *Campylobacter jejuni* subspecies *jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*). *C. jejuni* accounts for approximately 95% of *Campylobacter* caused human gastroenteritis, and *C. coli* is responsible for approximately 3-4% of the human illness.

Table 1. Bacteria that may be transmitted by a waterborne route

Bacterial Family	*Bacterial Genus*	*Bacterial strains (examples)*
Aeromonadacae	*Aeromonas*	*A. hydrophila*
Campylobacteraceae	*Campylobacter*	*Campylobacter* spp.
Enterobactereriaceae	*Escherichia*	*E. coli*
Enterobactereriaceae	*Salmonella*	*S. enterica*
Enterobactereriaceae	*Shigella*	*Shigella spp.*
Enterobactereriaceae	*Enterobacter*	
Enterobactereriaceae	*Klebsiella*	
Enterobactereriaceae	*Yersinia*	
Vibrionaceae	*Vibrio*	*V. cholerae*
Mycobacteriaceae	*Mycobacterium*	*M. bovis* *M. avium-intracellulare* complex
Legionellaceae	*Legionella*	

Other species causing human gastroenteritis include *C. lari*, *C. upsaliensis*, *C. fetus* subsp. *fetus*, and subsp. *venerealis*, *C. hyointestinalis* subsp. *hyointestinalis*, *C. concisus*, *C. jejuni* subsp. *doylei*. For the purpose of this document, *C. jejuni* and *C. coli* are collectively referred to as *Campylobacter* in the following text. Although usually a food borne illness, water is now regarded as an important route of transmission of *Campylobacter*. Waterborne disease outbreaks have been reported in several Scandinavian countries, UK, USA and Canada (Auld *et al.*, 2004; Kassa *et al.*, 2004; Schuster *et al.*, 2005).

Campylobacter infection world-wide displays a marked seasonality, peaking in late spring and early summer. This may be due to changes in the shedding/colonisation of *C. jejuni* by the natural hosts and changes in human behaviour such as swimming and seasonal farm animal contact

Figure 2 shows notification rate per 100,000 of the population in both Australia (excluding New South Wales) and Victoria. Campylobacter is not reported for NSW because it is only notifiable as foodborne disease or gastroenteritis in an institution. Data is for 2005 and shows highest incidence during summer months. Around 5000 cases of campylobacter enteritis are reported annually in Victoria (Anonymous, 2004). This figure grossly underestimates the actual incidence of the illness as most adults would not report the illness or have it investigated by a doctor. *Campylobacter* cells are mostly slender, spirally curved rods, with a single polar flagellum at one or both ends of the cell, and typically motile with a characteristic rapid darting corkscrew-like mobility

(Smibert, 1984; Vandamme, 2000). They are Gram-negative and non-spore forming bacteria. Their cells are 0.2-0.8 *μ*m wide and 0.5 to 5 *μ*m long.

Campylobacters are normal intestinal flora of young cattle, sheep, goats, dogs, rabbits, monkeys, cats, chickens, turkeys, ducks, seagulls, pigeons, blackbirds, starlings and sparrows (Smibert, 1984), pigs (Nielsen et al., 1997), and in blood and faecal material from humans with *Campylobacter* enteritis. Healthy puppies and kittens, rodents, beetles and houseflies may also carry campylobacters (Hartnett et al., 2002). *C. jejuni* is predominantly associated with poultry and *C. coli* is found predominantly in pigs.

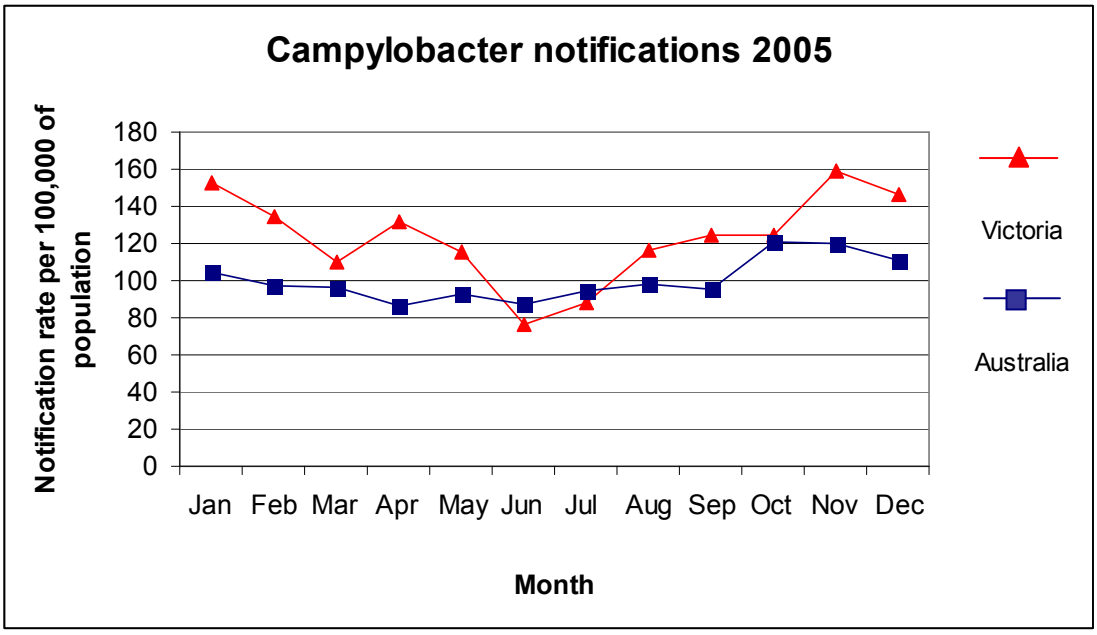

Figure 2 Notification rate per 100,000 of the population in both Australia (excluding New South Wales) and Victoria.

Campylobacters are highly sensitive to desiccation and do not survive well on dry surfaces (Fernandez, 1985). *Campylobacter* appear to be well adapted to the avian gastrointestinal tract and for this reason are most commonly found in birds. Animals that harbor these bacteria include cattle and poultry such as chickens and ducks. Nearly all surface water is said to contain species of *Campylobacter*, even remote areas, where contamination is probably from birds (Leclerc *et al.*, 2002).

Due to their sensitive nature to environmental conditions and inability of growth at temperatures below 30°C and under air, the ability of campylobacters to multiply outside of an animal host is severely restricted. Milk and water are the most common vehicles associated with transmission of *Campylobacter.* Raw (unpasteurised) milk is largely responsible for dairy-related transmission. Surveys in other developed economies, including the United Kingdom, Sweden, Germany, New Zealand, Denmark, US and Norway, indicate milk is the most frequent cause of foodborne *Campylobacter* infection (Friedman et al., 2000). Published information suggests that among the major routes of *Campylobacter* transmission to humans are bathing or swimming in a *Campylobacter* spp. contaminated lake or pool and direct contact with infected farm animals, such as cattle, sheep, chicken, etc. These

possible routes of transmission of *Campylobacter* spp. are summarised in Figure 3.

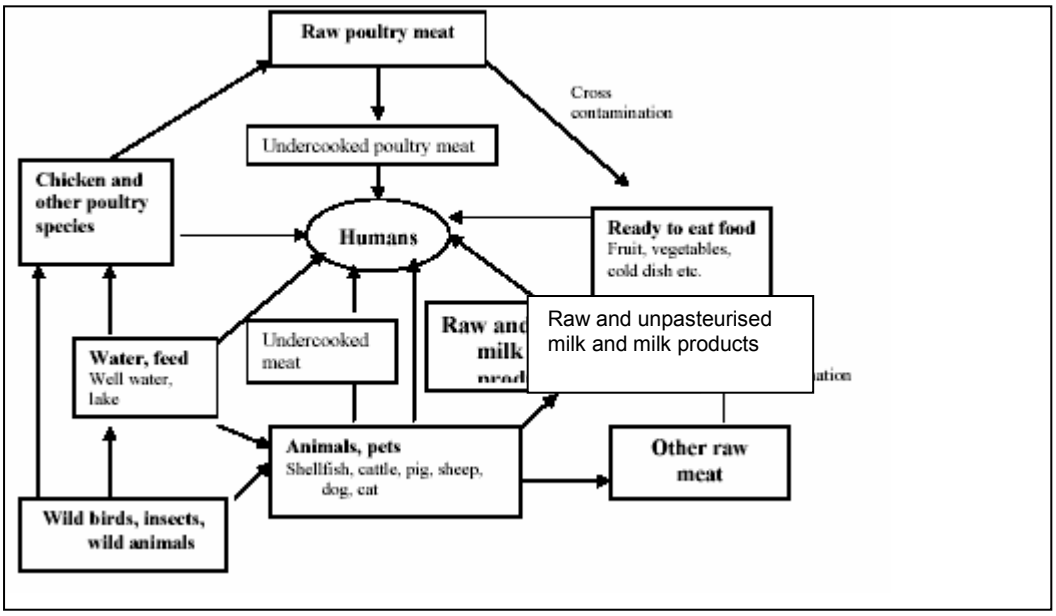

Figure 3. Transmission routes of Campylobacter species. Adopted from Friedmann et al., (2000) and "Campylobacter" data sheet of Ministry of Health, New Zealand (May 2001).

In any case of the mentioned routes of transmission, *Campylobacter* infection is a result of oral ingestion of *Campylobacter* through food or water or animals, and faecal contamination is the common source of campylobacter transmission.

3.2.1.2 Salmonella

Salmonellae are associated with the gastrointestinal tracts of humans, cattle, sheep, pigs, poultry, wild birds, domestic pets, rodents and snakes (Lightfoot, 2004; Schuster *et al.*, 2005). Animal reservoirs, including cattle, pigs and poultry, are often asymptomatic carriers and may show no or little sign of disease and most species of animals and birds have been found to have had the organisms at one time or another. Salmonella are transmitted by the faecal-oral route. Sources of transmission include person-to-person, food-borne, waterborne (drinking water and direct contact with faecally contaminated water) and direct contact with infected animals.

The genus *Salmonella* consists of two species; *S. enterica* and *S. bongori* but within the genus there are over 2500 serotypes and these are often named after the place they were first isolated e.g. *Salmonella* Newport.

The presence of these organisms in waterways is commonly due to faecal contamination. This can be exacerbated by the use of animal excreta on farmland (muckspreading) or intensive farming where there is run-off from dairy farms and feed lots after long periods of wet weather. *Salmonella* belong to a family of bacteria, the Enterobacteriaceae which live in the gut of animals.

Salmonella are a problem because they cause salmonellosis. Salmonellosis is a leading cause of enteric illness, with symptoms ranging from mild

gastroenteritis to systemic illness such as septicaemia and other longer-term conditions. The most common form of the illness is gastroenteritis which occurs usually 12-36 hours after ingestion of the infected food. This illness, which although very unpleasant at the time, is mostly short-lived and recovery occurs in 1-2 days, but more serious problems can occur such as enteric fever and meningitis which may be fatal. Infants and elderly have a much higher incidence of salmonellosis and *Salmonella* has emerged as an important pathogen in patients with AIDS. The number of notifications of Salmonellosis, in Australia, in the period of 1991 to 2006 is shown in Figure 4

Of recent concern worldwide is the emergence of multiple antibiotic resistant strains of *Salmonella*, an example being *S*. Typhimurium definitive phage type 104 (DT104). Multiresistant *S*. Typhimurium DT104 is a significant human and animal pathogen, with high morbidity observed in cattle and poultry (Crerar *et al*., 1999). To date, this organism is not endemic in Australia, although it is a significant health problem in European countries, North America, the Middle East, South Africa and South-East Asia (Jay *et al*., 2003). *S*. Typhimurium DT104 constitutes 8–9% of human *Salmonella* isolates in the USA. Sporadic human cases are reported in Australia, although these are commonly acquired overseas (Blumer *et al*., 2003).

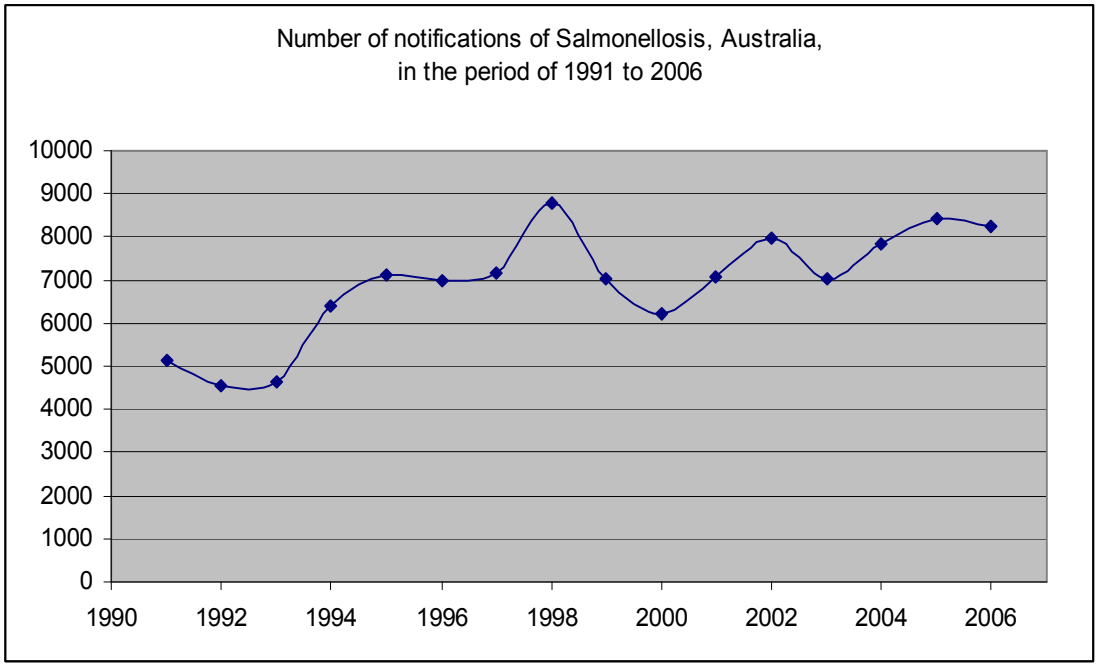

Figure 4. The number of notifications of Salmonellosis, in Australia, in the period of 1991 to 2006

Studies of contamination of lettuce by *E. coli* 0157: H7 and *Salmonella* Typhimurium have shown that both these bacteria can colonise the internal part of lettuce leaves, and it is unlikely that washing by the consumer would remove the bacteria, prior to consumption (Franz *et al*., 2007). *Salmonella* sp. have also been associated with alfalfa sprouts, clover sprouts and bean sprouts (Barak et al. 2002). Alfalfa sprouts and other seed sprouts have been implicated in numerous outbreaks of salmonellosis and the source of these epidemics appears to have been low-level contamination of seeds by

Salmonella bacteria that developed into clinically significant populations during the seed germination process (Howard *et al*., 2003). This demonstrates the risk of using partially treated sewage as irrigation water as some studies have shown that even after effluent treatment *Salmonella* can still be detected in discharge water in downstream samples (Gopo and Chingobe, 1995)

3.2.1.3 Pathogenic *Escherichia coli*

E. coli are members of the family Enterobacteriaceae and are a common part of the normal intestinal flora of humans and other warm-blooded animals. The organisms are described as gram-negative, facultatively anaerobic rod shaped bacteria (Desmarchelier and Fegan, 2003). Although most strains of *E. coli* are considered harmless, the species does contain certain strains that can cause severe illness in humans (Bell and Kyriakides, 1998). Strains of *E. coli* are differentiated serologically, based on O (somatic) and H (flagella) antigens (Lake et al., 2003).

Human pathogenic *E. coli* are characterised into specific groups based on virulence properties, mechanisms of pathogenicity and clinical syndromes (Doyle et al., 1997). These groups include;

- enteropathogenic *E. coli* (EPEC),
- enterotoxigenic *E. coli* (ETEC),
- enteroinvasive *E. coli* (EIEC),
- enteroaggregative *E. coli* (EAEC) and
- enterohaemorrhagic *E. coli* (EHEC).

EPEC causes illness primarily in infants and young children in developing countries. Symptoms include watery diarrhoea, with fever, vomiting and abdominal pain. The diarrhoea is usually self-limiting and of short duration, but can become chronic (more than 14 days). EPEC is also recognised as a food and water-borne pathogen of adults, where it causes severe watery diarrhoea (with mucus, but no blood) along with nausea, vomiting, abdominal cramps, fever, headache and chills. Duration of illness is typically less than three days (Doyle and Padhye, 1989; Dalton et al., 2004).

ETEC is another major cause of diarrhoea in infants and children in developing countries, as well as being recognised as the main cause of ‘travellers diarrhoea’ (Doyle and Padhye, 1989). Symptoms include watery diarrhoea, low-grade fever, abdominal cramps, malaise and nausea. In severe cases, the illness resembles cholera, with severe ‘rice-water’ diarrhoea and associated dehydration. Duration of illness is from three to 21 days (Doyle and Padhye, 1989).

EIEC cause a dysenteric illness similar to shigellosis. Along with profuse diarrhoea, symptoms include chills, fever, headache, muscle pain and abdominal cramps. Onset of symptoms is usually rapid (<24 hours), and may last several weeks (Doyle and Padhye, 1989).

Many synonyms are used to describe EHEC, including Shiga toxin-producing *E. coli* (STEC), Shiga-like toxin-producing *E. coli* (SLTEC), and verocytotoxin-producing *E. coli* (VTEC). *E. coli* O157:H7 is the best known and most widely studied serotype of *E. coli.* One of its natural habitats is the intestines of

cattle, which creates the potential for contamination of milk and dairy products.

EHEC infection normally results in diarrhoea like symptoms. Haemorrhagic colitis, an acute illness caused by EHEC organisms, is characterised by severe abdominal pain and diarrhoea. This diarrhoea is initially watery but becomes grossly bloody. Symptoms such as vomiting and low-grade fever may be experienced. The illness is usually self-limiting and lasts for an average of 8 days. The duration of the excretion of EHEC is about one week or less in adults, but it can be longer in children (ICMSF, 1996). Complications resulting from EHEC infections vary. About 5 per cent of haemorrhagic colitis victims may develop haemolytic uremic syndrome (HUS) (European Commission, 2000). This involves the rupture of red blood cells (haemolysis), subsequent anaemia, low platelet count and kidney failure. The case-fatality rate of HUS has been reported to be as high as 8% in food borne outbreaks (Rangel *et al*., 2005). Shiga toxins produced by EHEC attack the lining of the blood vessels throughout the body, predominantly affecting the kidney. However other organs such as the brain, pancreas, gut, liver and heart are also affected and may result in further complications such as thrombotic thrombocytopenic purpura. Pathogenic *E. coli* are transmitted by the faecal-oral route. Sources of transmission include person-to-person, food-borne, waterborne (drinking water and direct contact with faecally contaminated water) and direct contact with infected animals.

ETEC stains are a major cause of diarrhoea in infants and young children in developing countries, particularly in the tropics, and are a leading cause of travellers' diarrhoea (Gross and Rowe, 1985; Doyle and Padhye, 1989; Nataro and Levine, 1994). Although uncommon, a number of food-borne outbreaks due to ETEC have occurred internationally. Mead *et al.* (1999) estimated that ETEC infection is responsible for approximately 0.4% of food-borne illnesses in the US. In 1983 a multi-state ETEC outbreak occurred in the US that was associated with consumption of imported Brie and Camembert cheese (Anon, 1984; MacDonald et al., 1985). More recently, contaminated parsley was implicated in two ETEC outbreaks in Minnesota, USA during 1998 (Naimi et al., 2003). The source of the contamination was believed to be inadequately chlorinated wash water used on-farm.

EPEC stains have caused infantile diarrhoea in hospitals and nurseries in the United Kingdom and the United States (Robins-Brown, 1987; Nataro and Levine, 1994). In developing countries, EPEC stains are still responsible for a high incidence of sporadic infant diarrhoea. Limited information is available on food-borne outbreaks associated with EPEC. Outbreaks associated with consumption of contaminated cold pork and meat pies have been reported in Britain (Doyle and Padhye, 1989).

Since its identification as a human pathogen in 1982, and implication in a number of outbreaks in the United States, *E. coli* O157:H7 has become identified as the most predominant cause of EHEC related disease (WHO/FAO, 2002). It is estimated that 85% of EHEC infections in the United States are food-borne (Mead et al., 1999).

In the United States, consumption of undercooked hamburger meat has been an important cause of EHEC outbreaks (Nataro and Kaper, 1998). A large

multi-state *E. coli* O157:H7 outbreak involving consumption of contaminated hamburgers occurred in December 1992 – January 1993 with 732 cases identified, of which 195 were hospitalised and 4 died (Nataro and Kaper 1998). Food-borne outbreaks of *E. coli* O157:H7 have also been associated with consumption of contaminated fresh produce. In the United States, outbreaks occurred in 1995 and 1996 (70 and 49 cases respectively), which were traced to consumption of lettuce (Tauxe, 1997). Studies have shown that *E. coli* O157:H7 can be transmitted to lettuce plant tissue from soil contaminated with manure and contaminated irrigation water (Solomon et al., 2002). Another large *E. coli* O157:H7 outbreak occurred in the US in 1996 which was linked to apple juice. Although the low pH of fruit juices will generally not allow the survival and growth of many Enterobacteriaceae, some strains of *E. coli* O157:H7 may survive due to their high acid tolerance. In 2002, an outbreak of E. coli 0157:H7 in Canada was attributed to the consumption of un-pasteurised Gouda cheese (Honish et al, 2005). Irrigation water remains a prime suspect in the outbreak linked to use spinach grown in the Salinas Valley and nearby St Clara County, California. Surface water has bacteria levels that make them inappropriate for irrigation of crops destined for the dining table and in California, in this 2006 outbreak, surface water may have been used to irrigate the spinach crop. A number of measures have been suggested to prevent contamination of the water including fencing creeks and changing feeding and watering patterns, so animals are kept further away from creeks. This would provide a natural barrier to prevent manure from getting close to the water.

Over 200 non-O157 STEC serotypes have been isolated from humans, with the WHO identifying O26, O103, O111 and O145 as the most important food-borne non-O157 serogroups worldwide (WHO, 1998). STEC has been a notifiable disease in most Australia States and Territories since August 1998 (Roche et al., 2001). During the period of 2001 – 2005, the notification rate for STEC (excluding HUS cases) in Australia has been 0.2 – 0.3 cases per 100,000 population (Ashbolt *et al*., 2002; OzFoodNet, 2003; OzFoodNet, 2004, OzFoodNet, 2005). *E. coli* O157 has been the most commonly reported serotype. Significant variations in notifications exists between states and territories, and part of this variation is likely to be a result of different practices employed by pathology laboratories when screening faecal samples for toxin producing *E. coli* (OzFoodNet, 2003).

A large EHEC outbreak occurred in South Australia during 1995, which resulted in approximately 200 cases of illness. Twenty-two people aged between 4 months and 12 years developed haemolytic uraemic syndrome (HUS) and were hospitalised and a 4-year-old child died. Investigations of the outbreak identified EHEC strain O111:NM (or strain O111:H-, NM for non-motile) as the principal cause of the outbreak. A locally produced uncooked, fermented mettwurst was identified as the vehicle for the pathogen. The product was found to contain a variety of EHEC strains in addition to O111 (Paton and Paton, 1998).

The major reservoir of EHEC organisms appears to be the intestinal tract of ruminants, in particular cattle and sheep (Desmarchelier and Fegan, 2003). *E. coli* O157:H7 and other EHEC species have been isolated from both healthy and diarrhoeic animals, and individual animals can carry more than one

serotype (Anon, 1998). Foods derived from these animals may become contaminated via exposure to faecal material during processing.

A variety of host factors may be important in the pathogenesis of specific *E. coli* serotypes. In general, the young and the elderly appear to be more susceptible to pathogenic *E. coli* infection. Epidemiological studies have identified that children are at higher risk of developing post-diarrhoeal HUS than other age groups (Cummings et al., 2002).

3.2.2 Minor bacterial causes of infection transmissible by a waterborne route in the Australian environment.

3.2.2.1 Aeromonadacea

Aeromonas spp. are ubiquitous and occur worldwide, but are most frequently isolated from treated and untreated water and animals associated with water such as fish and shellfish. They may be pathogenic to amphibians, reptiles and fish. *Aeromonas hydrophila* is a gram-negative, facultatively anaerobic, non-spore forming rod-shaped bacterium that is present in all freshwater environments and in estuarine environments. It is also found in a wide range of foods, including seafood products and shellfish, raw foods of animal origin (for example, poultry, ground meat, raw milk), and raw vegetables and salads.

Aeromonas spp. cause a broad spectrum of infections in humans, usually in immunocompromised patients. While identified as waterborne pathogens, *Aeromonas* spp. have not been definitively implicated as a significant cause of food-borne illness. *A. hydrophila* may cause gastroenteritis in healthy individuals or septicaemia in individuals with impaired immune systems or various malignancies. Two distinct types of gastroenteritis have been associated with *A. hydrophila*: a cholera-like illness with a watery (rice water) diarrhoea; and a dysenteric illness characterised by loose stools containing blood and mucus. Symptoms associated with *Aeromonas*-related gastroenteritis include diarrhoea, abdominal pain, nausea, chills and headache, dysentery-like illness and colitis. Symptoms usually occur within 24–48 hours of exposure and generally last from one to 7 days (Kirov, 2003). On rare occasions, the dysentery-like syndrome is severe and may last for several weeks (Anon 2003). Human pathogen members of the family Aeromonadacea include *A. hydrophila*, *A. sobria* and *A. cavae.* These bacteria are widespread in surface waters and some reported densities include levels of up to10^3 cfu/ml in river water and 10^8 cfu/ml in raw sewage. High population densities in water ways are probably related to faecal contamination (Theron and Cloete, 2002).

3.2.2.2 *Shigella*

Shigella infections are highly communicable and several reports have been made of large-scale outbreaks associated with drinking water and public use of recreational waters such as freshwater swimming lakes (Craun *et al.*, 2005; Iwamoto *et al.*, 2005; Koutsotoli *et al.*, 2006). The infectious dose of these organisms can be as few as a 10 viable cells and infections can be caused by an infected person defecating in swimming or drinking water, passage of the

organisms through wild birds and animals into potable water or swimming areas or overflow of raw sewage into the waterway. This organism is usually associated only with humans and higher apes and there is no known reservoir in agricultural, wild or domestic animals.

Outbreaks of *Shigella sonnei* gastroenteritis have also been associated with food products. In 1983 at two Texas university campuses that were nearly 100 km apart an outbreak of illness was associated with tossed salads. Both schools had received the salad ingredients from the same company, the previous week.

3.2.2.3 *Enterobacter*

The genus *Enterobacter* consists of 13 species and can be found on human skin and plants as well as in soil, water, sewage, intestinal tracts of humans and animals, and some dairy products. Some species of *Enterobacter*, such as *E. sakazakii,* are opportunistic human pathogens. *E. cloacae* A-11 and similar bacteria can be found on cucumber and radish seeds as well as peas, soybeans, sunflowers, and sweet corn seeds. Strains of this complex have been isolated from water, papermill-process water, soil, and decaying wood. They are frequently isolated from damaged plant tissues and lesions although they are rarely considered to be pathogens. The presence of these organisms in water is likely to indicate faecal contamination but these organisms are unlikely to be associated with large waterborne disease outbreaks.

3.2.2.4 *Klebsiella*

Klebsiella spp. are ubiquitous in nature. Their habitats encompass the gastrointestinal tract of mammals as well as environmental sources such as soil, surface waters, and plants (Bagley, 1985). The genus *Klebsiella* is subdivided into five species; *K. pneumoniae, K. oxytoca*, *K. ornithinolytica, K. terrigena*, and *K. planticola*. *K. planticola* and *K. terrigena* are considered to be environmental species (Podschun et al., 2001).

Klebsiella pneumoniae is known as a resident of the intestinal track in about 40% of man and animals. Environmental isolates have been described as being indistinguishable from human clinical isolates with respect to their biochemical reactions and virulence. While the medical significance of *Klebsiella* obtained in the natural environment is far from clear, such habitats are thought to be potential reservoirs for the growth and spread of these bacteria which may colonize animals and humans.

3.2.2.5 *Yersinia*

Bacteria of the genus *Yersinia* are common environmental isolates including waterways such as lakes and dams. Certain strains of *Y. enterocolitica* (e.g.,

serotypes O3, O5, O8, and O9) are known to have virulence factors and are pathogenic to humans, causing gastrointestinal infections. The routes of infection have not been elucidated completely, but contaminated food and water are the most likely sources. Most of the environmental isolates of *Y.*

enterocolitica are non-pathogenic serotypes. *Y. enterocolitica* is able to grow at very low temperatures (4°C) and may survive for a long time in the environment and would be expected to be found in Victorian rural waters. Only a few outbreaks of gastrointestinal illness have been reported to be associated with *Y. enterocolitica* in the environment, in sewage, in contaminated surface water, and in drinking water (Leclerc *et al.*, 2002; Theron and Cloete, 2002) .

3.2.2.6 Vibrionaceae

Although the epidemic diarrhoeal disease cholera is known to be transmitted by water contaminated with pathogenic *Vibrio cholerae,* it only a major public health concern in developing countries where outbreaks occur in a regular seasonal pattern (Leclerc *et al.*, 2002; Ashbolt, 2004). Between 1817 and 1994 seven distinct pandemics of cholera have occurred and last century the disease was confined mainly to Southeast Asia. The disease has now been identified in Europe, Africa, South America and the US and is predominantly associated with riverine and esturine waters. In general this organism demonstrates a preference for saline environments but it is endemic in freshwater plankton in some of the warm regions of the world. The current climatic conditions in Victoria make it unlikely for this organism to ever become endemic in the waters of the area serviced by G-MW.

3.2.2.7 Mycobacteriaceae

Mycobacterium spp. are, in general, slow growing bacteria. They can be strict human or animal pathogens (such as *M. tuberculosis* and *M. leprae*) or free-living organisms that grow within the environment. Mycobacteria can be recovered from a wide variety of environmental niches and members of the *Mycobacterium avium* complex (MAC) have been recovered from both fresh water (ponds, lakes, rivers, bogs and swamps), brackish, sea water and wastewater, sometimes in high numbers (WHO, 2004). Contact with water or aquatic environments is probably important in the exposure of man and animals to environmental mycobacteria and plays a role in the development of an effective immune response.

In 1997, Hunter reported that about eight species of mycobacteria had been associated with waterborne transmission of human disease (Hunter, 1997). These species included *M. avium* complex*, M. fortuitum, M. gordonae, M. marinum, M. scrofulaceum, M. terrae, M. ulcerans,* and *M. xenopi*. This list has been extended to include *M. chelonae, M. immunogenum, M. abscessus, M. kansasii, M. ulcerans, M. szulgai, M. simiae, M. palstre* and *M. avium* subsp. *paratuberculosis* (MAP)*.*

Mycobacterium avium complex (MAC)

The *Mycobacterium avium* complex (MAC) is a group of related organisms, including *M. intracellulare*, *M. avium* and its subspecies *avium*, *paratuberculosis* and *silvaticum*. Because *M. avium* subsp. *paratuberculosis* (MAP) is very slow growing, fastidious and requires special media for its isolation, the term MAC is usually used to indicate members of the complex

other than MAP, although strictly speaking it is a member. The transmission routes of human MAC infection remain unclear, but there is no evidence of person-to-person transmission and environmental sources of infection are likely. Infection is thought to occur through colonisation of the gastrointestinal or respiratory tract and exposure probably occurs by inhalation or ingestion. There have been a number of publications suggesting that treated and untreated water may be a possible primary source of infection (Pickup *et al.*, 2005; Whan *et al.*, 2005).

***Mycobacterium avium* subsp. *paratuberculosis* (MAP)**

M. avium subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease (also known as paratuberculosis), a chronic inflammatory disease of the intestines of herbivores (cattle, sheep, goats, rabbits, deer), macaques and other wild animals (Mackintosh *et al.*, 2004; Pavlik *et al.*, 2005). MAP has also been implicated as a possible cause of Crohn's disease in humans. Transmission is thought to occur mainly by the faecal-oral route (Biet *et al.*, 2005). MAP can be ingested in large numbers when calves suckle on teats that have been contaminated by the faeces of shedding animals. Food sources are likely to represent a bigger source of human exposure to MAP than drinking water because the numbers likely to be present in food may be higher. The organism can survive for protracted periods in the environment and hence could be present in catchment areas receiving agricultural runoff and as with other potentially zoonotic pathogens rain falling onto pastures contaminated with *M. avium* subsp. *paratuberculosis* would be expected to wash these organisms into surface waters and rivers. MAP in untreated water poses a possible human health risk through contact with recreational water sources. There is an un-quantified risk that MAP maybe transmitted to humans where such waters are sourced for domestic supply.

3.2.2.8 Legionellaceae

The genus *Legionella* has over a hundred named species. *L. pneumophila* is the most frequent of the human pathogens and is commonly found in aquatic habitats including groundwater but the organisms are very dependant upon warm temperatures for growth and survival. Disease outbreaks of *L. pneumophila* are relatively common and are associated with cooling towers, spas and hot water tanks where biofilms containing high numbers of the organism can be aerosolized and inhaled into human lungs (Ashbolt, 2004). No reports of outbreaks caused by this organism have been associated with lakes, dams, channels or rivers.

3.2.2.9 Leptospira

Leptospirosis is an epidemic-prone zoonotic bacterial disease that can be transmitted by direct contact with contaminated water. It affects humans and animals. It is caused by bacteria of the genus *Leptospira*. In humans it causes a wide range of symptoms, and some infected persons may have no symptoms at all. Symptoms of leptospirosis include high fever, severe

headache, chills, muscle aches, and vomiting, and may include jaundice (yellow skin and eyes), red eyes, abdominal pain, diarrhea, or a rash.

Leptospires are long, thin motile spirochetes. They may be free-living or associated with animal hosts and survive well in fresh water, soil, and mud in tropical areas (Rathinam and Namperumalsamy, 1999). Organisms are antigenically complex, with over 200 known pathogenic serologic variants. Transmission occurs through direct or indirect transmission from a mammalian host. Rodents and wild animals are the most common reservoirs for this widespread zoonosis and they shed large amounts of leptospires in their urine. Outbreaks of leptospirosis are usually caused by exposure to water contaminated with the urine of infected animals. Indirect transmission via contact with *Leptospira* contaminated water or soil, is thought to be responsible for most cases. In 2003 a case of Leptospirosis was reported in a kayaker on the Yarra river (Worth and Biggs, 2003) and water sports are considered a risk factor in the transmission of this disease (Haake *et al.*, 2002). Transmission is also thought to occur through contact of the skin and mucous membranes with water, damp soil or vegetation (such as sugar cane), or mud contaminated with rodent urine. Flooding facilitates spread of the organism because of the proliferation of rodents and the proximity of rodents to humans on shared high ground. Outbreaks of leptospirosis occurred in Taiwan, Republic of China, associated with Typhoon Nali in 2001; in Mumbai, India, after flooding in 2000; in Argentina after flooding in 1998; and in the Krasnodar region of the Russian Federation in 1997. After a flooding-related outbreak of leptospirosis in Brazil in 1996, spatial analysis indicated that incidence rates of leptospirosis doubled inside the flood-prone areas of Rio de Janeiro.

3.2.2.10 Listeria

Listeria monocytogenes is a food-borne pathogen that is capable of causing a food-borne illness called Listeriosis. It presents a hazard to particular groups of consumers such as: the elderly, new born, pregnant women and those whose resistance to infection is weakened (*eg*. those with HIV, leukaemia etc). Listeriosis has a mortality rate of about 30%.

Listeria spp are commonly found in many environments including soil, dirt, water and can be carried by both domestic and wild animals. The *Listeria* bacterium is widespread throughout the environment and is thus a contaminant of raw foods.

There are six known species of *Listeria*. These are:

- Listeria monocytogenes
- Listeria innocua
- Listeria ivanovii
- Listeria seeligeri
- Listeria welshimeri
- Listeria grayi

On the farm, the environmental conditions for the growth of *Listeria* is ideal. The organism can grow in soil, muddy and dusty conditions, in water and in

Virus Family	*Virus Genus*	*Virus Type*
Picornoviridae	*Enterovirus*	*Polio 1-3*
		Coxsackie A
		Coxsackie B
		ECH01-31
		Enteroviruses
	Hepatovirus	*Hepatitis A*
	Hepatitis E-like	*Hepatitis E*

dams. Cows can be carriers and mastitis can result from a *Listeria* infection. The feeding of poor quality silage is another problem area, as the organism can grow in poorly fermented silage.

3.3 Waterborne pathogens and causes of zoonosis - Viral

3.3.1 Viruses

Viruses have an absolute requirement for living cells in which to replicate, and cannot be found free living in the environment. A list of viruses that may be transmitted by a waterborne route is shown in Table 2. Typically human enteric viruses enter waterways via sewage systems, subsequent passage through wastewater treatment and discharge to receiving waters or by direct contamination. Direct contamination of water ways can also occur from houseboats, recreational boats, and septic tanks leaking into waterways as well as manure sludge running into irrigation channels. In raw faecal material viral numbers can exceed 10^9 particles per ml while numbers from sewage can exceed 10^4 plaque forming units (PFU) per litre.

Reoviridae	*Rotavirus*	*Rotavirus 1-4*
Caliciviridae	*Norovirus*	*Norwalk*
	Sapovirus	*Sapporo*
Astroviridae	*Astrovirus*	*Astrovirus 1-5*
Adenoviridae	*Mastadenovirus*	*Adenovirus 1-47*

Table 2. Viruses that may be transmitted by a waterborne route

3.3.2 Picornoviridae (*Enterovirus* and *Hepatovirus*)

Enterovirus and *Hepatovirus* are members of the Picornoviridae family of RNA viruses. Enteroviruses are ubiquitous, enterically transmitted viruses that cause a wide spectrum of illness that can be particularly severe in young children (Vasickova *et al.*, 2005). Enteroviruses are able to persist in the environment, where they can survive for several weeks, and have been associated with infection from eating soft fruits, vegetables and shellfish. They have also been detected in marine sediments (Vasickova *et al.*, 2005).

Hepatitis A virus (HAV) is the only member of the genus *Hepatovirus*. They are most commonly associated with inadequate water supplies and poor hygiene. The viruses cause inflammation of the liver (hepatitis) and the effects can be long-lasting. The virus is transmitted via the faecal-oral route, usually through ingestion of contaminated food or water (Carter, 2005). Hepatitis A and E are also transmitted by the fecal-oral route, in association with lack of access to safe water and sanitation. Hepatitis A is endemic in most developing countries, and most children are exposed and develop immunity at an early age. As a result, the risk for large outbreaks is usually low in these settings. In hepatitis E–endemic areas, outbreaks frequently follow heavy rains and floods; the illness is generally mild and self-limited, but for pregnant women case-fatality rates can reach 25%. After the 2005 earthquake in Pakistan, sporadic hepatitis E cases and clusters were common in areas with poor access to safe water. Over 1,200 cases of acute jaundice, many confirmed as hepatitis E, occurred among the displaced. Clusters of

both hepatitis A and hepatitis E were noted in Aceh after the December 2004 tsunami.

3.3.3 Reoviridae (*Rotavirus*)

Rotaviruses belong to the family Reoviridae and are double stranded RNA viruses (Vasickova *et al.*, 2005). Young children are particularly at risk and in developing countries over 800,000 deaths each year result from rotavirus infection (Ashbolt, 2004). The genus *Rotavirus* is divided in five groups (A-E) but only A, B, and C are human pathogens and it is readily transmissible via the faecal-oral route (Carter, 2005).

3.3.4 Caliciviridae (*Noroviruses* and *Sapoviruses*)

The calicivirus famly includes 2 genera the *Noroviruses* and *Sapoviruses*. *Noroviruses* (genus *Norovirus*, family Caliciviridae) are a group of related, single-stranded RNA, nonenveloped viruses that cause acute gastroenteritis in humans (Hansman, 2007). Norovirus was recently approved as the official genus name for the group of viruses provisionally described as “Norwalk-like viruses”. This group of viruses has also referred to as caliciviruses (because of their virus family name) and as small round structured viruses, or SRSVs (because of their morphologic features). Another genus of the calicivirus family that can cause gastroenteritis in humans is Sapovirus, formerly described as “Sapporo-like virus”.

Infections with these viruses occur around the world and appear to be more common in winter (Carter, 2005). Current estimates suggest that upwards of 95-96% of nonbacterial gastroenteritis outbreaks of unidentified etiology in adults may be due to human caliciviruses (Huffman *et al.*, 2003). Water (swimming areas and contaminated drinking water) and food are likely vectors in transmission of the disease (Vasickova *et al.*, 2005). Noroviruses have also been detected in oysters and other shellfish, water from drinking fountains, ice and community drinking water.

3.3.5 Astroviridae (*Astrovirus*)

Astroviruses are a single stranded RNA virus and are a leading cause of infantile viral gastroenteritis worldwide (although it is considered to be a milder disease compared with *Rotavirus*) (Vasickova *et al.*, 2005). Swimming areas contaminated with *Astrovirus* are one of several likely transmission sources.

3.3.6 Adenoviridae (*Mastadenovirus*)

Adenoviruses are double-stranded DNA viruses and human adenoviruses are members of the genus *Mastadenovirus*. There are around 50 immunologically distinct types (6 subgenera: A through F) that can cause human infections although only types 40 and 41 induce gastroenteritis. Adenoviruses are unusually stable to chemical or physical agents, meaning that they can survive prolonged periods outside of the body and they are frequently found in faecally polluted waters (Vasickova *et al.*, 2005).

3.4 Waterborne pathogens and causes of zoonosis – Parasitic

Water borne parasites include quite a diverse range of organisms. Included in this group are unicellular organisms such as amoebae through to complex and large organisms such as liver flukes and worms. They can have complex lifecycles, often free living in the environment, and have hardy reproductive stages that can include spores, cysts, oocysts, ova, larval and encysted stages. Transmissible stages of parasites can enter waterways directly, via the voiding of faeces into water by animals that have direct access to that water, see Figure 5.

Giardia and *Cryptosporidium* have become the major parasites associated with water borne zoonotic infection. Both of these parasites are indigenous in many species and their cysts and oocysts are known to be excreted in high densities from infected animals. They are also extremely hardy and can survive the use of common disinfectants including chlorine treatments used in water treatment.

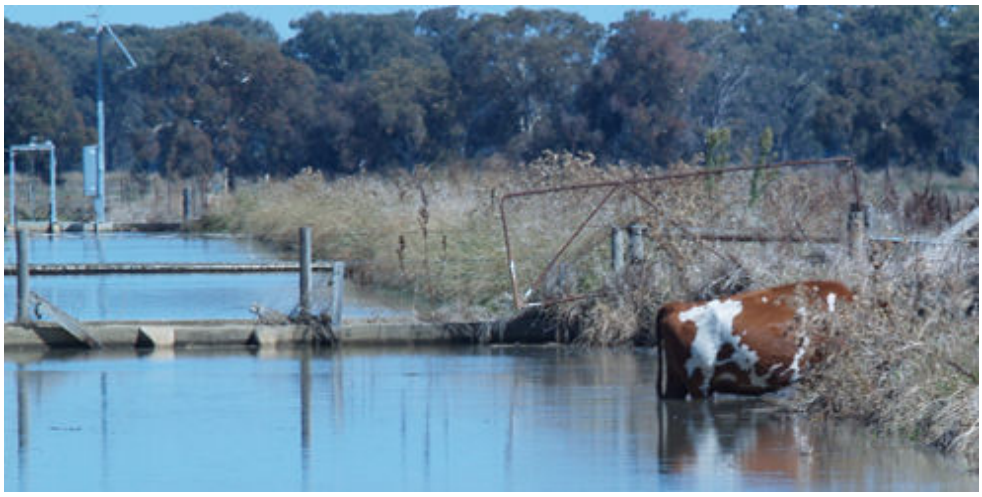

Figure 5. Dairy cow grazing sides of irrigation channel.

Another significant group of water borne parasites are the Helminths. Helminth is a general term for a parasitic worm. The helminths include the Platyhelminthes or flatworms (flukes and tapeworms) and the Nematoda or roundworms.

The Department of Health does not require all parasitic disease to be notified. The data that is collected for Cryptosporidium and Giardia suggests that enteric disease caused by these parasites is not a significant contributor to the total infectious disease burden in the state of Victoria as only around a 1000 cases are notified per year. However as with all enteric disease only people who have extreme illness seek medical assistance and only a small proportion of these are investigated for parasites (Anonymous, 2004). Table 3 shows parasites that may be transmitted by a waterborne route.

3.4.1 Microspora

Microsporidia are obligate, intracellular spore forming protozoa (although it has been suggested that they should be re-classified as fungi rather than

protozoa) (Didier *et al.*, 2004). The phylum contains over 1200 species of which 14 species are currently known to infect humans (Ghosh *et al.*, 2006). Microsporidia can also infect protozoa, invertebrates and vertebrates such as fish. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the most common causes of human infections. They are particularly associated with diarrhea and systemic disease although relatively few episodes of waterborne human illness have been reported worldwide (Slifko *et al.*, 2000; Didier *et al.*, 2004; Gajadhar and Allen, 2004; Didier, 2005; Ghosh *et al.*, 2006).

Species of microsporidia infecting humans have been identified in water sources as well as in wild, domestic, and food-producing farm animals, raising concerns for waterborne, foodborne, and zoonotic transmission. Studies have shown that microsporidia species infecting humans appear to lack host specificity (allowing cross-species-infectivity), have spores that are environmentally hardy and the infectious dose is regarded as 'probably low' (Didier *et al.*, 2004). However, this family of parasites is not considered a threat with regard to contamination of Australian waterways.

Table 3. Parasites that may be transmitted by a waterborne route

Parasite Family	*Parasite Genus*	*Parasite Type*
Microspora-		
Enterocytozoonidae	*Enterocytozooan*	*E. bieneusi*
	Encephalitozooan	*E. cuniculi*
		E. intestinalis
Protozoa-		
Crytosporidiiae	*Crytosporidium*	*C. parvuum*
Hexamitidae	*Giardia*	*G. lamblia*
		G. duodenalis
Sarcocystidae	*Toxoplasma*	*T. gondii*
Balantidiidae	*Balatidium*	*B. coli*
	Naegleria	*N. fowleri*
Trematodes-		
Opisthorchiidae	*Opisthorcis*	*Opisthorcis spp.*
Heterophydae	*Metagonimus*	*M. yokogawai*
Echinostomatoidea	*Echinostoma*	*Echinostoma spp.*
Fasciolidae	*Fasciola*	*F. hepatica*
Troglotrematidae	*Paragonimus*	*Paragonimus spp.*
Schistosomatidae	*Schistosoma*	*Schistosoma spp*
Cestodes-		
Diphyllobothriidae	*Diphyllobothrium*	*D. latum*
Taeniidae	*Taenia*	*T. saginata*
		T. solium
Nematodes-		
Anisakidae	*Ascaris*	*A. suum*

No cases of human waterborne illness have been reported in Australia although human disease associated with Human Immunodeficiency Virus has been described.

3.4.2 Protozoa

The transmission of *C. parvum G. lamblia*, and *G. duodenalis* in waterborne disease outbreaks has been widely documented (Slifko *et al.*, 2000; Ferguson *et al.*, 2003; Chalmers and Casemore, 2004; Grundlingh and de Wet, 2004; Kassa *et al.*, 2004; Craun *et al.*, 2005; Schuster *et al.*, 2005). Hundreds of waterborne disease outbreaks have been reported within the last decade, including the Sydney water quality issues of 1998. Although speculative, the causes of the these outbreaks are said to be primarily associated with cattle farming, including activities such as muck spreading, but are also associated with run-off from contaminated farm land into waterways and drains. In countries other than Australia, indigenous animals such as beaver and bear have been thought to be the cause of local disease outbreaks. Apart from farm animals, reservoirs in Australian animals can include most native marsupials and cryptosporidium has been isolated from eastern gray kangaroos around Sydney watersheds (Power *et al.*, 2005). The life cycles of *Cryptosporidium* and *Giardia* are shown in Appendices 1 and 2.

3.4.2.1 *Cryptosporidium*

Cryptosporidium is an intestinal protozoan parasite that induces gastrointestinal symptoms when ingested by humans. Being an obligate parasite, the organism requires a host to reproduce, and is transmitted to humans via ingestion of the environmental stage of its life cycle, the oocyst. The oocysts are approximately 4 – 6 µm in diameter and are shed in the faeces of infected hosts in large numbers.

Cryptosporidosis, the disease caused by infection from *Cryptosporidium*, is a severe diarrhoeal disease. In immunocompetent individuals the disease is self-limiting, usually lasting less than ten days. It is often accompanied with abdominal pain, nausea, vomiting, general malaise and low-grade fever. For

immunocompromised individuals, however, the disease can be prolonged and life threatening (Duffy and Moriarty, 2003).

The two main species of cryptosporidium that infect humans are *C. hominis* and *C. parvum*. The main symptom of cryptosporidiosis is diarrhoea, which may be accompanied by dehydration, weight loss, abdominal pain, fever, nausea, and vomiting. Disease, although lasting for up to two weeks, is usually self-limiting in immunocompetent. C. hominis primarily infects humans but has recently been reported to infect a dugong and a lamb. Rare occurrences of low-level natural infection of cattle by C. hominis have also been reported. By contrast, *C. parvum* naturally infects several animal species that serve as reservoirs for zoonotic infection, including cattle, sheep, goats, and dear.

C. parvum oocysts have been shown to be able to survive up to 176 days in drinking water or river water stored at 4°C, with inactivation between 89% and 99% of the population (Robertson et al., 1992). Desiccation is detrimental to oocyst survival and low water activity has been reported to result in reduced viability (Rose and Slifko, 1999). A study by Robertson et al (1992) showed air-drying at room temperature resulted in 97% inactivation within 2 hours and 100% inactivation within 4 hours (Robertson et al., 1992).

Symptomatic cryptosporidiosis is usually characterised by profuse watery diarrhoea, often leading to rapid weight loss and dehydration. Other symptoms can include abdominal cramping, nausea, vomiting, low grated fever and headache (Smith, 1993). The disease is usually self-limiting, with symptoms normally lasting for two to four days.

Cryptosporidium is transmitted via the faecal-oral route. Person-to-person contact to oocysts is of particular concern in settings such as childcare centres. The majority of documented cryptosporidiosis outbreaks have been associated with waterborne transmission. Cryptopsoridiosis became a notifiable disease in Australia in 2001. A total of 3,255 (16.6 cases per 100,000 population) cases were notified to health authorities during 2002 (Yohannes et al., 2004). Children under the age of four have the highest cryptosprodiosis notification rate (129 cases per 100,000 population). This may reflect an increased susceptibility of children to *Cryptosporidium* and/or increased likelihood of exposure. The most prominent waterborne outbreak occurred in Milwaukee in 1993 and resulted in an estimated 403,000 cases of illness (MacKenzie et al., 1994). *Cryptosporidium* oocysts are resistant to many disinfection techniques. It is for this reason that conventional water treatment plants are not always effective in removing the oocysts.

Although the majority of reported cryptosporidiosis outbreaks are waterborne, a number of food-borne outbreaks have occurred. For example an outbreak was observed in Maine, US that was associated with consumption of fresh-pressed apple cider (Millard *et al*., 1994). *Cryptosporidium* oocysts were detected in the apple cider, on the cider press and in the stool specimen of a calf on the farm that supplied the apples. The secondary transmission rate to other household members was 15%. Outbreaks have also been linked to consumption of unwashed green onions (Anon, 1998).

3.4.2.2 *Giardia*

Giardia cysts have been detected in 81% of raw water samples and 17% of filtered water samples in the United States (Marshall *et al.*, 1997). *G. lamblia* continues to be the most frequently identified etiologic agent in water borne disease outbreaks. As in the past, outbreaks of giardiasis are associated with ingestion of unfiltered and inadequately chlorinated. Waterborne transmission has been implicated as a cause of giardiasis in travelers. Backpackers who drink unfiltered stream water are also at risk (Marshall *et al.*, 1997).

Giardia is the most commonly isolated intestinal parasite throughout the world and is especially prevalent in children in developing countries. The incubation period is usually one to two weeks. Symptoms include watery diarrhoea, abdominal distinction, and gastric cramps.

The life cycle of giardia is composed of two stages; and actively multiplying trophozoite and a resistant cyst. Cysts survive in food and water. When ingested, the cyst passes through the stomach, where the acid environment triggers excystation, which usually takes place in the duodenum. Cyst formation takes place as the trophozoite moves through the colon.

3.4.2.3 *Toxoplasma*

Infection with the protozoan *T. gondii* is common worldwide. Although not a serious disease in adults, children can suffer retardation and blindness. It has only been recently that toxoplasmosis has begun to be considered a waterborne illness and internationally a few cases of disease *T. gondii* in water have been reported. Water has been the suspected vehicle of *T. gondii* dissemination in toxoplasmosis outbreaks in Brazil, Canada and Panama (Slifko *et al.*, 2000; Dubey, 2004; de Moura *et al.*, 2006). No cases of waterborne toxoplasmosis have been reported in Australia.

3.4.2.4 *Naegleria*

Naegleria is an ameba commonly found in the environment, particularly in water and soil. Only one species of *Naegleria* has been found to infect humans, *N. fowleri*. *N. fowleri* is found worldwide. Most commonly, the ameba is found in warm bodies of fresh water, such as lakes, rivers, and hot springs, warm water discharge from industrial plants, under-chlorinated swimming pools and soil. Infection with this organism is relatively rare but could occur during the summer months when water temperatures rise. *Naegleria* sp. are found in increased in numbers in waters contaminated with coliforms as filamentous cyanobacteria and eubacteria serve as a food source to these organisms.

3.4.3 Trematodes

The trematodes are flatworms that may affect various organs. They have at least two suckers, one oral and one ventral. The oral sucker surrounds the

mouth and the intestinal system has a blind ending. Along with the cestodes, the trematodes (Class Trematoda) belong to the Phylum Platyhelminthes. There are two recognized classes, the *Monogenea*, which includes important ectoparasites of fish, and the *Digenea*, including species parasitic in the bile ducts, alimentary and respiratory tracts, and blood vessels of vertebrates. The most important trematodes from a veterinary point of view are the liver flukes, which cause severe disease and production loss in farmed ruminants in particular. The trematodes which are of importance in human pathology belong to the Digenea (derived from the greek di = two, and generis = generation). This name refers to the obligatory change of host during the life cycle of the parasite. Although details differ depending on the species of parasite, eggs are produced by the adult parasite and arrive in the outside world via faeces or urine of the host. The eggs hatch, miracidia emerge and penetrate snails. There they change into sporocysts, rediae and cercariae. Cercariae emerge from the snail, swim around and then penetrate the next intermediate host to form metacercariae. Cercariae from the *Fasciolidae* encyst on water plants. Cercariae from schistosomes penetrate the final host directly. Secondary intermediate hosts include fish, crabs, snails and insects. After they have been eaten by the final host the metacercariae grow into adult parasites and the life cycle then continues. Flukes generally use snail intermediate hosts, and are more of a problem where conditions favour high snail densities, e.g. on wet land or in wet years. Another family, the Opisthorchiidae, use fish as second intermediate hosts, and can cause liver disease in people who inadvertently eat poorly cooked infected fish.

Table 4 shows the localization of various *Digenea* within the body. Most of the medically and veterinary significant cestodes are not endemic in Australia but are regularly detected in travelers, foreign nationals and refugees. However Schistosome dermatitis, known as swimmer's itch, a common global problem for users of recreational swimming areas, can be contracted in fresh, brackish and salt waters and has been detected in Queensland, SA and the waters of the Murray River (Hurley *et al.*, 1994).

Table 4. Localization of *Digenea* group of Cestodes within the body.

Intestinal lumen	Large intestinal fluke (*Fasciolopsis buski*) Small intestinal flukes (*Metagonimus* and *Heterophyes*)
Lungs	Lung fluke *(Paragonimus*)
Bile ducts	Large liver flukes (*Fasciola hepatica* and *F. gigantica*) Small liver flukes (*Opisthorchis, Clonorchis, Dicrocoelium*)
Blood vessels	Blood flukes (*Schistosoma* sp.)

3.4.4 Cestodes (*D. latum* and *Taenia spp.*)

Worms in the Class Cestoda are generally flat, segmented and ribbon-like, and are popularly known as tapeworms. They have no gut and absorb nutrients across the body wall. The adults of most tapeworms of vertebrates live in the alimentary tract. *Diphyllobothrium latum* is the largest human tapeworm. It can reach 10 meters in length, and live in a host for up to 25 years. It causes disease in populations who consume raw or undercooked freshwater fish. Disease is especially seen in the Great Lakes, Scandinavia, W. Europe, Japan and South America (Gajadhar and Allen, 2004). Infection with *Diphyllobothrium latum* is usually asymptomatic, but they can cause pernicious anaemia.

Two species from the genus *Taenia* are common parasites of man, these being *Taenia solium* (the Pork tapeworm) and *T. saginata* (the Beef tapeworm). If *T. solium* eggs are ingested (from fecally contaminated water or by anus-to-mouth transfer of infective eggs), they may hatch in the gut and spread systemically, causing human cysticercosis. Cysticercosis is a dangerous but rare disease that affects the central nervous system. *T. solium* infection has the ability to develop both adult and larval stages in humans. Humans accidentally become the intermediate host by ingestion of faecally contaminated food or water (most common) or autoinfection (eggs from anus to hand to mouth) (Slifko *et al.*, 2000). In developing countries *T. saginata* eggs may also be transmitted by ruminants consuming contaminated water and vegetation (Nithiuthai *et al.*, 2004) and this form of transmission is highly unlikely in Australia.

3.4.5 Nematodes

Ascariasis is an infection of the small intestine caused by *Ascaris lumbricoides*, a large roundworm. Although more commonly associated with tropical regions including the Northern Territory, it has been associated with death and morbidity where sewage is used as a fertilizer on crops or humans have been exposed to water contaminated with human faecal material. The size of the ova is usually quite large, causing them to settle out of water quite quickly. For this reason, and particularly their association with tropical environments, this organism is unlikely to be a major pathogen in the region serviced by G-MW.

3.5 Other potential zoonotic and water borne pathogens

The potential for water ways to be inadvertently or deliberately contaminated by microbes not normally found in a distribution system should be considered in any discussion of zoonotic and water borne pathogens. This section covers selected biological agents that may be of public or animal health concern but are not covered elsewhere. Table 5 lists organisms that are considered in this section.

3.5.1 *Burkholderia*

Members of the genus *Burkholderia*, were first defined in 1992 and were previously described as belonging to the genus *Pseudomonas*. There are at least 20 validly named species in the genus but the medically important species are *B. cepacia*, *B. pseudomallei* and *B. mallei* (Sprague and Neubauer, 2004).

This is an important pathogen of humans (melioidosis) and farm animals in tropical and subtropical areas of SE Asia and Northern Australia, where it is endemic in rodents and is found in moist soil, on vegetables and on fruit (Wiersinga *et al.*, 2006). A closely related but non-pathogenic species, *B. thailandensis*, has been described from environmental samples. Although an unlikely pathogen in Victoria it should be considered as a possible agent of waterborne disease.

3.5.2 *Pseudomonas aeruginosa*

Currently there are 34 known *Pseudomonas* species and they are all able to colonise low-nutrient habitats such as mineral water. One of those species is *Ps. aeruginosa*, a ubiquitous bacterium that inhabits water, soil and plants. It has also been isolated from carrots, onions and lettuce (Leclerc *et al.*, 2002).

In humans this organism is an opportunistic pathogen and is intrinsically resistant to commonly used antibiotics. While this group of organisms have not been associated with reported outbreaks of waterborne disease, the presence of high numbers of this organism in a rural water supply that is used, for example, as bathing water, could present a serious health risk to a person who has medical devices that pass through the skin, has cystic fibrosis or is immuno-compromised.

Table 5. Potential zoonotic and water borne pathogens not considered in previous sections.

Family	*Genus*	*Type*
	Pseudomonas	
	Burkholderia	*B. pseudomallei*
	Francisella	*F. tularensis*
	Chlamydia	*C. psittaci*
	Coxiella	*C. burnetti*
	Anatoxin A and Microcystins	From freshwater cyanobacteria
	Prions	

3.5.3 Francisella tularensis

Tularemia (also known as deerfly fever or rabbit fever) is an infectious disease caused by the bacterium *Francisella tularensis*. It is found naturally in small mammals such as rabbits, rodents, and hares, as well as the insects that feed on these animals. The bacteria can survive for weeks at low temperatures in water, moist soil, hay, straw, or decaying animal carcasses. Tularemia was first described by scientists in 1911. Its ability to infect whole populations was seen during outbreaks of waterborne disease in Europe and the Soviet Union in the 1930s and 1940s. This organism is not endemic in Australia and is most often seen in the US and Europe.

3.5.4 *Chlamydophila psittaci*

Psittacosis, also known as parrot fever and ornithosis, is a bacterial infection of humans that can cause severe pneumonia and other serious health problems. It is caused by *Chlamydophila psittaci*, formerly known as *Chlamydia psittaci*. Parrot fever is a rare infectious disease that causes pneumonia in humans. It is transmitted from pet birds or poultry.

Human infection with *C. psittaci* usually occurs through the inhalation of the organism aerosolized from respiratory secretions or dried feces of infected birds. Other sources of exposure can include bird bites, mouth-to-beak contact, and handling the plumage and tissure of infected birds, however parrot fever can also be transmitted from contaminated water where sick birds have been drinking.

3.5.5 *Coxiella burnetii*

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a species of bacteria that is distributed globally. Q fever is an illness that leaves a person incapacitated for several weeks with a debilitating headache and fever. The organism is found in cattle, sheep, and goats. It can be turned into an aerosol from water that has been contaminated with animal urine and is considered a potential biological weapon.

3.5.6 Anatoxin A and Microcystins

Cyanobacteria are common in fresh water throughout the world and are known to produce several types of toxins: microcystins which are heat-stable, cyclic heptapeptide hepatotoxins that also promote tumor growth and inhibit protein phosphatases; anatoxin, a neurotoxin, which inhibits acetylcholinesterase; and paralytic shellfish poisoning (PSP) toxins, such as saxitoxin, which are also neurotoxic. *Lyngbya wollei*, commonly found in some lakes and reservoirs in the southeastern USA, and one strain of *Aphanizomenon flos-aquae* also produce potent neurotoxins, related to PSP toxins.

3.5.7 Prions

Prions are small folded protein molecules containing no genetic information, which are made up of amino acids, the essential building blocks of all

proteins. Prion-like proteins that are found naturally in many (perhaps all) plants and animals, are folded differently than normal proteins due to slight amino acid changes in certain regions of the protein. They have the ability to affect other proteins, causing them to change from the normal form to the abnormal form. In their normal, non-infectious state, prions are believed to be involved in cell-to-cell communications and other important cell functions. Prions are associated with, and may cause transmissible spongiform encephalopathy (TSE) diseases in animals and humans. Brain tissues affected by these diseases have tiny holes in them making them resemble a sponge when viewed under a microscope. TSE diseases are untreatable and fatal. The most common examples of TSE diseases are Chronic Wasting Disease (CWD) in deer/elk/moose, BSE or Mad Cow Disease in cattle, and Scrapie in sheep. Prions are thought to cause a variant form of Creutzfeldt-Jakob Disease (vCJD), a fatal neurodegenerative illness, and Kuru in humans. Prions are of interest as a possible waterborne disease agent as there is concern that carcasses of infected animals can leach the prions into groundwater which could then be used as a source of drinking water by humans and other animals. Research is on-going in this area and at this point is not a concern in Australia.

3.6 Specific factors affecting transfer of zoonotic agents

3.6.1 Farmed animals

Increased risks for the transmission of water borne and zoonotic diseases can be related to the close proximity of farmed, domestic and feral animals. For example, *Cryptosporidium parvum* and *Giardia duodenalis* in surface water have been found to be associated with the presence of high density of domestic and wild animals in the surrounding areas. Young calves are frequently identified as the source *Giardia* and *Cryptosporidium* and they usually shed more pathogens compared to adults. Other studies have revealed that *Cryptosporidium* spp. are frequently detected in surface water in dairy farming areas, whereas *Giardia* cysts are more predominantly associated with urban sewage (LeChevallier *et al.*, 1997; Kistemann *et al.*, 2002).

Surface runoff and point source pollution from pastoral agriculture can introduce pathogenic micro-organisms such as *Campylobacter*, *Cryptosporidium* and *Giardia* into streams and rivers (Geldreich, 1996). Investigation carried out in rural streams in New Zealand surrounded by dairy farms found a constant presence of *Campylobacter* which researchers believe could be due to cycling of *Campylobacter* in farm animals. Another study in New Zealand found that *E. coli* concentrations increased more than 100 times background level after cattle crossed a stream, which could be attributed to direct faecal deposition, wash-off from legs and disturbance of sediments by cattle hooves (Davies-Colley *et al.*, 2004). Two cow crossings produced plumes of turbid water associated with very high concentrations of faecal indicator bacteria (*Escherichia coli*) and high suspended solids and total nitrogen. On the first crossing, towards the milking shed, the cows were tightly-bunched and produced a sharp spike of contamination (*E. coli* peaking

at 50,000 cfu/100 ml). After milking, the cows wandered back across the stream as individuals or small groups, and contaminants were less elevated, albeit for a longer period. The cows defecated approximately 50 times more per meter of stream crossing than elsewhere on the raceway (Davies-Colley *et al.*, 2004).

Cattle and sheep are asymptotic natural reservoirs of *E. coli* O157:H and it is believed that about 30-80% of cattle are carriers of this pathogen (Callaway *et al.*, 2003). Furthermore, in countries where cattle are raised in high densities, for example feedlots, there is an association with higher rates of human Vero toxigenic *E.coli* infection. Other reservoirs of EHEC are goats, red deer, horses, dogs, birds, and flies. In 2000, (Walkerton, Canada) an estimated 2000 people became ill and 7 died from exposure to EHEC contaminated drinking-water (Hrudey *et al.*, 2003). The bacteria can survive in liquid manure, non-liquid manure and drinking troughs. Foods that are irrigated, washed or prepared with polluted water are also a common cause of infection (Doyle, 1990).

Salmonella enterica and *Campylobacter jejuni* infections are very common in a number of domestic and wild animal species but the incidence of infections in poultry is high, in particular in chickens (Sahin *et al.*, 2002). Newly introduced farmed birds (ostriches, emus) may also contaminate soil and water with pathogens such as *Mycobacterium* spp and *Salmonella* spp.

Adenoviruses and enteroviruses have been found in lakes and rivers whose catchment areas were influenced by poultry and sheep farming (Till *et al.*, 2000). Swine/ pig farming has also been implicated as a possible source of Hepatitis E (Worm *et al.*, 2002).

Mass production of animals and development of large meat factories and international trade of meat products and animals are believed to be the reasons for the increased prevalence of *Yersiniosis* in humans (Neubauer *et al.*, 2001). Wastes from animals at slaughter (in abattoirs), on farm deaths and the mass culling of infected animals are also potential source of water borne zoonoses. Discharge or release of inadequately treated wastewater or solid wastes (*e.g.* gastrointestinal contents or animal carcasses into the environment can also lead to contamination of water sources (Gannon *et al.*, 2004).

3.6.2 Wild birds and animals

Wild animals and birds are considered to be a major contributor to zoonotic disease. Australia is home to 13 bird families and approximately 700 endemic bird species as well as native fauna including vertebrates such as kangaroos and fish. These agents are considered in the following sections.

3.6.2.1 Migratory birds

Bird migration has been defined as the predictable seasonal movement of birds from breeding grounds to distant wintering grounds and back again within a single year. Of the 700 endemic bird species about 145 are known to undertake large-scale migratory movements but this occurs predominantly within Australia, and only occasionally are species known to travel as far north

as New Guinea. None are known to reach the mainland of Asia (Dingle, 2004).

Migratory birds also travel from the Northern Hemisphere to Australia each year along the East Asian-Australasian flyway. Around 2 million birds shorebirds alone are said to visit Australia annually and although they migrate vast distances they return to the same wintering habitat year after year (Clarke, 2000). There is a possible risk of these birds carrying diseases such as Avian flu into the country.

Relatively few reports have been made of outbreaks of zoonotic infection in wild migratory birds. However, relatively close to Australia, New Zealand has had one major reported outbreak of Salmonellosis, caused by *Salmonella enterica*, serotype Typhimurium DT160, in which mass mortality of birds was reported (Alley *et al.*, 2002). Throughout New Zealand, human cases of Salmonellosis were seen to have increased gradually over the period of the outbreak. *Salmonella* bacteria are commonly found in the intestine of wild birds. These organisms are maintained within bird populations by several mechanisms; raptors eating *Salmonella*-infected prey, scavenging or carrion eating birds such as crows eating infected prey and birds living in a contaminated environment picking up the bacteria. This is the case with domestic pigeons and colonial water-birds. The most significant outbreaks of wild bird Salmonellosis occur, however, in passerines (or perching birds). Although only a few healthy passerines harbor *Salmonella* in their intestine, these birds often gather in very large numbers at bird feeders and easy feeding sites such as grain spills on farms. Finches, house sparrows, and cow-birds appear to be especially at risk. These infected birds may transmit infection to humans, either directly as a result of handling, or more commonly, as a result of exposure to domestic cats infected by preying on sick and moribund birds. The risks associated with this type of outbreak occurring in Australia are difficult to determine but would not be negligible.

Highly pathogenic avian influenza H5N1 virus or avian influenza is also spread via migratory birds. The East Asian-Australasian flyway and the West Pacific flyway of winter migratory birds include Australia, and while it is possible that H5N1 virus could be brought to Australia by migratory birds, it is unlikely, as only shorebirds tend to travel to Australia. No birds with the H5N1 strain of avian influenza have ever been found in Australia.

3.6.2.2 Water birds

Wild water birds are extremely common throughout Australia's inland wetlands, rivers and lakes. Water birds can be found in diverse, shallow, fresh water habitats ranging from lakes and rivers to sewage ponds, irrigation channels, and floodplains. Figure 6 shows wild and domestic dusks feeding together in an irrigation channel.

Deep water (e.g. large, deep dams) is said to discourage feeding and breeding (Kingsford and Norman, 2002). Ninety-three water bird species are known to be found in Australia (Kingsford and Norman, 2002). These species are included in six major orders shown in Table 6. Due to the highly social nature of these birds it is relatively easy for transmission of avian disease to pass through a flock and affect all local birds.

Figure 6 Wild and domestic dusks feeding in an irrigation channel upstream of a dairy farm outside Cobram, Victoria. Wild birds can be the source of zoonotic pathogens.

Table 6. Australian water birds found predominantly in freshwater habitats

Order	*Representative species*
Anseriformes	*Ducks, geese and Black swan*
Podicipediformes	*Grebes*
Pelecaniformes	*Australian pelican and cormorants*
Ciconiiformes	*Herons, ibis, spoonbills and bitterns*
Gruiformes	*Cranes, rails, crakes and gallinules*
Charadiiformes	*Waders and terns*

3.6.2.3 Wild animals

In dry eucalypt open-forest and woodlands, such as in Australia's Central and North Central regions, waterways such as creeks and rivers provide a unique habitat for native fauna such as reptiles, marsupials, arboreal animals like possums and small terrestrial mammals such as the yellow-footed antechinus.

Several factors influence the presence of Australia native animals in watersheds and along water ways, these include;

- land use (e.g. agricultural land or national park),
- presence of non-native species and their diversity,
- human population size and
- climatic conditions (e.g. drought).

The role of native animals in the transmission of waterborne disease is not fully understood and there is a scarcity of published information in this area.

Examples of published works include;

- the relationship of marsupials in the transmission of *Giardia* and *Cryptosporidium* (Buckley *et al.*, 1997; Power *et al.*, 2005),
- Presence of *Chlamydia* in Australian frogs and Koalas (Pospisil and Canderle, 2004)
- how toxoplasmosis is a common cause of death in wild and captive Australian marsupials (Eastern barred bandicoots, wallabies, kangaroos and wombats) (Hartley, 2006) and

- the presence of non-human strains of Cryptosporidium in Bilbies (Warren *et al.*, 2003).

3.6.3 Land use

Australia is a predominantly arid country and relies heavily on irrigation for agricultural sustainability. Large increases in demand for meat, milk and eggs are expected as the world population increases (Bradford, 1999) and irrigation is seen as a means to increase agricultural production while the amount of land used for agricultural purposes stays at the same level. Australian Bureau of Statistics data shows that between 10 and 25% of all land in north-central Victoria is irrigated and in general between 5 and 10 megalitres of water per hectare is used annually on that irrigated land (ABS, 2002-3). The Australian average for irrigated land is 4.4 megalitres per hectare (ABS, 2006). While irrigation is critical to providing fresh and affordable food, this form of intensive agriculture is associated with an undetermined increase in zoonotic disease, particularly where animal agriculture is involved. As intensive agricultural practices are said to be increasing at the rate of 4.3% per year (Bradford, 1999) the role of livestock in waterborne bacterial outbreaks must be better defined. In the past this role has been difficult to define as both humans and various wildlife species can shed the same microorganisms and thereby serve as sources of infection.

3.7 Identification of Zoonotic agents in water

Currently, there is no simple, reliable, universal method to collect, process, and analyse a water sample for all pathogenic microorganisms of interest (viruses, bacteria, protozoa). There are many difficulties in developing a universal method and these include the physical differences between the major pathogen groups (e.g. size, nutrient requirements etc.), efficiently concentrating large volume water samples to detect low target concentrations of certain pathogen groups, removing co-concentrated inhibitors from the sample (e.g. PCR inhibitors and agents that affect cell viability), and standardizing culture-independent detection methods. Integrating the technologies into a single, universal, simple method and detection system would represent a significant advance in public health and microbiological water quality analysis. Recent advances in sample collection (tangential flow), on-line sample processing and purification (robotic sample handling to extract and process DNA), and advanced DNA technologies (DGGE and microarrays) may form the basis of a universal method to detect known and emerging waterborne pathogens and these methodologies are presented here.

3.7.1 Sample collection

Traditional methods of water sample collection and microbe concentration use dip cups in combination with membrane filtration methods. In general water samples are collected using a stainless-steel weighted bottle sampler. This weighted bottle sampler holds up to a 1-liter bottle made of either Teflon or

polyethylene or glass. The sampler is lowered to the water by using a hand reel and synthetic rope (nylon or polyethylene) configuration. The sample is then filtered using gravity or suction techniques to collect microbes on the filter for further analysis. This technique commonly referred to as Normal Flow Filtration (NFF) may be used for filtration of clean streams, however, if the water is turbid due to a high microbial load and/or mud then the filtration can fail due to membrane fouling. Membrane-based Tangential Flow Filtration (TFF) differs from traditional method as the fluid passes tangentially along the surface of the membrane rather than being forced directly down and through it. An applied pressure serves to force a portion of the fluid through the membrane to the filtrate side. As in NFF particulates and macromolecules, that are too large to pass through the membrane pores, are retained on the upstream side. However, in this case the retained components do not build up at the surface of the membrane. Instead, they are swept along by the tangential flow and collected as a concentrate. This feature of TFF makes it an ideal process for finer sized-based separations and allows large volumes of water to be filtered at the collection site. The difference between NFF and TFF is shown in Figure 7.

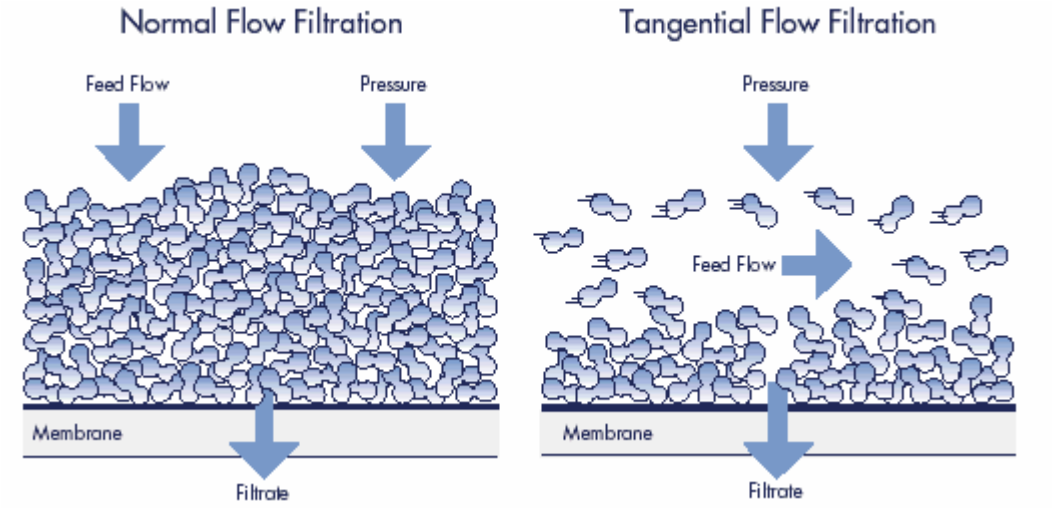

Figure 7. Comparison of NFF and TFF (from http://www.millipore.com/publications.nsf/docs/tb032)

3.7.2 Review of traditional testing methods for detecting waterborne pathogens

Traditional testing methods for water supplies rely on the detection of microbial indicators that indicate the presence of faecal pollution and the potential presence of other human pathogens, (see Appendix 7 for Microbiological Water Quality Guidelines). These methods are generally agar based and require a minimum of 24 hours for cultures to grow. Evidence suggests however, that in any sample, only 0.01-1.0 % of the viable bacteria may be detected using culture methods. It has also been shown that unfavourable conditions for growth promote a state where organisms are 'viable but non-culturable', which also results in these low detection rates. Conventional testing methods for detecting indicator and pathogenic organisms in water, may underestimate the actual population due to sub-

lethal environmental injury, inability of the target organism to take up nutrients and other physiological factors which reduce bacterial cultivability. The current Australian standard testing protocol is based on AS 4276. The International Organization for Standardization also provides methods for the detection and enumeration of indicator organisms under 07.100.20 – Microbiology of water. The use of indicator organisms is based on the assumption that indicator organisms, such as *E. coli*, are only found in mammals such as cattle, dogs and humans and their presence in water indicates recent contamination with faecal material. Indicator tests are still in current use as no single procedure has proved suitable for the identification of all pathogens present in water. The high costs of testing and a requirement for trained personnel are also a limiting factor in extending water testing to unusual pathogens. Table 7 shows current and alternative testing methods. There are several other drawbacks to the use of these methods. In particular, it should be noted that absence of indicator organisms does not mean that the water is free of pathogens including those pathogens not associated with faecal contamination like *Mycobacterium ulcerans*. *M. ulcerans* is the cause of 'Bairnsdale ulcer' and is known in the broadsheet press as the 'flesh eating bacteria'. Equally a decrease in the numbers of indicator organisms does not necessarily point towards a decrease in pathogenic viruses.

3.7.3 Review of molecular testing methods for detecting water borne pathogens

Molecular biology techniques may be used to detect pathogens in water supplies. One method that has proved particularly useful is used to identify unknown bacteria isolated from traditional agar culture plates. The method relies on sequencing the 16S rRNA gene, a region of DNA approximately 1500 bases in length which can then be compared to a database of all known microbes. However the techniques used to study bacterial communities in environmental samples are rapidly developing, particularly in the area of molecular methods for the identification and monitoring of microorganisms in natural ecosystems (Hoefel *et al.*, 2005; Innok *et al.*, 2005; Lyautey *et al.*, 2005; Lyautey *et al.*, 2005; Ball and Crawford, 2006; Niva *et al.*, 2006). The trend is towards culture independent methods because they are believed to overcome problems associated with selective cultivation and isolation of bacteria from natural samples.

Table 7. List of Standard laboratory based methods

The following methods are commonly used by laboratories to test water to AS 4276

Name of Test	Basis of method	Costs per test[a]	Training /expertise required	Comments
1. Cultivation techniques	The most probable number (MPN) test estimates the total numbers of indicator bacteria in a given sample of water.	Low	Minimal. Tests are often performed by technicians	Costs are generally low but precision is often poor. Readily available test.
2. Enrichment techniques	These techniques are used to detect specific pathogens that are not identified in standard cultivation techniques.	Medium	Interpretation of results usually requires personnel with microbiology degree	Costs are usually much higher than standard cultivation techniques and in general take several days longer to obtain a result. Readily available test.
3. Filtration methods	Bacteria and parasites may be recovered (and numbers estimated) from the surface of a membrane filter.	Low	Minimal. Tests are often performed by technicians	Performed in addition to 1 & 2 above. Readily available test.
4. Immuno-capture	This method is based on a concentration procedure using antibodies attached to magnetic beads. The beads are exposed to a large-volume water-sample and the antibodies attached to the bead capture the microbes to which they have an affinity. The magnetic beads are then captured and placed in a suitable growth medium at the testing laboratory	High	Minimal. Tests are often performed by technicians	Performed in addition to 1 & 2 above
5. Flocculation	Viruses and parasites may be concentrated by adsorbtion to flocculants such as aluminium hydroxide and calcium carbonate.	Medium	Minimal. Tests are often performed by technicians	The drawback of this technique is that it has been shown to affect the viability of *Crytosporidium* sp.
Continuous flow centrifugation	This technique allows continuous concentration of samples..	High	Minimal. Tests are often performed by technicians	This test would be used with a cultivation or identification technique
Alternative methods (Research methodology that may not yet be associated with any international water testing standards)				
Acridine Orange stain	Live bacteria are concentrated by membrane filtration and visualised using a fluorochrome stain that causes bacteria to glow. Dead bacteria do not fluoresce in the same manner as live bacteria.	High	Interpretation of results requires personnel with microbiology degree	This method cannot determine the identification of the bacteria present

Physiological activitiy detection in bacteria	Electron transporter indicators, such as 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride [INT], may be used to indicate respiratory activity of any bacteria in a sample.	High	Interpretation of results requires personnel with microbiology degree	This gives a 'viable bacteria present? - yes or no' result, but does not identify bacteria.
Molecular techniques for the identification of pathogens	PCR- using single primers, multiplex or real-time methods 16S rRNA sequencing for pathogen identification Micro-arrays	High	Interpretation of results requires personnel with microbiology degree or PhD	These tests requires a high level of operator competence and associated costs reflect this

[a] Costs described as low would be around a few dollars per test, medium between $10-100 and high costs are over $100 per test.

The principal reason for the use of culture-independent techniques is the lack of knowledge of the real conditions under which most of bacteria are growing in their natural habitat and the difficulty to develop media for cultivation accurately resembling these conditions. Genetic fingerprinting techniques are able to provide a profile representing the genetic diversity of a microbial community from a specific environment. Denaturing gradient gel electrophoresis (DGGE) is perhaps the most commonly used among the culture-independent fingerprinting techniques. PCR-DGGE of ribosomal DNA was introduced into microbial ecology by Muyzer (Muyzer *et al.*, 1993; Muyzer and Smalla, 1998) and is based on the separation of polymerase chain reaction (PCR) amplicons of the same size but different sequences, see figure 8.

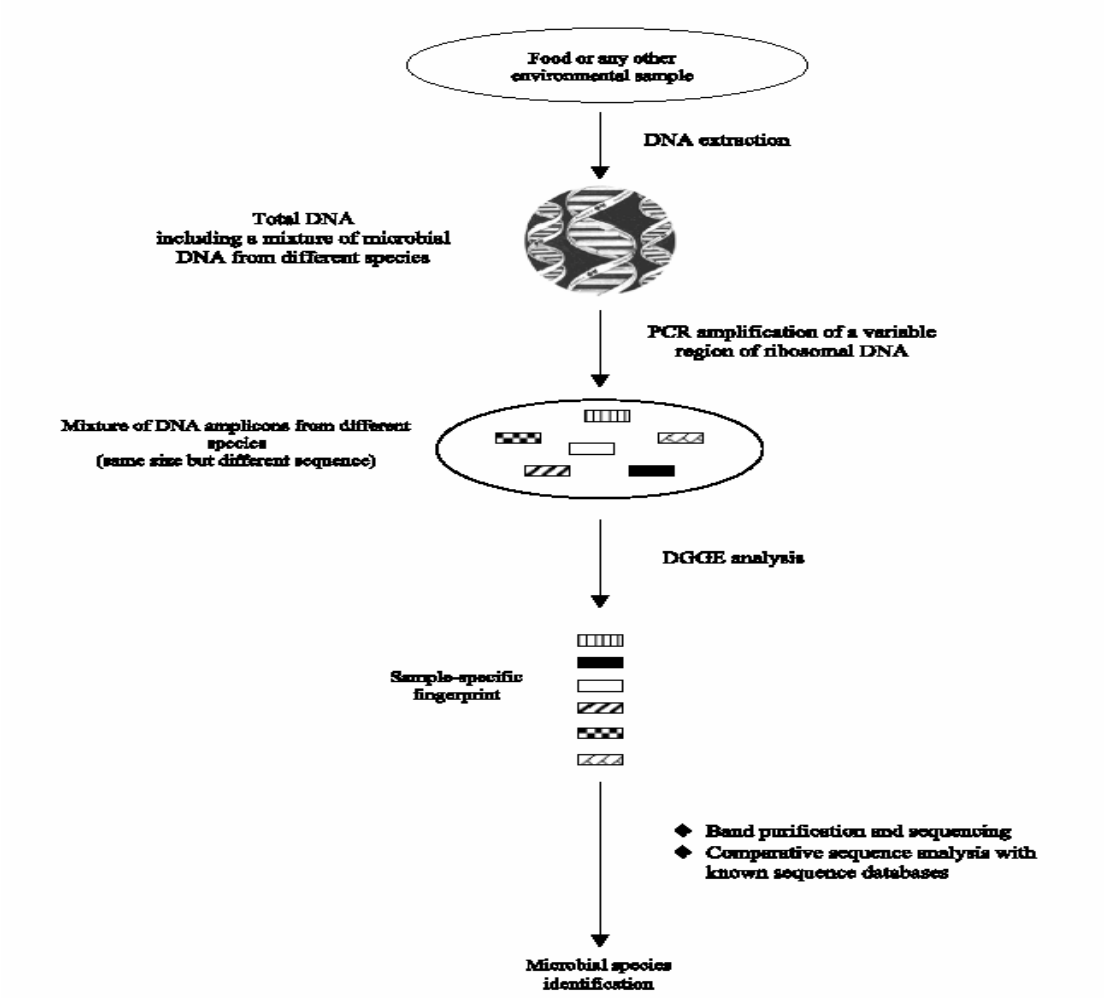

Figure 8 Flow diagram of the application of PCR-DGGE in food and environmental samples, after Ercolini (Ercolini, 2004)

PCR-DGGE is usually employed to assess the structure of microbial communities in environmental samples without cultivation, and to determine the community dynamics in response to environmental variations.

3.7.4 Review of currently available commercial kits for identification of water borne pathogens

Several commercial test kits are available for field testing of water for the presence of pathogens. These tests can be divided into several types including:

- Agar coated plastic slides that are dipped into the water and returned to the laboratory for incubation. These tests are primarily designed to detect *Legionella* sp., *Enterobacteriacea* and sulphate reducing bacteria.
- Screening tests for *E. coli* and based on the use of chromogenic compounds in a dehydrated growth media. This test requires a sample of water to be added to a bag containing a dehydrated growth medium. After overnight incubation the bag is observed for colour changes that indicate a positive result.
- Screening tests for other faecal indicators are available including such tests as Enterolert (IDEXX) which provides results in 24 hours
- Magnetic bead kits. These kits are based on the immuno-capture technique described above. Samples are still required to be returned to a laboratory for completion of the test.

The target market for these tests includes farmers and land holders who may want to test dam or other stored water for the presence of enteric bacteria e.g. *E. coli*. These tests have a number of limitations and any positive test may require further testing for confirmation and identification purposes, therefore they are not a good basis for risk management of waterways.

4. Summary

It is now, and has been in the past, a major challenge to water suppliers such as the Goulburn-Murray Rural Water Authority to be able to identify all bacterial, viral and parasitic pathogens when they contaminate water supplies in moderate and high risk numbers. This challenge can only be harder when trying to identify the same pathogens in a low risk situation, for example, where only a few viable cells have been released in a single episode of contamination.

Economic sustainability of agriculture and protection of human and animal health is dependant upon good quality water and one of challenges of the future may well be the requirement of rapid detection and removal of new waterborne pathogens. These could include identification of *Burkholderia* and *Francisella* spp not previously described in Victorian waterways.

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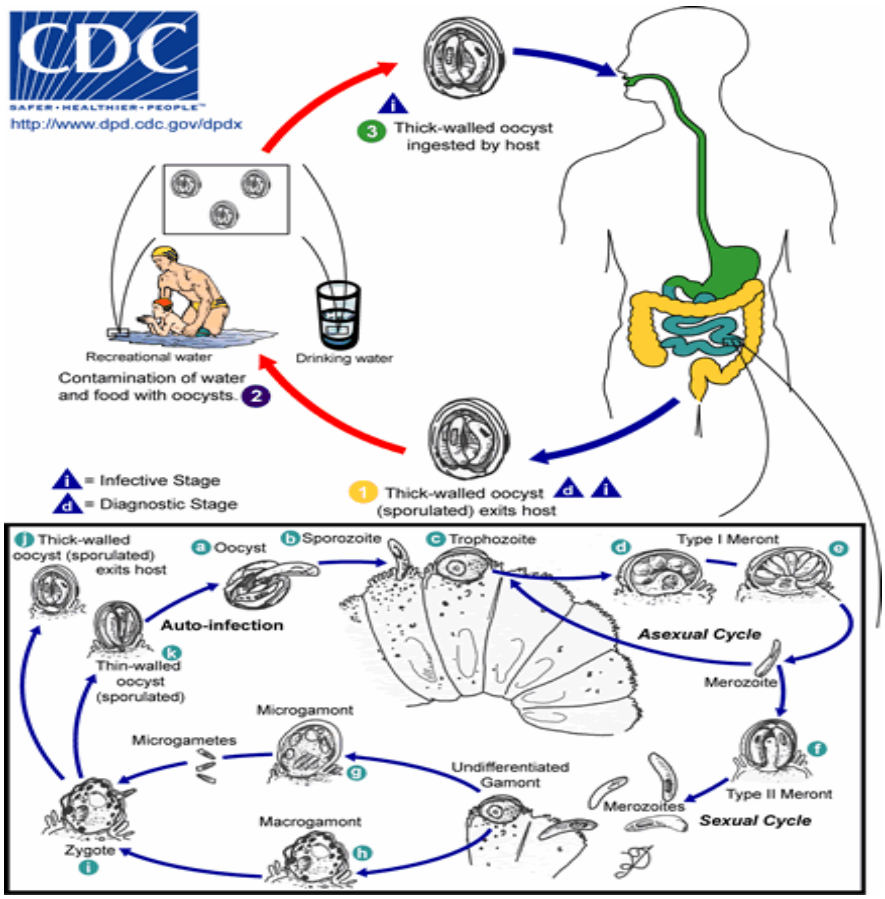
Appendix 1 Life cycles of significant parasites- Cryptosporidium



Life cycle of *Cryptosporidium parvum* and *C. hominis*. *Cryptosporidium* stages were reproduced from Juranek DD. Cryptosporidiosis. In: Strickland GT, editor. Hunter's Tropical Medicine and Emerging Infectious Diseases, 8th ed. Philadelphia: WB Saunders; 2000. Originally adapted from the life cycle that appears in Current WL, Garcia LS. Cryptosporidiosis. Clinc Microbiol Rev 1991;4:325-58.

Sporulated oocysts, containing 4 sporozoites, are excreted by the infected host through feces and possibly other routes such as respiratory secretions (1). Transmission of *Cryptosporidium parvum* and *C. hominis* occurs mainly through contact with contaminated water (e.g., drinking or recreational water). Outbreaks in the United States have occurred in waterparks, community swimming pools, and day care centers. (2). Following ingestion (and possibly inhalation) by a suitable host (3), excystation (a) occurs. The sporozoites are released and parasitize epithelial cells ((b), (c)) of the gastrointestinal tract or other tissues such as the respiratory tract. In these cells, the parasites undergo asexual multiplication (schizogony or merogony) ((d), (e), (f)) and then sexual multiplication (gametogony) producing microgamonts (male) (g) and macrogamonts (female) (h). Upon fertilization of the macrogamonts by the microgametes ((i)), oocysts ((j), (k)) develop that sporulate in the infected host. Two different types of oocysts are produced, the thick-walled, which is commonly excreted from the host (j), and the thin-walled oocyst (k), which is primarily involved in autoinfection. Oocysts are infective upon excretion, thus permitting direct and immediate fecal-oral transmission. Note that oocysts of *Cyclospora cayetanensis*, another important coccidian parasite, are unsporulated

at the time of excretion and do not become infective until sporulation is completed. Refer to the life cycle of *Cyclospora cayentanensis* for further details.

Appendix 2 Life cycles of significant parasites- Giardia

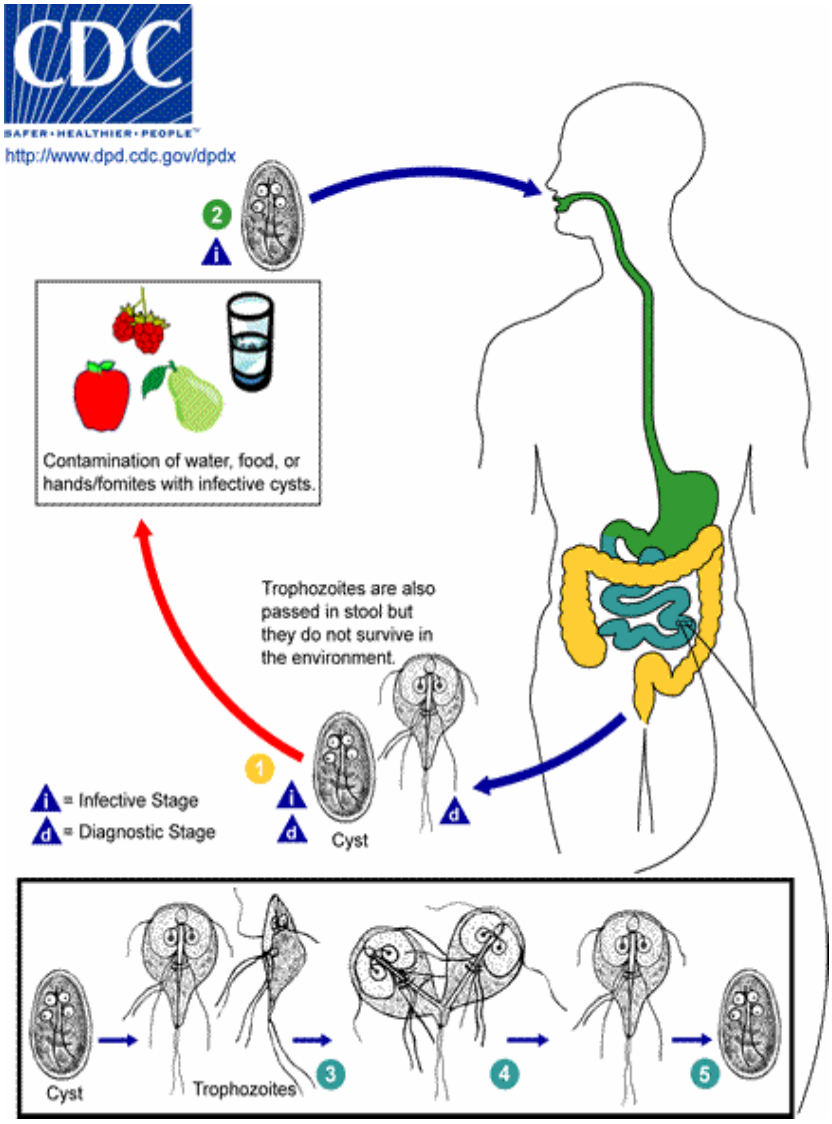


Figure reproduced from
http://www.dpd.cdc.gov/dpdx/HTML/Image_Library.htm

Cysts are resistant forms and are responsible for transmission of giardiasis. Both cysts and trophozoites can be found in the feces (diagnostic stages) 1. The cysts are hardy, can survive several months in cold water. Infection occurs by the ingestion of cysts in contaminated water, food, or by the fecal-oral route (hands or fomites) 2. In the small intestine, excystation releases trophozoites (each cyst produces two trophozoites) 3. Trophozoites multiply by longitudinal binary fission remaining in the lumen of the proximal small bowel where they can be free or attached to the

mucosa by a ventral sucking disk 4. Encystation occurs as the parasites transit toward the colon. The cyst is the stage found most commonly in non-diarrheal feces 5. Because the cysts are infectious when passed in the stool or shortly afterward, person-to-person transmission is possible. While animals are infected with *Giardia*, their importance as a reservoir is unclear.

Appendix 3 : Classification of Water Related Zoonotic Diseases (Moe, 2004)

Category	**Zoonotic example**	**Relevant control strategies**
Waterborne via drinking water	Salmonellosis, E.coli O157:H7, cryptosporidiosis, giardiasis, campylobacteriosis, microsporidiosis, toxplasmosis, balantidiasis, yersinosis, tularaemia, cysticercosis	Protect drinking water sources from contamination by animal faeces Improve microbiological water quality through water treatment
Water borne via recreational water contact	Leptospirosis, cryptosporidiosis, giardiasis	Protect water source from animal contamination
Water washed	Cryptosporidiosis, giardiasis, balantidiasis, hepatitis E virus?	Increase water quantity to improve hygiene Promote hand washing
Water-based	Schistosomiasis (*Schistosoma japonicum*)	Protect user Control aquatic hosts Surface water management
Water-related insect vectors	West Nile virus, Rift valley fever virus, yellow fever virus, sleeping sickness (African trypanosomiasis)	Protect user Control aquatic hosts Surface water management
Inhalation water/waste water aerosols	Mycobacteria	Protect individual who have occupational exposure Limit human exposure to geographic areas impacted by aerosols
Aquatic food	Paragonimiasis	Avoid ingestion of raw or under cooked crustaceans Prevent faecal contamination of freshwater crab and crayfish habitats Control snails by molluscicides

Appendix 4 : Examples of Some of Known Water Borne Zoonotic Pathogens & Their Characteristics (Carr and Bartram, 2004)

Pathogen	**Characteristic**
Cryptosporidium parvum (protozoa)	Widespread domestic and wild animal reservoirs; environmentally persistent; resistant to treatment processes; low infectious dose; limited immune response in 1993 the Crypto outbreaks in Milwaukee, USA believe to have caused 54 deaths
Toxoplasma gondi (Protozoa)	Widespread domestic and wild animal reservoirs; environmentally persistent; resistant to treatment processes; resistant to antibiotics; low infection dose
Campylobacter jejuni (Bacteria)	Widespread domestic and wild animal reservoirs; antibiotic resistant strains; low infectious dose
Eschericha coli O157:H7 (Bacteria)	Widespread domestic and wild animal reservoirs; severe outcomes in children; low infectious dose; resistant to antibiotics;
Mycobacterium avium ssp. *Paratuberculosis* (Bacteria)	Infects wide range of animal hosts, both domestic and wild, and is wide spread in various regions; cause sever illness in susceptible organisms; evade the immune system by surviving in macrophages; environmentally persistent; resistant to heat and chlorination and can survive in bio-films and distribution systems; difficult to isolate , culture and identify; transmission route includes drinking water, recreational water contact and inhalation of aerosols
Hepatitis E virus (HEV) (Virus)	Geographic spread and frequency of animal reservoirs unknown; severe outcomes in pregnant women; low infectious dose; detection and cultivation difficult;
Fasciola hepatica (Parasite-Liver fluke)	Widespread in domestic and wild animals in certain regions; disease of moderate to high severity; low infection dose; limited immune response; human variant has a novel properties that facilitate the transmission through drinking water

Appendix 5. : Pathogens found in **irrigation waters** and wastewaters that may adversely affect human health (Metcalf & Eddy 1991)

	Organisms	Disease	Remarks
	Bacteria		
	Escherichia coli	Gastroenteritis	Diarrhoea
	Legionell pneumophila	Legionellosis	Acute respiratory illness
	Leptospira	Leptospirosis	Jaundice, fever
	Salmonella sp.	Salmonellosis	Food poisoning
	Salmonella typhi	Typhoid fever	Diarrhoea, fever
	Shigella	Shigellosis	Bacillary dysentery
	Vibrio cholerae	Cholera	Diarrhoea, dehydration
	Yersinia enterolitica	Yersinosis	Diarrhoea
	Viruses		
	Adenovirus	Respiratory disease	
	Enterovirus	Gastroenteritis, meningitis	
	Hepatitis A	Infectious hepatitis	Jaundice, fever
	Norwalk agent	Gastroenteritis	Vomiting
	Reovirus	Gastroenteritis	
	Rotavirus	Gastroenteritis	
	Protozoa		
	Balantidium coli	Balantidiasis	Diarrhoea, dysentery
	Cryptosporidium	Cryptosporidiosis	Diarrhoea
	Entamoeba hystolica	Amebiasis	Amoebic dysentery
	Giardia lamblia	Giardiasis	Diarrhoea, nausea
	Helminthis		
	Ascaris lumbricoides	Ascariasis	Roundworm infestation
	Enterobius vericularis	Enterobiasis	Pinworm
	Fasciola hepatica	Fascioliasis	Sheep liver fluke
	Hymnolepis nana	Hymenolepiasis	Dwarf tapeworm
	Taenia saginata	Taeniasis	Beef tapeworm
	Taenia solium	Taeniasis	Pork tapeworm
	Trichuris trichiura	Trichuriasis	whipworm

Appendix 6 : Examples of plant pathogens found in irrigation water in particular in recycled irrigation water (Dutky 1995)

Viruses	Tomato mosaic virus Cucumber green mottle virus Pelargonium flower break virus Camion motile virus
Fungi	*Phytophthora cryptogea* *Phytophthora nicotianae* *Plasmopara lactuae-radicis* *Phythium asphanidermatum* *Phythium dissotocum* *Phythium intermedium* *Phythium myriotylum* *Fusarium oxysporum*
Bacteria	*Pseudomonas solanacearum* *Xanthomonas campestris*

Appendix 7: Microbiological Water Quality for Different Water Usages

7.1. Guidelines Trigger Values for Thermotolerant Coliforms/faecal coliform for Irrigation Water (ANZECC and ARMCANZ, 2000)

Cfu=Colony forming units

Intended use of water	Level of thermotolerant coliforms (median value)
Thermotolerant Coliforms Raw human food crops in direct contact with irrigation water (via sprays, irrigation of salad vegetables)	<10 cfu/100mL
Raw human food crops not in direct contact with irrigation water (edible product separated from contact with water eg. by peel, use of trickle irrigation) ; or crops sold to consumers cooked or processed	<1000 cfu/100mL
Pasture and fodder for dairy animals (without holding period)	<100 cfu/100mL
Pasture and fodder for dairy animals (with holding period of 5 days)	<1000 cfu/100mL
Pasture and fodder (cattle, sheep and goats)	<1000 cfu/100mL
Silviculture, turf, cotton	< 10 000 cfu/100 mL
Helminths Protection of crops where it is endemic	<1 helminth egg / L

7.2 Guidelines Trigger Values for Thermotolerant Coliforms/faecal coliform for Recreational Water (ANZECC and ARMCANZ, 2000)

Intended use of water	Level of thermotolerant coliforms, enterococci and protozoa (median value)	Comments
Drinking water for livestock	<100 /100 mL	
Recreation-Primary contact	< 150 /100mL faecal coliform	***Faecal coliform :*** minimum of five samples taken at regular

	or 35 enterococci organisms/100mL Pathogenic protozoa -nil	intervals not exceeding one month, with four out of five samples containing less than 600 orgnaims/100 mL ***Enterococci :*** maximum number in one sample : 60-100 organisms/100mL
Recreation-Secondary contact	< 1000 /100mL faecal coliform or 230 enterococci organisms/100mL Pathogenic protozoa -nil	***Faecal coliform :*** minimum of five samples taken at regular intervals not exceeding one month, with four out of five samples containing less than 4000 orgnaims/100 mL ***Enterococci :*** maximum number in one sample : 450-700 organisms/100mL

Primary contact includes swimming, bathing and water skiing where there is a likelihood of swallowing water
Secondary contact includes fishing, canoeing and boating where there is less contact with the water body

7.3 Guidelines Trigger Values for Thermotolerant Coliforms/faecal coliform for **Drinking Water** (NHRMC and ARMCANZ, 1996; ANZECC and ARMCANZ, 2000)

Intended use	**Thermotolerant coliforms**	**Comments**
Drinking water for livestock	<100 /100 mL	
Drinking water for human	No *E. coli*	

Appendix 7.4 : . Summary of Water Quality Criteria for Microbiological Indicators (source **:**
http://www.env.gov.bc.ca/wat/wq/BCguidelines/microbiology/microbiology.htmlupdated **2001)**

Water Use	Escherichia coli	Enterococci	Pseudomonas aeruginosa	Fecal coliforms
Raw Drinking Water - no treatment	0/100 mL	0/100 mL	0/100 mL	0/100 mL
Raw Drinking Water - disinfection only	less than or equal to 10/100 mL 90th percentile	less than or equal to 3/100 mL 90th percentile	None applicable	less than or equal to 10/100 mL 90th percentile
Raw Drinking Water - partial treatment	less than or equal to 100/100 mL 90th percentile	less than or equal to 25/100 mL 90th percentile	None applicable	less than or equal to 100/100 mL 90th percentile
Raw Drinking Water - complete treatment	None applicable	None applicable	None applicable	None applicable
Aquatic Life - shellfish harvesting	less than or equal to 43/100 mL 90th percentile	less than or equal to 11/100 mL 90th percentile	None applicable	less than or equal to 43/100 mL 90th percentile
Aquatic Life - shellfish harvesting	less than or equal to 14/100 mL median	less than or equal to 4/100 mL median	None applicable	less than or equal to 14/100 mL median
Wildlife	None applicable	None applicable	None applicable	None applicable
Livestock - free range animals	None applicable	None applicable	None applicable	None applicable

Livestock - general livestock use	200/100 mL maximum	50/100 mL maximum	None applicable	200/100 mL maximum
Livestock - closely confined (no treatment)	0/100 mL maximum	0/100 mL maximum	None applicable	0/100 mL maximum
Livestock - closely confined (disinfection only)	less than or equal to 10/100 mL 90th percentile	less than or equal to 3/100 mL 90th percentile	None applicable	less than or equal to 10/100 mL 90th percentile
Livestock - closely confined (partial treatment)	less than or equal to 100/100 mL 90th percentile	less than or equal to 25/100 mL 90th percentile	None applicable	less than or equal to 100/100 mL 90th percentile
Livestock - closely confined (complete treatment)	None applicable	None applicable	None applicable	None applicable
Irrigation - crops eaten raw	less than or equal to 77/100 mL geometric mean	less than or equal to 20/100 mL geometric mean	None applicable	less than or equal to 200/100 mL geometric mean
Irrigation - public access - livestock access	less than or equal to 385/100 mL geometric mean	less than or equal to 100/100 mL geometric mean	less than or equal to 10/100 mL 75th percentile	None applicable
Irrigation - general irrigation	less than or equal to 1000/100 mL geometric mean	less than or equal to 250/100 mL geometric mean	None applicable	less than or equal to 1000/100 mL geometric mean
Recreation - aesthetics - non contact	None applicable	None applicable	None applicable	None applicable
Recreation - secondary contact - crustacean harvesting	less than or equal to 385/100 mL	less than or equal to 100/100 mL	less than or equal to 10/100 mL	None applicable

	geometric mean	geometric mean	75th percentile	
Recreation - primary contact	less than or equal to 77/100 mL geometric mean	less than or equal to 20/100 mL geometric mean	less than or equal to 2/100 mL 75th percentile	less than or equal to 200/100 mL geometric mean
Industrial Water (dairy, food processing) - no treatment	0/100 mL	0/100 mL	None applicable	0/100 mL
Industrial Water (dairy, food processing) - disinfection only	less than or equal to 10/100 mL 90th percentile	less than or equal to 3/100 mL 90th percentile	None applicable	less than or equal to 10/100 mL 90th percentile
Industrial Water (dairy, food processing) - partial treatment	less than or equal to 100/100 mL 90th percentile	less than or equal to 25/100 mL 90th percentile	None applicable	less than or equal to 100/100 mL 90th percentile
Industrial Water (dairy, food processing) - complete treatment	None applicable	None applicable	None applicable	None applicable
Industrial Water - other industries	less than or equal to 385/100 mL geometric mean	less than or equal to 100/100 mL geometric mean	less than or equal to 10/100 mL 75th percentile	None applicable

1. Fecal coliform criteria which presently exist will apply on an interim basis until use of the other preferred indicators is adopted.
2. For the dairy industry there is an additional criterion of less than or equal to 5/100 mL lipolytic and/or proteolytic bacteria.
3. Medians and geometric means are calculated from at least 5 samples in a 30-day period. Ten samples are required for 90th percentiles.
4. These recreation and shellfish harvesting criteria are applicable to fresh and marine waters, except the E. coli criteria, which apply only to fresh water.
5. Only a few salad greens which cannot be adequately washed to remove adhering or trapped pathogens are of concern under the crops eaten raw section of irrigation. Examples include lettuce, cabbage, broccoli, cauliflower and similar crops.
6. These primary contact recreation criteria may be subject to revision depending upon the future results of a federal/provincial study group on Canadian recreational water quality.

Appendix 7.5 : Guidelines for the microbiological quality of various ready to eat foods

TABLE I Guidelines for the microbiological quality of various ready-to-eat foods

Food category (see table 2)	Criterion	Microbiological quality (CFU per gram unless stated)			
		Satisfactory	Acceptable	Unsatisfactory	Unacceptable/ potentially hazardous*
	Aerobic colony count† 30°C/48h				
1		$<10^3$	10^3-$<10^4$	$\geq 10^4$	N/A
2		$<10^4$	10^4-$<10^5$	$\geq 10^5$	N/A
3		$<10^5$	10^5-$<10^6$	$\geq 10^6$	N/A
4		$<10^6$	10^6-$<10^7$	$\geq 10^7$	N/A
5		N/A	N/A	N/A	N/A
	Indicator organisms‡				
1-5	Enterobacteriaceae§	<100	100-$<10^4$	$\geq 10^4$	N/A
1-5	*E. coli* (total)	<20	20-<100	≥100	N/A
1-5	*Listeria* spp (total)	<20	20-<100	≥100	N/A
	Pathogens				
1-5	*Salmonella* spp	not detected in 25g			detected in 25g
1-5	*Campylobacter* spp	not detected in 25g			detected in 25g
1-5	*E. coli* O157 & other VTEC	not detected in 25g			detected in 25g
1-5	*V. cholerae*	not detected in 25g			detected in 25g
1-5	*V. parahaemolyticus*¶	<20	20-<100	100-$<10^3$	$\geq 10^3$
1-5	*L. monocytogenes*	<20**	20-<100	N/A	≥100
1-5	*S. aureus*	<20	20-<100	100-$<10^4$	$\geq 10^4$
1-5	*C. perfringens*	<20	20-<100	100-$<10^4$	$\geq 10^4$
1-5	*B. cereus* and other pathogenic *Bacillus* spp#	$<10^3$	10^3-$<10^4$	10^4-$<10^5$	$\geq 10^5$

* Prosecution based solely on high colony counts and/or indicator organisms in the absence of other criteria of unacceptability is unlikely to be successful.
† Guidelines for aerobic colony counts may not apply to certain fermented foods – for example, salami, soft cheese, and unpasteurised yoghurt. These foods fall into category 5. Acceptability is based on appearance, smell, texture, and the levels or absence of indicator organisms or pathogens.
‡ On occasions some strains may be pathogenic.
§ Not applicable to fresh fruit, vegetables and salad vegetables.
¶ Relevant to seafood only.
# If the *Bacillus* counts exceed 10^4 CFU/g, the organism should be identified.
** Not detected in 25g for certain long shelf-life products under refridgeration
NA Not applicable

source : http://www.hpa.org.uk/cdph/issues/CDPHvol3/No3/guides_micro.pdf