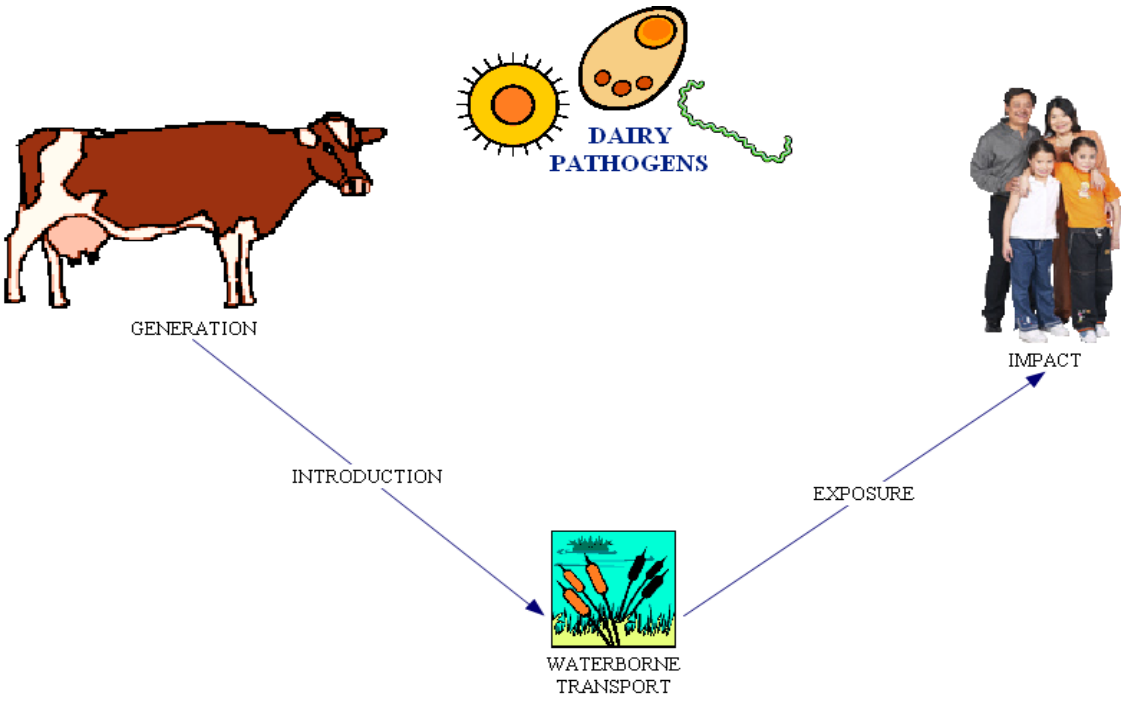
Waterborne Dairy Pathogens.

An analysis of industry research needs.



Dairy Australia

Your Levy at Work

The Waterborne Dairy Pathogens project is a Dairy Australia initiative funded by the Dairy Service Levy and the Australian Government.

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Waterborne Dairy Pathogens
An analysis of industry research needs

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WATERBORNE DAIRY PATHOGENS

Introduction

Pathogens

Overview

Pathogen is a general term to describe disease causing organisms. Zoonoses are infectious diseases that can be transferred between animals and humans. Pepper et al (2006) and Birchall et al (2008) provide information on a wide range of pathogens, including bacteria, parasites, viruses, fungi and prions. Examples of each, together with a brief overview, are presented below.

Bacteria e.g;

- *Escherichia coli* – causing diarrhoea
- *Campylobacteraceae jejuni* – causing gastroenteritis
- *Salmonella sp* – a cause of food poisoning
- *Listeria monocytogenes* – potentially leading to meningitis or miscarriage
- *Mycobacterium paratuberculosis* – Johne's Disease; which may be linked to Crohn's Disease

Many waterborne bacterial pathogens are ingested, reproduce in the gut of a host and are excreted to the environment in faeces. They can be transmitted by direct contact (e.g. human to human) or by contaminated food or water. Some bacteria survive reasonably well outside a host, especially in an aquatic environment (Haydon, 2006). Most bacteria are inactivated at temperatures above 60^{o}C. Bacteria range in size from 0.1 to 20 microns (*u*m – a millionth of a metre).

Parasites, including;

- Protozoa, e.g;
  - *Cryptosporidium parvum* – causing diarrhoea
  - *Giardia lamblia* – causing diarrhoea and nausea
- Multi-cellular worm like organisms, e.g;
  - Liverfluke – a disease mainly affecting farmed animals
  - Tapeworms

Protozoa exhibit a variety of life cycles. In simple cycles (e.g. *Giardia*) cysts are ingested by a host resulting in infection and reproduction within the host, and the formation of new cysts that are excreted to the environment. More complex cycles (e.g. *Cryptosporidium*) involve additional stages within the host prior to the release of oocysts. Parasitic worms (helminths) may also involve further stages in other host species as well. Young animals, without immunity, are more prone to infection by parasites and shed large numbers of cysts and oocysts – which can lead to seasonal variations in parasite populations (Haydon, 2006). Most can persist in the environment for many months. The survival of protozoa decreases with rising temperatures, although freezing is also fatal for *Giardia*. Protozoa range in size from 4 to 10 *u*m.

Viruses, e.g;

- Rotaviruses – causing diarrhoea in infants and young children.
- Foot and mouth disease.

Many viruses are host-specific. They are ingested by a host, replicate in the host, and are excreted in faeces. Transmission is via contaminated food or water (often from partially treated sewage or septic tanks) and direct contact. They can persist for long periods in the environment and some (e.g. rotavirus and Hepatitis A) can accumulate in shellfish (e.g. oysters) due to their filter feeding action and the protective environment inside the shell. Many viruses can survive for years at low temperatures (below 5^{o}C) and survival decreases with temperature (Haydon, 2006). Viruses are very small, ranging in size from 20 to 100 nm (nanometers – or milli-microns).

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Fungi, e.g;
- Ringworm

Prions, e.g;
- Bovine spongiform encephalitis (BSE or mad cow disease) – a fatal brain disease - that has not occurred in Australia.

Some of these agents are hosted by dairy cows (and often by other organisms as well), and some are resident in Australia.

Transmission

Zoonotic disease can be spread by faeces, urine, saliva, blood and milk. Pathogens that live in the gut of animals (enteric) may be excreted in faeces and transported further afield by water – referred to as waterborne enteric pathogens. Pepper et al (2006) report the conclusion of other research that, globally, 75% of infectious diseases are zoonotic and that 80% of those are waterborne.

To cause waterborne disease, pathogens from animals must be introduced to water, remain viable in water (in sufficient numbers), and be introduced to a prospective host (exposure). Parasites and viruses cannot multiply in water when distant from a host, but in the right conditions, bacteria may.

Understanding

The Understanding Dairy Catchments Tool (Pollino, 2008) proposes six key questions to explore knowledge about waterborne contaminants. With regard to dairy pathogens, the general answers are well known:

- Impacts? Human health issues, and industries and activities affected by water quality (e.g. oyster growing and recreation involving direct contact with water).
- Causes? Zoonotic organisms, as described above.
- Sources? Dairy cows, other livestock, native animals, humans and foods.
- Pathways? Waterborne (and other).
- Dairy farm management? Stock access to waterways, effluent recycling, dams and other 'sinks', and buffers such as vegetated (riparian) filter strips.
- Driving changed management? Environmental regulations (effluent), riparian programs, stock health management.

A conceptual model of that understanding about dairy pathogens is illustrated overpage. It involves:
- the generation of pathogens by cows (e.g. in dung and urine),
- their movement (introduction) to a water-body,
- survival and transport in a waterway,
- exposure to humans (directly or via contaminated products) and
- the onset of disease, or curtailment of economic or recreational activity (impact).

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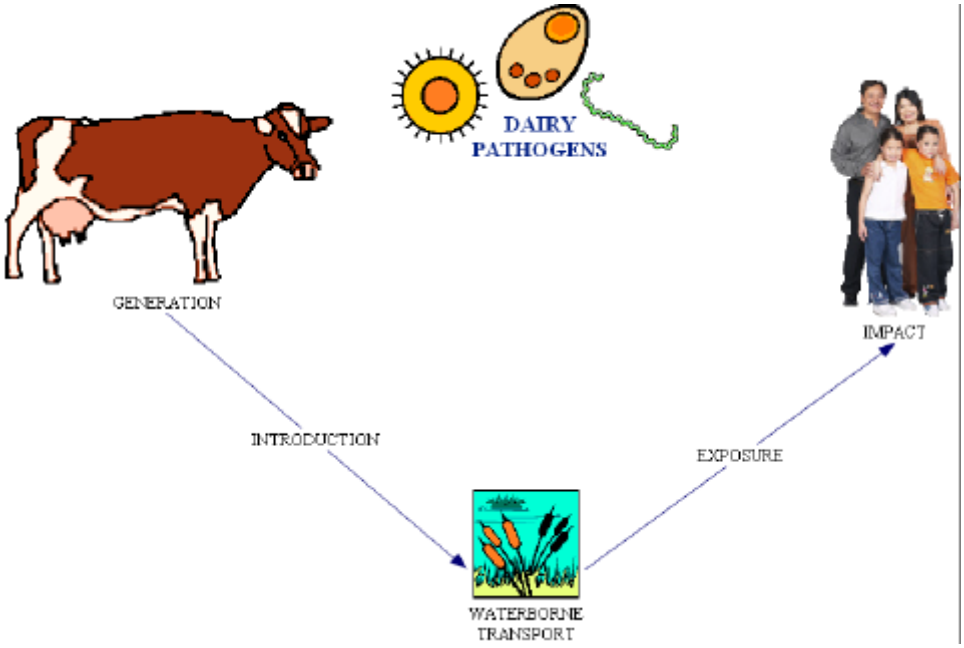
Conceptual model



Monitoring pathogens

It is not usually recommended practice to monitor for specific pathogens in the environment as it is not particularly accurate, is time consuming and expensive (Haydon, 2006). *E. coli* has been considered to not generally reproduce in water and is the dominant organism in faeces; and testing for it is relatively cheap, fast and accurate – hence its use as a standard indicator of faecal contamination.

However, there is increasing evidence of re-growth of *E. coli* in some waters and sediments (Mandeville, 2006). Furthermore, given that different pathogens move through the environment along different pathways and at different rates (as well as having different decay functions) it's very high value as a universal indicator may not persist in future thinking. Adcock (2001) notes that *Campylobacter* do not replicate in the environment yet they are a significant pathogen and persist in drainage waters from irrigated dairy pastures suggesting they could be a useful indicator species for dairy.

Industry implications

Dairying has the potential to contribute to public health risks through the contamination of water by pathogens. The industry and farmers must therefore understand that risk and take appropriate measures to manage the risk.

However, the issue is not straight forward. Complexity arises because there are multiple potential sources and pathways of pathogens affecting humans and waterways. It is often difficult to isolate the causes of disease outbreaks – and immediate perceptions may not prove accurate. The dairy industry is open to quick judgments that may be erroneous; yet, in some instances, it could be the cause of human health impacts.

It is essential that the industry has a thorough understanding of all issues affecting farming and the potential for health (and economic) impacts to others; and that it is able to apply that knowledge to reduce risk and to ensure it is not unjustly blamed for any outbreaks.

WATERBORNE DAIRY PATHOGENS

About this report

This report describes current knowledge about waterborne dairy pathogens and the risk they pose to human health and economic activity. It comments on current research initiatives in Australia and identifies knowledge priorities for the dairy industry.

The report:

- Describes the pathogens of most interest,
- Discusses policy options to manage the risks posed by waterborne pathogens,
- Reviews literature about dairy pathogens – their generation on farms, introduction to water and transport, and their impact on human health,
- Assesses knowledge gaps, and
- Presents conclusions regarding recommended management practices and priorities for dairy research.

Pathogens and policies

Dairy pathogens

Pepper et (2006) and Birchall et al (2008) both provide an extensive overview of pathogens that may be associated with dairy farms and cows. WHO (2006) provides an extensive coverage of all waterborne pathogens.

Bacteria

Coliform bacteria is a term for bacteria of the *Enterobacteriaceae* family, such as *Escherichia coli*, that are detected by a standard 'coliform' test, that is used as an indicator of the presence of bacteria.

Escherichia coli are commonly found in humans and other vertebrate animals. They are generally 'harmless' but some strains cause diarrhoea and severe illness, especially in infants. In extreme cases, some strains can be fatal. *E. coli* 0157:H7 is a virulent strain capable of stimulating 'Shiga-like toxins' that are important in the onset of haemorrhagic colitis and hemolytic-uremic syndrome (HUS) affecting the kidneys, intestines and central nervous system (Ibekwe, 2002).

E. coli are transferred by food and water and are commonly found in ruminants, as well as other vertebrate species. *E. coli* is often used as an indicator of the presence of faecal bacteria, so it is important to have a good understanding of them and their movement in the environment.

Campylobacteraceae jejuni cause gastroenteritis in humans. *Campylobacteraceae* are the most frequently identified cause of bacterial diarrhea in industrialized countries (Pepper et al, 2006), with *Campylobacteraceae jejuni* being most common. They are part of the normal intestinal flora of birds (including ducks, pigeons, poultry, seagulls, blackbirds, starlings and sparrows) and pigs, but can also be found in cattle (and other animals) and be transmitted in milk. Faecal contamination is the main form of transmission and they are widespread in surface waters; even though they are sensitive to desiccation and do not grow at temperatures below 30^{o}C. Sheep, goats, dogs, rabbits, cats – and possibly rodents, kittens, beetles and flies – also carry campylobacters. Oral ingestion of contaminated water or food (including milk) is the main cause of infection.

Salmonella *sp* cause gastroenteritis through food poisoning. They can survive for prolonged periods in the environment forming a 'reservoir' that may be a source of on-going re-infection of livestock, contributing to 'chronic herd infection' (Edrington, 2007). *Salmonella* are associated with humans, cattle, sheep, pigs, poultry, wild birds, pets, rodents and snakes. Antibiotic resistant strains are emerging and multi-drug-resistance has been linked to dairy herds (Tait & Cullen, 2006). Humans can contract the disease by ingesting contaminated food stuffs, including fresh vegetables.

Listeria monocytogenes has little impact on healthy people but can be fatal to those with suppressed function of their immune system (resulting in meningitis and blood poisoning), and to unborn babies (resulting in miscarriage or still birth). Humans usually come into contact with *Listeria* via food-borne pathways, but it is widespread in the environment (being able to grow in soil and water) and is carried by domestic and wild animals. Raw foods may be contaminated from any of those sources, including irrigated stock effluent. The organism can grow in poorly fermented silage and cows can develop mastitis as a result of infection by *Listeria* (Pepper et al, 2006).

Mycobacterium avium sub-species ***paratuberculosis (MAP)***, causes Johne's Disease in cattle and there has been speculation that it may be linked to Crohn's Disease in humans. Cows with

Johne's excrete the bacteria in faeces (and there is evidence of it being secreted in milk). MAP is resistant to chlorine, has been found in tap water and is thought to grow in bio-films that form on the inside of water pipes. An American study (Pierce, 2009) documents a cluster of unrelated individuals with Crohn's Disease and identifies a possible link to treated drinking water. Although a causal link has been proposed between MAP and Crohn's Disease the Chief Medical Adviser to the Australian Government has concluded that there is no substantive evidence to support the suggestion (McClintock, 1998). There is evidence that a small proportion of MAP organisms in milk can remain viable following pasteurization.

Leptospira cause Leptospirosis in humans, which begins with flu-like fevers and vomiting, but may progress to meningitis, liver damage and renal failure in later stages. It is found in humans and animals – rodents and wild animals are considered to be primary 'reservoirs', shedding the disease in their urine. The bacteria may be free-living and survive well in water, soil and mud in tropical areas.

Parasites

Parasite is a generic term for organisms that live on, or in, another (host) species and gain food from them. Two main forms of pathogenic parasites are microbial protozoa and a range of worm like organisms.

Two common protozoan pathogens are *Cryptosporidium* and *Giardia*.

Cryptosporidium parvum is an intestinal protozoa causing diarrhoea in humans, especially infants and children. The high incidence of disease in children under four may reflect their vulnerability as well as an increased likelihood of exposure through poor hygiene (it being water and food borne). It is reasonably persistent in water and is resistant to many disinfection techniques (including chlorine), so water treatment plants are not always effective in its removal. It is naturally present in cattle (especially young calves), sheep and goats and in native animals, including kangaroos – although the forms found in kangaroos are generally not infectious to humans (Bryan et al, 2009). Many species or genotypes of *Cryptosporidium* appear to be host specific and western grey kangaroos predominantly shed a marsupial genotype that has not been found in humans – although it is noted that host-specific forms should not be excluded as potential human pathogens (Power et al, 2005).

Giardia lamblia causes diarrhoea and nausea, and is the most commonly found intestinal parasite throughout the world, especially in children in developing countries. It is waterborne and has a cyst stage (oocyst) that survives in water and food before becoming active in the acidic environment of the stomach. It is frequently identified in young calves and is also often associated with urban sewage.

Parasitic worms ('helminths') that can infect cattle include roundworms (nematodes) and flatworms such as tapeworms (cestodes) and flukes (trematodes) (Pepper et al, 2006).

Liver fluke (**Fasciola hepatica**) cause disease and production loss in farmed animals (cattle and sheep) and can also infect humans, native animals (kangaroos and wombats) and rabbits. Eggs from the parasites are excreted in dung and hatch into larvae that infect snails where they multiply and form another larval stage – which emerges from the snail, attaches to vegetation and forms cysts that are ingested by grazing animals (AWI, 2010).

Ascaris suum (roundworm) is a parasite of pigs that occasionally infects cattle and is capable, on rare occasions, of infecting people (Collick, 2007). Known as a hardy organism, the eggs are more resistant to stress than the oocysts of *Cryptosporidium*, but have been inactivated by heat (>55^{o}C) and aridity in biodrying compost systems used for dairy manure.

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Viruses

Viruses are responsible for many human diseases, such as influenza, mumps, measles and chicken pox. Rotavirus is a common group of viruses that cause severe gastroenteritis with vomiting and diarrhoea in infants and young children who are yet to establish immunity. They are highly infectious and can be spread easily by faecal – hand - oral pathways.

Noroviruses (a major source of food-borne gastroenteritis) may be found in livestock (cattle and pigs) and humans – each with their own strain. However, human strains have been found in dairy cattle raising the prospect of indirect zoonotic infections – and bovine/human recombinations (Mattison et al, 2007)

Prions

Bovine spongiform encephalitis (BSE or mad cow disease), is a fatal brain disease caused by prions. Prions are made up of protein molecules. They do not possess nucleic acid but are able to cause other proteins to change into an abnormal form. It is thought that prions could leach from the carcasses of dead animals into water supplies. BSE has not been detected in Australia.

Impacts

The pathogens of most concern to the US dairy industry as a result of confirmed or suspected links to outbreaks of disease in humans, have been listed as bacteria *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, *Mycobacterium paratuberculosis*, and the protozoan parasites *Cryptosporidium parvum*, and *Giardia* spp (Birchall et al, 2008). In New Zealand, *Campylobacter jejuni* is reported to be the principal bacterial hazard for drinking water and recreational water users (Birchall et al, 2008), although *Giardia* is the most commonly notified waterborne disease, with high incidence rates when compared to other developed countries (Tait & Cullen, 2006).

Campylobacter bacteria are the most common reported cause of gastroenteritis in South Australia (Adcock, 2001).

Major outbreaks of serious waterborne diseases are comparatively rare in Australia but enteric pathogens are frequently present in rural waterways and thought to be responsible for low-level incidences of waterborne microbial disease (Pepper et al, 2006).

Pathways

Waterborne dairy pathogens usually arise from dung or urine and may:

- Be excreted directly into streams,
- Flow from inadequate effluent treatment (such as pond overflow),
- Be carried in paddock run-off either from faeces deposited by grazing cattle of from recycled effluent applied to paddocks, or
- Flow from wetlands or dams (that may act as 'sinks' as well as 'sources').

Humans may be exposed to waterborne pathogens by direct contact (e.g. water sports such as swimming), by drinking it (either with or without prior treatment), or by consuming food contaminated by poor quality water (e.g. oysters or fresh vegetables contaminated by irrigated effluent). The impact may be an immediate one of public health or an economic penalty on industries reliant upon un-contaminated water.

Many of the pathogens carried by dairy cows are also carried by other animals (including native wildlife such as birds and kangaroos), and may be introduced to waterways along similar paths.

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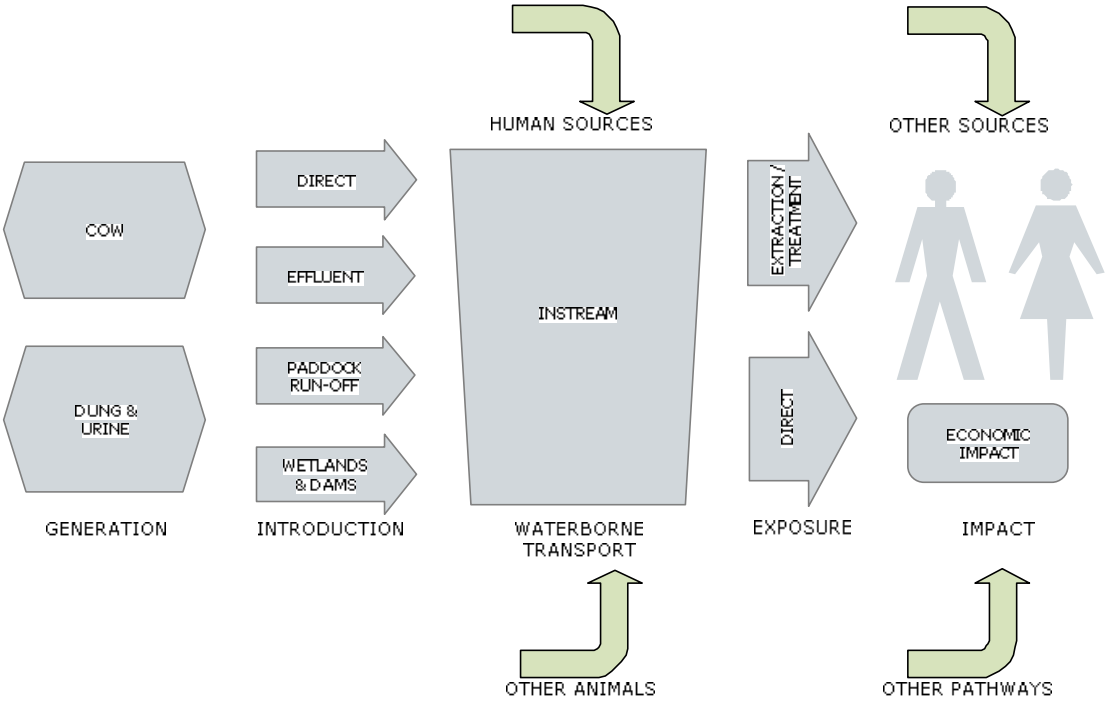
Thanks to improved genetic testing techniques, it is becoming easier to identify specific strains from different types of animals and to assess their impact on human health. Pathogens from human sources may also be introduced to streams (e.g. via sewage and inadequate sanitation).

There are also pathways other than by water between humans and dairy farms for pathogens (e.g. direct infection to farmers or to the consumers of contaminated milk products). Furthermore, humans may also be infected by pathogens from other sources as many pathogens common in dairy cattle are prevalent in other animals, the environment, and humans; and may be conveyed via contaminated non-dairy foods or poor personal hygiene.

This report does not consider those other pathways; nor does it consider the fouling of water by other sources such as wildlife, other domestic or feral animals, or human sewage.

Pathogen pathways



Policy options

Risk Management

Policies in Europe and the United States of America focus on not exceeding prescribed maximum levels of contaminants in waters for drinking, bathing and shellfish harvesting. In the USA, the Clean Water Act regulates by way of Total Maximum Daily Loads for water with 'impaired' quality. In the EU, the Water Framework Directive requires the identification of 'pressures and impacts' and 'programmes of measures' to achieve specified water quality outcomes (Kay et al, 2007). It is supported by the Bathing Water Directive that reinforces water quality standards from the World Health Organisation.

These approaches focus attention on quantifying the dynamics of faecal indicator bacteria (mainly coliforms and enterococci), such as source loadings, transport rates and inactivation.

These quantitative policy approaches have several short-comings (Kay et al, 2007 and Deere & Davison, 1998) such as:

- There is a tendency to focus on matching the concentration of contaminants in point source discharges with water quality standards – without consideration of spatial and temporal (event based) variations or total loads.
- There is insufficient data available on land use effects, seasonal impacts on catchment-scale fluxes (and concentrations of contaminants from different sources) to confidently establish meaningful local targets or programs of measures.
- There isn't the capability to apportion loadings of contaminants to individual sources within catchments.
- Inadequate support from sampling programs that fail to capture information during episodic events, and detection methods that are not suitable for routine policing.
- An assumption that adequate regulatory processes exist to deal with diffuse sources of contamination, such as imposing changes in land use or introducing 'altered farm support structures'.
- Insufficient consideration of the feasibility of achieving water quality targets.
- The detection of abnormal levels of contaminants in drinking water occurs too late to protect consumers.

Deere & Davison (1998) reported on legislation in the United Kingdom that required water providers to test for *Cryptosporidium* and to not exceed a specified concentration. The Act was in response to an outbreak and was introduced to aid prosecutions (and defences) following an unsuccessful prosecution.

Given the difficulty of quantitative regulations there have been calls for a 'new regulatory paradigm for drinking water protection' and encouragement for 'water safety planning' – a risk management approach (Kay et al, 2007). Hazard Assessment Critical Control Point (HACCP) styled approaches require an assessment of risks within contributing catchments – an approach driven by Australian researchers and water managers.

The World Health Organisation (2006) advocates a framework for safe drinking-water that includes health based targets and risk-based water safety plans.

The risk-based approach results in more attention to protecting the 'gathering grounds' (source areas) in catchments and invites a 'multiple barrier' approach to public health protection (Kay et al, 2007). There will be more emphasis on process performance at critical control points and less on end-of-treatment contaminant concentrations (Ashbolt). A principal of 'tolerable disease burden' will be founded on quantitative risk assessments, at each of the 'barriers'. Risk assessments will require data on the generation of pathogens from different sources within catchments, on inactivation rates, and on rates of human exposure to pathogens.

The HACCP approach aims to control risks as near to their source as possible and requires a detailed evaluation of each 'step' in a process or pathway. Critical factors that can be measured and managed are monitored and reported upon (Deere & Davison, 1998). It involves:

- A detailed analysis of the system,
- Identification of critical control points,
- Development of target levels and critical limits,
- A monitoring system,
- Corrective action,
- Verification procedures, and
- Documentation.

A risk-based approach will address some of the short-comings of water quality concentration targets, but will still be reliant on an adequate understanding of how pathogens move through catchments and impact upon people, as well as adequate information in the form of hard data.

Water quality and public health policies will give direction and momentum to the sort of programs and research questions that are relevant to industry but there will also be need to respond to emergencies. Health authorities faced with a disease outbreak will want quick answers to some basic questions; where is it coming from, how is it getting here, and how/where can it be stopped? There may also be a question from various stakeholders of; who can we blame, or get compensation from?

Models and other tools

Most policy responses will inevitably rely to some degree upon models. A range of models have been developed to describe how pathogens are generated and move through catchments, and there are also several risk identification tools available.

Hipsey et al (2008) – Centre for Water Research, University of WA (and others, in SA) – developed a generic process based model of microbial pollution in aquatic systems (e.g. reservoirs). The model fits with CAEDYM (Computational Aquatic Ecosystem Dynamics Model) and can couple with multi-dimensional hydrodynamic models. It is a generic model, allowing different key coefficients to be adjusted for different organisms and different environments. The variables include growth rates, mortality (including temperature, salinity and pH), inactivation by sunlight, predation and grazing, plus sedimentation, survival and re-suspension.

Ferguson et al (2005 & 2007) developed a process based model to predict pathogen catchment budgets for Sydney drinking water catchments. It uses a mass balance approach and predicts total loads of *Cryptosporidium*, *Giardia* and *E. coli* generated and exported from sub-catchments. Its modules include a land budget, in-stream transport, sewage treatment, and on-site systems. The outputs of this model may be used as input to a hydrodynamic model (e.g. as developed by Hipsey et al) to predict the fate of the organisms. The model can identify priority sub-catchments in terms of the generation of pathogen loads, prioritize management options and assess the consequences of changes in land use. It could be augmented with economic information to perform cost-benefit assessments of alternative catchment risk reduction programs. Bryan et al (2009) applied this model in the Myponga catchment, in South Australia. Haydon (2006) notes the hydrological component of the model is relatively simple – and in effect a steady-state model for three rainfall states; dry, intermediate wet, and large wet.

Haydon (2006) developed a model to predict pathogen concentrations in catchment run-off as a time series for a PhD thesis at Monash University. A 'simple lumped conceptual modeling approach' was used, coupling a pathogen model to an existing model of rainfall run-off. The model is run on an hourly timestep. Haydon notes that few existing models have been 'adequately calibrated or tested'. His model was tested in the O'Shannassy (Vic), Myponga and

Aldgate (SA) catchments, calibrated, sensitivity tested and run through an uncertainty analysis of the model inputs.

Hart et al (2006) promote the use of Bayesian models for catchment analysis, noting that process based models:

- Are not able to deal with multiple stressors in a systematic way,
- Rarely treat uncertainty explicitly, and
- Rarely link the contaminant with downstream impacts.

Oliver et al (2010) developed a risk assessment tool for application on-farm in the United Kingdom; a faecal indicator organism risk indexing tool (FIORIT) – to prioritize fields most at risk of contributing faecal indicator organisms to waterways. It aims to combine risk assessment with risk communication. Its trial application (ten farms and 162 paddocks) revealed that only 1-3% of paddocks (less than 10% of the area) were at risk – primarily due to strong hydrological links between source areas and watercourses. Further testing in a range of environments is required for a more robust tool.

Kay (2007) reports a catchment simulation model developed in New Zealand by Collins and Rutherford to predict coliform concentrations. It incorporates daily livestock data, simulated surface and sub-surface flows and faecal indicator fluxes, and direct deposition where livestock have access to streams.

The Water Research Centre, University of NSW has modeled pathogens (viruses and parasitic protozoa) and the nutrient performance of on-site systems (Katrina Charles PhD) and developed a Decision Support System to differentiate between human, herbivore and other sources of faecal pollution by load or percentage.

Key points

- There is a wide array of waterborne pathogens present in the environment and a wide array of associated illnesses.
- Humans may be exposed to pathogens from a variety of sources, via diverse pathways.
- Major outbreaks of waterborne illness are rare in Australia, but enteric pathogens are common in the environment and have been linked to low-level incidences of disease.
- There is a sound general understanding about pathogens in catchments but far less confidence in detailed aspects regarding pathways, fluxes and causal links. It is easy to make broad generalizations but very difficult to establish proofs.
- Multi-barrier risk management is likely to be a more effective policy tool than regulated targets regarding the incidence of pathogens.
- Risk management will require modeling and while models exist, they highlight a lack of knowledge about processes and pathways for different pathogens and a lack of Australian data on matters such as pathogen generation, transport and survival rates.

Generation of pathogens

Sources of pathogens

Cows and calves

For many dairy pathogens, their incidence is greatest amongst juvenile stock which have not established immunity. A detailed literature review of pathogens in faecal matrices and sewage effluent (Ferguson et al, 2009) showed that, in terms of both prevalence and concentration, juvenile animals were higher than adults and domestic animals were higher than wildlife. There were extensive variations in concentrations (even up to four orders of magnitude from the same matrices). Reducing the levels of infection in domestic animals was noted as an option to reduce pathogen numbers in streams, along with improved treatment of domestic sewage.

Newman et al (2003) report evidence that calves can produce huge numbers of *Cryptosporidium* oocysts (ten billion/day for several weeks) and act as reservoirs of infection. Adult cows are generally resistant to the disease but may occasionally shed small numbers of oocysts. Similarly, a survey of cattle in southern Alberta (Canada) found 23% of calves to be infected with *Giardia*, but none of the surveyed adult cattle. Another Canadian study found the peak concentration of *Cryptosporidium* oocysts in a stream to coincide with calving (Newman et al, 2003).

In an American study of 30 calves over their first two years of life, Santin et al (2008) found that nearly all were infected with *Cryptosporidium parvum* in the first three weeks after birth. They noted that humans working with calves were at risk of being infected and becoming a source of infection to other people. Their study highlighted the importance of knowing the genotypes present to determine the zoonotic implications.

*Mycobacterium paratuberculosis (*Johne's Disease in ruminants*)* affects many animals, with young ones being most susceptible (McClintock, 1998). Calves may be infected by ingesting feed, milk or water that is contaminated with faecal matter from infected stock. Some infected adults may develop clinical disease symptoms (chronic diarrhoea, wasting and even death). Johne's Disease is endemic in south-eastern Australia (infecting from 3-20% of herds) but is absent from WA, Queensland and the Northern Territory.

Cows may become infected by ingesting pathogens with feed or water that is contaminated with faeces. Pathogens may be excreted in faeces (that may contaminate milk), as well as in milk by infected cows with mastitis (Oliver, 2006). They can also be transmitted in urine, respiratory secretions, sloughed skin etc, and be collected in waste management systems. Birchall et al (2008) report a long list of pathogens found in dairy effluent.

A New Zealand study of faeces from 19 herds of cattle and sheep (Bailey et al, 2003) found *Campylobacter* species (the leading causative agent of acute diarrhoea in humans in many industrialized countries) in 6% of dairy herds, 58% of feedlot cattle, 2% of pastured beef cattle, less than 1% of mutton and 8% of prime lambs – implying that production systems may influence disease rates. *Listeria ivanovil* was cultured from one dairy cow.

Leptospirosis is widespread on Victorian dairy farms that do not vaccinate against it (Chappel 1997).

Ferguson et al (2007) reported that wildlife excrete significant loads of *E. coli*, but they had lower prevalence and concentrations of *Cryptosporidium* and *Giardia* (and the genotypes exhibited by wildlife were unlikely to be infectious to humans). They also reported evidence that oocysts were easily freed from cow manure and readily transported by surface run-off. Cows may also deposit faeces directly into streams.

WATERBORNE DAIRY PATHOGENS

Water troughs

Stock water troughs may become a source of pathogens on dairy farms. *E. coli* 0157:H7 can survive for extended periods in soil and water, and in freezing, acidic and dry conditions (Islam et al, 2004). It has persisted at high levels in cattle faeces and for at least 245 days in sediments in a simulated water trough.

Surface drains

An examination of drainage water from surface irrigated dairy pastures on the Lower River Murray (Saint, 1999) found stable populations present of *E. coli*. Levels would drop rapidly from peaks following irrigation or rainfall to a standing population of around 10^5/100mL that could persist for an extended period of time. The bacteria appeared to survive longer at 10°C than at 19°C. Water samples in this study provided no evidence of *Cryptosporidium* or *Giardia* although a related study (Adcock, 2001) detected *Cryptosporidium* twice, *Giardia* once and *Campylobacter* regularly. *Salmonella* were not detected in irrigation water but were found in drainage water.

Some water samples from the drains were tested for faecal sterols however it was difficult to determine if they were from human or animal sources – although contributions from birds were consistently identified (Saint, 1999).

Effluent

It has been postulated that grazing of pastures irrigated with effluent, without an adequate withholding period, may promote ongoing cycles of re-infection and lead to high levels of campylobacters in NZ dairy herds (Tait & Cullen, 2006). Islam et al (2004) recommended composting or aging manure to eliminate pathogens before using it as a fertiliser. Oliver (2006) reported that *E. coli* 0157:H7 has been isolated on vegetables exposed to bovine manure compost and contaminated irrigation water, and that the pathogen persisted in soil for more than five months. Ravva et al (2006) reported that *E. coli* failed to proliferate in dairy effluent but did persist (possibly in viable but non-culturable states).

Aarons (2006) reports that bacteria deposited on pastures have been shown to survive for up to six days and Kay (2007) reports field plot trials showing *E. coli* to survive for five to six months in deposited faecal matter. Die-off is more rapid at higher temperatures and if manure is left on the surface and not incorporated into the soil.

An American study of 20 farms found 1.4% of cows bedded on sand carried *E. coli* 0157:H7, while twice that rate, 3.1%, was found in cows on saw-dust beds (LeJune, 2005). It was suggested the drier sand also contained less carbon on which bacteria could feed. Airborne transport on dust particles was noted as one possible means of transport. Airborne transport may be an issue for cows in feedlots where dust is more common.

Survival

Haydon (2006) reports the survival of pathogens in fields to largely be a factor of how hot and dry conditions become. Pathogen decay is a function of evapotranspiration and soil moisture. Risk assessments and models often adopt a standard 'daily deposition rate' per cow and multiply it by stocking rates; and the estimate adopted for deposition rates can have a significant bearing on model outcomes (along with rainfall and catchment area). Temperature, desiccation and UV light influence pathogen survival, and decay is often exponential and up to forty days (or longer) in wet environments – less if dry and/or hot.

Many human pathogens are 'stenothermal' – they can only grow around a small temperature range (37°C); but may survive for long periods outside that range (Haydon, 2006).

Cryptosporidium can only survive a few hours in dry conditions, but they may be protected within dung in the field. Oxygen, nutrient and pH levels also affect microorganisms.

Davies et al (2004) used 'fluorescence in situ hybridization' (FISH) to estimate the viability of *Cryptosporidium* oocysts and concluded that (in the absence of sunlight – the most important single factor affecting bacterial inactivation) temperature and soil type were the most significant of influences. Temperatures of 35°C may result in enhanced inactivation. They stressed the importance of using inactivation rates that were appropriate to local soils and climate when modeling the fate of pathogens.

Jiang (2008) notes that bacterial die-off is exponential and common survival times are in the order of months; although there is considerable variability. Jiang summarizes the factors affecting bacterial survival as:

- Sunlight. The sterilizing effect of ultraviolet light (and drying) lowers the survival rates of bacteria, although it has little penetration into soil.
- Temperature. Microorganisms survive longer in cold soils than warm soils – die-off rates double with each 10°C between 5 and 30°C. *E. coli* have been reported to survive for 42-49 days at 37°C, 49-56 days at 22°C, and 63-70 days at 5°C.
- Soil moisture. Microorganisms can survive very dry conditions, but *E. coli* and *Enterococcus* survive best in soils close to field capacity.
- pH. Bacteria survive a range of pH levels but die-off is least in the 6-7 range. Growth and activity are less in acidic conditions.
- Organic matter. Coliform bacteria survival is enhanced by higher levels of organic matter.
- Soil Texture. Bacteria survive longer in fine textured soils.
- Nutrient availability. *E. coli* can replicate and migrate in soil cores and prosper most in soils with higher nutrient levels.

Fact sheets summarizing findings from the Cooperative Research Centre for Water Quality and Treatment report that temperature is the most influential factor for *Cryptosporidium* inactivation in soils and faeces; with higher inactivation at higher temperatures. There was no significant difference in die-off rates between the surface and interior of cow pats.

Little is known of predation upon pathogens in the field but, if soil moisture is adequate, it may be significant within the soil (Haydon, 2006). Soils with high levels of carbon usually support more soil biota which may increase predation. Bryan and Kandula (2009) report that dung beetles can digest and effectively inactivate *Cryptosporidium* oocysts. In a modelled scenario it was assumed that dung beetles could reduce manure export by 50%, and reduce *Cryptosporidium* export by 21.7% in wet weather. No benefits were achieved in dry weather as there was no run-off or transport of pathogens in those conditions (Bryan et al, 2009).

In terms of pathogen budgets, some pathogens would also be ingested by grazing herbivores – although likely rates are unknown (Haydon, 2006).

Cryptosporidium in water bodies were found to be subject to significant levels of predation by other microbes and to suffer inactivation at higher temperatures – due to increased metabolic activity (King et al, 2007).

Other sources

The above discussion focuses on waterborne pathogens in the context of dairy farms, but it must be remembered that many of the species present on farms can reach humans from other sources as well, and via transport mechanisms other than water.

Wild birds and animals (including kangaroos and fish) are considered to be a major contributor to zoonotic disease (Pepper et al, 2004). Many bird species are highly sociable, as well as mobile,

and may spread pathogens quickly. This is especially so with migratory species. Gulls, pigeons, sparrows, finches and starlings have all be found to carry bacteria such as *E. coli* and are suspected of transmitting disease long distances as well as being reservoirs supporting re-infection (Pedersen & Clark, 2007).

Other native animals have also been reported as hosts of pathogens, but their role is 'not fully understood' and there is a 'scarcity of published information in this area' (Pepper et al, 2006). Ferguson et al (2007) reported that wildlife excrete significant loads of *E. coli*, but they had lower prevalence and concentrations of *Cryptosporidium* and *Giardia*, and the genotypes (strains) exhibited by wildlife were unlikely to be infectious to humans.

Management

Saint (1999) suggested that to reduce the number of *E. coli* transported from flood irrigated dairy pastures by surface drains it could be effective to not irrigate immediately following the removal of cattle and to hold run-off in drains for several days to allow bacterial populations to return to normal levels.

Adcock (2001) noted that recycling irrigation drainage water could recycle pathogens as well and increase the risk of stock infection. High irrigation efficiency – with low run-off – would be one way to reduce the risk of contamination to waterways, as would avoiding the application of animal waste slurries to flood irrigated paddocks. As calves can be readily colonized by *Cryptosporidium* and *E. coli*, they should not be grazed on pastures at high risk of contributing run-off to drains and waterways. Grassed buffers could also be useful barriers.

Key points

- The incidence of many pathogens is highest in calves; although overall there is little data on infection rates in Australia or on the impacts of infection on stock performance and health.
- Pathogens may survive in troughs, drains, effluent and soil; leading to ongoing cycles of re-infection.
- The survival of pathogens is affected by sunlight, temperature and soil moisture, along with other soil parameters.
- The degree of predation, or the impact of dung beetles, is largely unquantified in Australian circumstances.
- Many of the pathogens found in dairy cattle also occur in other animals. Whenever possible it is important to identify the specific strains that are present and to assess their specific impact (direct or indirect) on human health.
- There are management options available that may be applied in many situations to reduce the exposure of stock to, and the incidence of, pathogens; e.g. keeping stock (especially calves) away from high risk fields and not producing irrigation run-off from fields that have recently been grazed by infected stock or sprayed with recycled effluent.

Introduction and transport

Pathways

Run-off driven

Pathogen loads in watercourses are driven by rain events, and are often directly proportional to run-off. *E. coli* counts have increased ten million times during moderate run-off events (Ashbolt). Commenting on the highly seasonal and highly episodic nature of faecal indicator loads in streams Kay et al (2007) noted the need for pathogen export coefficients for high and low flows and for winter and summer for different land uses, to support GIS based models and risk assessments. They also concluded that the models needed to accommodate episodic fluxes during storm events (the key risk period for public exposure) to be of value to policy audiences.

A study in north-west Tasmania (Holz, 2007) found bacterial concentrations to increase as did flow rate. Some variations in concentrations were thought to be caused by direct deposition by cows into surface drains. Concentrations also increased during and immediately after grazing. In this cool region, numbers were observed to increase during warmer months in one year when there was summer run-off and it was speculated that bacterial growth in drains or higher rates of survival may be contributing factors.

Haydon (2006) notes that storms carry large numbers of pathogens compared to baseflow conditions and that surface flow tends to dominate sub-surface flow during such events. The levels of pathogens are highest at such times, along with other contaminants affecting water colour and turbidity. Water treatment plants are under most stress when dealing with poor quality water – when their performance is most critical in terms of protecting public health.

In a study of eight catchment systems in southern Australia, Roser and Ashbolt (2007) also found the majority of pathogen impacts on reservoirs to arise from rainfall induced run-off. They found as much as 300 years worth of dry weather pathogen contamination could be exported in one day during a single small run-off event.

Surface run-off

Rainfall and splash mechanisms are assumed to be the main process to liberate pathogens from dung (Haydon, 2006) – analogous to the generation of sediments. Once liberated, they may move with water or attach to vegetation or soil particles (at a rate that is influenced by soil moisture), and then move with them.

The degree to which soil filters and traps pathogens is influenced by the size and shape of the pathogen (and that of any particles to which they adsorb). Haydon (2006) emphasizes that smaller pathogens (e.g. viruses) are less likely to be trapped and will have different transport mechanisms (pathways and rates) than larger pathogens. The partitioning between free, attached and clumped forms will vary – and there is great uncertainty about the percentages in each form. Most models treat attached pathogens like sediments; and un-attached forms as a function of concentrations and flow.

Viruses may attach to fine clays (less than 2*u*m), bacteria to silts under 10*u*m and protozoa to silts under 40*u*m (Haydon, 2006).

The Cooperative Research Centre for Water Quality and Treatment found that *Cryptosporidium* oocysts did not aggregate, indicating they would be mobilized and transported as single entities.

Stout et al (2005) examined the transport of phosphorus and faecal coliforms across simulated vegetated buffer strips and found them to be very similar. Short distances were involved in this laboratory experiment under conditions of near saturation.

Kay et al (2007) report a New Zealand study on sloping pastures (18 degrees) that found the time since grazing (and manure deposition) was the most significant predictor of *E. coli* concentrations in overland flow. They also reported a British study that concluded the most protection to water quality would come from preventing surface run-off. Newman et al (2003) also reported that faecal coliform concentrations in streams increased during storms and were partially dependent upon the length of time since grazing had occurred.

Journeaux (2005) reports other New Zealand research that showed reductions in pathogen yields from dung to surface flows of at least an order of magnitude (from high levels) after 14 days in the field. Part of the reduction was attributed to the formation a water-stable skin on dung that 'greatly reduces entrainment in surface run-off'.

Collins (2004) notes that, in hill country, the topography promotes the convergence of surface and sub-surface flows in wetlands which collect pathogens from upland areas and are attractive grazing sites for cattle – resulting in the direct deposition of additional dung in these areas. The wetlands contribute high numbers of faecal microbes to waterways during rain events. A model incorporating solar radiation and flow explained 87% of the variance observed in *E. coli* concentrations across five winter rainfall events. The cold New Zealand weather appeared to aid pathogen survival while the solar radiation was too weak to promote die-off.

Aarons (2006) reported a drastic increase in observed levels of nutrients and *E. coli* in a Gippsland stream receiving overflow from and effluent pond as surface flow.

Sub-surface

Microbes are transported in the soil by water and tend to move more in fine textured soils (predominantly through macropores) than course soils (Newman et al, 2003). Jiang (2008) notes that while fine texture enhances processes like inactivation straining, filtration and air-water trapping (suggesting less leaching and potentially greater surface run-off), soils with fine texture may be well structured with micro and macropores; which substantially enhance the sub-surface movement of pathogens.

Microorganisms move through soil with water in two fractions; one through the soil matrix and one through by-pass (or 'preferred') pathways – such as macropores (Jiang, 2008). Macropores (e.g. structural gaps or invertebrate holes) may only represent a small percentage of total porosity in a soil, but contribute the vast majority of soil-water flow. Soils under grasslands tend to have a larger proportion of macropores than arable fields. Soil moisture also influences water and pathogen movement – microbes travel much shorter distances in dry soil (Jiang, 2008). There is less by-pass flow under spray irrigation than under flood irrigation (Journeaux, 2006).

A study of *E. coli* levels in water sourced from the River Murray, applied as flood irrigation and captured in drains (Adcock, 2001) found relatively low levels in sub-surface drains that were assumed due to natural filtration by the soil and dilution from groundwater infiltration. Recorded median values were:

200/100mL – inlet channel
1,800/100mL – irrigation area
4,600/100mL – sub-surface drain
200,000/100mL – surface run-off.

A US study of *Cryptosporidium* movement found the parasites (from applied liquid manure) moved more readily in no-till fields than conventionally tilled cropping land, due to the presence of macropores created by earthworms or plant roots (Ramirez, 2009). In fields with tile drains there

was a ready conduit to watercourses. In no-till fields there was infiltration from the manure even before rainfall, but none moved in tilled soils. Farmers were encouraged to conduct light tillage over tile drains to disrupt the macropores and to abstain from applying liquid manure as fertiliser at least two days prior to anticipated heavy rainfalls.

A comparison of *E.coli* and enterococci using grassed soil in boxes under simulated rainfall found them to exhibit different release rates from manure and supported previous conclusions that the two vary considerably in size, morphology, motility and surface chemistry; resulting in substantive differences in their propensity for attachment to surfaces within soils and aquifers (Guber et al, 2007). Spherical enterococci have been reported to transport better in fractured rock than rod shaped *E. coli*.

Bolster et al (2009) reported a large diversity in the cell shape and size of different strains of *E. coli* – and subsequently a large diversity in their transport behaviour (which was most strongly correlated with width and shape – or 'sphericity'). Larger cells and rod shaped cells were not transported as freely as smaller cells or more spherical cells. They noted that conclusions (and models) of *E. coli* transport based on the evidence from single strains should be treated with caution and that ' the modeling of *E. coli* in the environment will require a distribution of bacterial attachment rates, even when modeling a single source.' They also observed that filtration rates would be affected by soil particle size, especially in comparison to the size of the microbes in question.

Mitigation

Collecting run-off and filtering out pathogens are two options for exploration to reduce the load of pathogens leaving dairy farms.

Dams, effluent ponds and wetlands can store water and become a sink for pathogens – but they may also be a source during flood or overflow events. The Sydney Catchment Authority has developed tools to help assess the risks from features that may be both sources and sinks (Whiffen, 2008).

Kay et al (2007) review several assessments of constructed wetlands that show very high levels of pathogen attenuation (generally above 90%) – however, it is noted that there is often an absence of data on the performance of such systems following storm events. Regulators in the United Kingdom have also expressed concerns that unlined constructed wetlands may lead to the pollution of groundwaters.

Vegetated filter strips (that may or may not be in riparian zones) aim to attenuate pathogen fluxes by trapping particles to which pathogens are adsorbed and filtering water as it infiltrates the soil. Different reviews of their effectiveness offer highly variable results (Kay et al, 2007). Results have ranged from reductions of up to 70% in faecal indicators, to no significant difference, to increased export of pathogens. Journeaux (2005) refers to research showing very low levels of entrapment of *E. coli* and *Campylobacter* in buffer strips during high run-off events, but very significant levels (more than 95%) during the slowest rates of water application – which may help account for the disproportionate increase of faecal microbes in streams following storm events.

The Cooperative Research Centre for Water Quality and Treatment found that grass was effective at reducing the transport of *Cryptosporidium*, but viruses – and to a lesser extent, bacteria – were easily mobilized. The high concentration of pathogens in faeces meant that cow pats contributed pathogens for prolonged periods, ranging from weeks in summer to months in winter. It was also found that subsequent rainfall could remobilize deposited oocysts.

When vegetated filter strips are combined with cattle exclusion in a riparian zone, the exclusion of cattle may be a significant factor. A study of cattle exclusion from streams in a 56.7ha catchment showed a 66% reduction in faecal coliforms and a 57% reduction in enterococci over a 7.5 year

period, and another study of riparian fencing and 'farm dirty water containment' in Scotland showed a 66% reduction in *E. coli* and 81% reduction in enterococci between 'paired catchments' (Kay et al, 2007). However, a study of more than 95% stock removal from a 255ha catchment in the UK showed only a 'surprisingly slow improvement in water quality'.

The New Zealand Ministry of Agriculture and Forestry has developed guidelines for the optimal design of riparian buffers to entrap faecal bacteria (Journeaux, 2006). They account for soil type, slope, the degree of bacterial attachment to soil and dung, and entrapment efficiency, but 'a large number of assumptions have been made' in their development (Journeaux, 2005).

Sediments

Stream bed sediments can be a significant reservoir of faecal coliforms; and, although they commence to die-off in sediments, there is potential for re-growth within freshwater stream environments (Kay et al, 2007). Sediment environments appear to increase the half-life of faecal coliforms and surviving pathogens can be released back into the water column as a result of increased (turbulent) stream flow or from cattle wading in streams (Newman et al, 2003). Several studies reported by Newman et al (2003) indicate that very high percentages of faecal coliforms entering a stream settle into sediments within a short distance of their entry – and that re-suspension can drastically increase concentrations above base levels.

New Zealand studies have shown that cattle within a stream increase *E. coli* levels by an order of magnitude or more due to direct defecation and stirring up sediments (Journeaux, 2005).

Direct access

Stock with access to waterways can be a direct source of pathogens – with cows crossing streams in New Zealand reported to result in spikes of *E. coli* (up to 50,000 cfu/100ml). It was also reported that cows defecated more per metre of stream crossing than elsewhere on the laneway (Pepper et al, 2006). Cows tend to defecate at similar rates over a property, except when in streams when they are five times more likely (Journeaux, 2005).

Pathogens may also enter streams indirectly in overland flow and potentially from wastes from slaughtered or dead animals or inadequately treated and managed effluent.

In-stream

Hipsey (2008) cites several reported cases of bacteria growing in aquatic environments. Maximum growth rates are mediated by temperature and nutrient availability. Examples include:

- enteric bacteria in fresh and saline water growing with the addition of nutrients from sewage,
- enteric bacteria growing in nutrient rich aquatic sediments,
- enteric bacteria multiplying in low-nutrient, low-temperature (mountain stream-like) water (but being suppressed by competition and predation), and
- faecal indicator bacteria growing in low nutrient waters.

Kay et al (2007) observed that field data suggests microbes remain viable for protracted periods in faeces and sediments, 'which might imply environmental regrowth', which is rarely considered in modelling.

The significance of in-stream processes, and our lack of knowledge about them, were highlighted by Haydon (2006) who reported an experiment that found pathogens took from 3 to 7 days to move downstream a distance that they were expected to traverse within 29 to 34 hours based on flow rates alone. Detection continued for 8 to 14 days, further underlining the complexity of transport mechanisms and likely interactions between deposition and re-suspension.

Management

Stock and effluent management

Drying, solar radiation and competition from soil bacteria following the application of animal wastes to soil and vegetation can all greatly reduce pathogen populations. However, without proper management, there is significant potential for pathogens to cause disease in grazing stock. Although cattle avoid grazing immediately around dung pats, they have little choice when effluent has been uniformly applied (Birchall et al, 2008).

Recommendations contained in existing state-based guidelines generally include the following strategies:

- Apply wastewater thinly and uniformly to recently grazed pasture so that pathogens can be exposed to maximum sunlight and desiccation.
- Exclude cattle from the reuse area for 2 to 5 weeks.
- Do not apply effluent to paddocks on which stock <12 months of age will graze.

Exclusion periods of 2 to 3 weeks are supported by New Zealand research. A trial in 2000 applied 14 mm of untreated wastewater (equivalent to 25 kg N ha^{-1}) to plots at intervals of 25, 20, 15, 10 and 5 days before grazing. Faecal coliform counts on pasture had decreased to background levels by 10 days, and cows offered a 'taste panel' of plots showed a dislike for pasture treated within the previous 10 days. There was no significant difference between the control and treated pastures at 15 days, but a minimum exclusion period of 20 days was recommended (Birchall et al, 2008).

To prevent the spread of Johne's Disease it is recommended that;

- Calf rearing areas are managed to ensure no effluent from susceptible species comes into contact with calves.
- Calves up to 12 months of age should not be reared on pastures that have had adult stock (or stock exposed to BJD) on them during the last 12 months.
- Contractors thoroughly clean all equipment that has been in contact with effluent or manure before leaving a farm (Birchall et al, 2008).

It has been concluded that there is insufficient research available to quantify the risk that effluent re-use poses to nearby residents due to aerosol transport, but separation distances of 125 to 300 metres were recommended to minimize risks from *Campylobacter* and *Salmonella* from irrigated pig effluent (Birchall et al, 2008).

The rate of adoption of sound effluent management by farmers has been questioned. A study involving dairy farmers in northern Victoria and the Waikato region of New Zealand concluded that, despite various extension programs and regulations, there was an unacceptable level of non-compliance in regard to effluent management (Davies et al, 2007). The researchers concluded that farmers tended to believe their management was adequate, while regulators were concerned with practices such as inadequate storage and application areas, stock-piling dried effluent after desludging, and using irrigation reuse dams for effluent containment. Farmers and regulators had different perspectives on risk. Farmers were often unsure about technical advice and cautious about taking initiatives that could expose them to regulatory review and prosecution. They also had concerns that, eventually, all systems face problems with pump breakdowns and other operational failures.

Wrigley (1992) made the observation that it was difficult to 'sell' effluent management systems on the basis of improved profitability. He also noted some regions had systematic difficulties in designing and operating sound systems due to land form, rainfall etc. The use of multi-purpose works, improvements in farm aesthetics, and farmer's strong resolve to be good environmental

custodians were suggested as means to encourage farmers to accept ownership and adopt sustainable waste management systems.

Fencing smaller wetlands to exclude stock will improve water quality (Journeaux, 2006), and the value of vegetated filter strips may be optimised by;

- Decreasing surface water flow (leading to less transport capacity and hence deposition of contaminants),
- Enhancing infiltration of water and pollutants into the soil, and
- Adsorbing pollutants onto litter, vegetation and the soil surface to lower outflow concentrations (Stout et al, 2005).

Key points

- The movement of pathogens to waterways is driven by rainfall events; with loads increasing exponentially with flow.
- Different pathogens will move along different pathways and at different rates (e.g. surface or sub-surface flow, either in suspension or attached to soil particles).
- Pathogen export rates are highest when there is least time between run-off occurring and the most recent grazing (and defecation) taking place.
- The sub-surface movement of pathogens is greatest through macropores in the soil, which tend to be well-developed under pastures.
- The ability of soils to filter pathogens is influenced by the relative size and shape of the pathogens and soil particles.
- Grassed filter strips have widely variable performance in terms of filtering pathogens (possibly influenced by flow rates and concentrations); ranging from significant reductions, to nil affect, to increasing pathogen loads.
- Areas of low-lying wet-soil tend to concentrate pathogens and attract greater deposition from grazing cattle.
- Stock defecate more when they are in streams and also stir up sediments, resuspending pathogens.
- There are management options, suitable for many situations, which may reduce the export of pathogens from dairy farms, such as; not recycling liquid effluent when rain and run-off is imminent, recycling effluent on grazed pasture and then with-holding stock, and excluding cattle from streams and wetlands.

Exposure and impact

Consequences

Many of the pathogens responsible for human illness occur regularly in society and result in only mild effects. *E. coli* (K-12 strain) actually plays a positive role in the human gut, producing vitamin K2 – but the virulent 0157:H7 strain can be fatal. An outbreak of *E. coli* 0157:H7 in Walkerton (Canada) was caused by low levels of contamination of drinking water – and the growth of the pathogen in biofilms in pipes (Ravva et al, 2006).

Cryptosporidium and *Giardia* were detected in Sydney drinking water supplies prompting 'boil water alerts' in 1998. Power et al (2005) note that there have not been any reported outbreaks of cryptosporidiosis in Sydney due to drinking water supply – but the risk of infection and severe illness is such that high levels of safeguards are warranted. The infectivity of *Cryptosporidium* is dramatically reduced by exposure to UV radiation (King et al, 2007).

The Cooperative Research Centre for Water Quality and Treatment (2005) reported that not all viable *Cryptosporidium* oocysts are capable of causing illness in humans. It is necessary to identify individual strains and geno-types to appreciate which ones are associated with health risks.

Two often cited cases which emphasize the potential impact of contaminated drinking water supplies are the Milwaukee (US) *Cryptosporidium* outbreak of 1993 (affecting 403,000 people, with 4,000 hospitalized and 54 associated deaths) and the Walkerton (Canada) *E. coli* 0157:H7 outbreak of 2000 (affecting 2,300 people with 7 associated fatalities) (Meinhardt, 2005).

In a documented case, children on a school excursion became ill with Cryptosporidiosis after visiting a dairy farm – and the cause was traced back to children tasting silage and pellets of cow feed (Shield et al, 1990). However, Casemore and Jackson (2005) have hypothesized that Cryptosporidiosis in humans is not usually a zoonosis – i.e. infection is via routes other than via animals.

A review of suspected drinking water contamination in British Columbia (Canada) concluded that there appeared to be a correlation between intensive livestock and the levels of *Cryptosporidium* and *Giardia* in water, but there was no scientific evidence verifying that pathogens from cattle resulted in human disease outbreaks (Newman et al, 2003). An outbreak in Cranbrook was cited as the only case in which there may have been a link between cattle and cryptosporidiosis in humans.

Cryptosporidiosis in humans is generally caused by either *Cryptosporidium hominis* or *C. parvum*. In cattle it is caused by *C. andersoni, C. bovis, C. ryanae*, or *C. parvum*. Studies to date indicate that *C. parvum* is found almost exclusively in pre-weaned calves, with the other forms found in older cattle (Nolan et al, 2009).

New Zealand has had the highest reported rates of campylobacteriosis in the world. It appears that up to 60% of cases originate from contaminated poultry, however overall numbers (especially those from poultry) are now declining steeply. A sizeable portion of non-poultry infection comes from ruminant sources, especially amongst rural residents - and children are most susceptible (McBride et al, 2009).

Oysters and other shellfish that are eaten raw can also be vectors for waterborne diseases to humans. Bacteria such as *Salmonella* and *Campylobacter* are of particular concern in the US. Oysters are filter feeders and acquire the organisms as they filter water for food. The bacteria may live in the environment created by the shell of the oyster and be incorporated into the meat

of the oyster. The oysters are then a public health risk if consumed without being cooked (McGinley). Both bacteria are common in poultry and can be found on the skin and in the meat of chicken. Contact with uncooked chicken meat is the most common form of human infection in the US. *E. coli* may be purged from oysters, but not *Salmonella* and *Campylobacter*.

Pathogens including *Giardia* and *Cryptosporidium* have been reported to remain viable (and likely to infect shellfish) in seawater for from several months up to a year (Graczyk et al, 2006).

In Australia, there was one case (affecting six people) of salmonellosis in 2001 associated with oysters – although it is reported that such outbreaks are not commonly linked with seafood (OzFoodNet Working Group, 2002). Incidents in subsequent years led to the prohibition of imported oysters from some countries due to contamination concerns.

A Western Australian study considered the impact of closure on oyster leases (which is regulated to occur when there may be a risk of contamination occurring – e.g. the onset of floods following heavy rains in the upstream catchment). The study considered the options of shifting oyster stock to a low risk site or depuration ('rinsing' pathogens from the oysters) and found the former unprofitable and the latter marginally profitable (Gibson, 2004). If closures were only for short periods their impact was more on cash flow than overall profit; and there may be management options to further reduce the economic impact, such as maintaining a portion of stock at a low risk site.

A wide variety of bacteria may be present in raw milk (NSW Food Authority, 2009). The type and level of microorganisms in the milk is influenced by animal health, the farm environment and production methods. Today, the main microbiological hazards associated with milk and dairy products include *Salmonella, Listeria monocytogenes*, pathogenic *Escherichia coli, Staphylococcus aureus, Campylobacter* spp*., Yersinia enterocolitica* and *Enterobacter sakazakii*.

Certain dairy products are deemed a high risk - unpasteurised milk, soft cheese, dairy desserts, fresh cheeses and dairy dips - as these products may support the growth of pathogenic (disease-causing) microorganisms. Between 1995 and 2008 there were fourteen disease outbreaks in Australia attributed to dairy products; mostly from consumption of unpasteurised milk (NSW Food Authority, 2009). During that period there were 600 foodborne disease outbreaks nationally, involving over 13,000 people – with 700 being hospitalized and sixteen deaths.

Detection

Detection of pathogens traditionally involved treating samples to concentrate the microbes, separating them and finally a means of detection (CRC for Water Quality and Treatment, 1998).

The difficulty in effectively monitoring for waterborne zoonoses was reported by Pepper et al (2006) who commented on challenges arising from the differences between pathogen groups (size, nutrient needs etc), detecting low concentrations in large volumes of water, standardizing culture-independent detection methods and removing agents that may inhibit pathogens from water samples. They also provided a review of molecular testing methods, which were developing rapidly.

The importance of sampling across the hydrograph in high-flow events was stressed by Roser & Ashbolt (2007). They also noted that either *E. coli* or enterococci could be used as general indicators of the presence of faecal matter (although there may be advantages with the latter); that *Cryptosporidium* were found more often and in higher concentrations than *Giardia* and may be a more useful pathogen to sample for; and that *Campylobacter* were universally present and easily detected. The World Health Organisation also classify Cryptosporidium as a 'reference pathogen' due to its relative ubiquity and resilience, and socio-economic impact (Jex et al, 2008).

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Difficulties in trapping and culturing waterborne pathogens were also noted by Newman et al (2003). They reported several studies concluding that faecal coliforms were generally considered a poor indicator of protozoans such as *Giardi* and *Cryptosporidium* due to differences in survival rates, transport mechanisms and in-stream settling patterns. They also reported on efforts to use differences in the ratio between faecal coliforms and faecal streptococcus to differentiate between alternative sources of water contamination – but found some overlap between ranges recommended for different sources by different authors and noted that health officials did not accept the approach.

Son et al (2009) analysed faeces from individual cows, composites of manure, water troughs, and milk and found a highly diverse range of *E. coli* on a single farm. There was little diversity within individual faecal samples but much diversity between them, manure composites and water from troughs. They noted that dairy farms are complex enterprises with cow movements and interactions, and they are 'open' systems – with bacterial inputs from humans, wildlife and purchased animals. Given this environment and the diversity of *E. coli* found, they considered it unlikely that molecular source tracking would prove successful in assigning pathogen isolates found in the environment back to a particular farm.

Kay et al (2007) summarized bacterial source tracking techniques as using either species associated with faecal matter from humans or animals; or chemical markers indicative of human sewage. They concluded there wasn't a single, definitive and exact approach available to apportion contributions of faecal indicators between human and animal sources. Although noting the development of a Screening Tool developed in Scotland it is hampered by a lack of empirical ground truth data on riverine faecal indicator levels.

In 2002 it was concluded that there was 'no single method capable of identifying specific sources of faecal pollution in the environment with absolute certainty' (Scott et al). A few years later Mandeville (2006) concluded that 'Bacterial Source Tracking' (BST) can reliably determine if faecal bacteria are from human or animal sources, and if they are from livestock or wildlife (although less reliably than the human/animal separation).

The office of the US Geological Survey presents a summary of microbial source-tracking and detection techniques at; http://water.usgs.gov/owq/microbial.html.

The Cooperative Research Centre for Water Quality and Treatment (2005) assessed six methods to identify individual genotypes of *Cryptosporidium* and found all to be valuable and worthy of further development. DNA (gene) sequencing methods were considered most valuable if discrimination was only required to the genotype level.

Jex at al (2008) provide a recent review of techniques to detect *Cryptosporidium* species. There are 15 recognized species of *Cryptosporidium* and numerous genotypic variants, with *C. parvum* and *C. hominis* the major causes of cryptosporidiosis in humans. The different species cannot be differentiated by morphology but can be by molecular (DNA) analytic and diagnostic tools. The authors note the importance of identifying specific strains of *Cryptosporidium* to understand and manage waterborne disease outbreaks.

New Zealand researchers have recently used Multi Locus Sequence Typing (MLST) to differentiate between wild bird, poultry and ruminant sources of pathogenic *Campylobacter* strains (McBride, 2009).

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Management

Multi-barrier, risk based approaches offer means to reduce the risks of waterborne pathogens infecting humans. Although proof of direct links between dairy farming and human disease outbreaks via waterborne pathogens is scarce the potential for harm is such that dairying must be involved in multi-stakeholder responses.

Key points

- Many pathogens are relatively benign; severe human health impacts can be due to specific virulent strains of widely occurring pathogens. When assessing risks, it is important to identify specific strains and genotypes.
- As an example, Cryptosporidiosis in humans is caused by *C. homins* or *C. parvum*. Cattle carry several forms of *Cryptosporidium* – but *C. parvum* is only found in pre-weaned calves. *C. bovis* from cows has not been associated with human illness.
- *Campylobacter* have been linked to human disease in New Zealand and to the contamination of oysters in the US.
- There is little evidence of waterborne pathogens from cows directly resulting in severe human health impacts; but the risk of that occurring cannot be dismissed.
- The type and level of micro-organisms in milk is influenced by animal health, the farm environment and production methods.
- Faecal coliforms are a poor indicator of *Cryptosporidium* due to differences in survival rates, transport mechanisms and in-stream processes.
- In regard to pathogens, dairy farms are complex and 'open' systems so, even with the best technology, it will be difficult for molecular source-tracking to link detected pathogens with individual farms. However, differentiation between human, livestock and wildlife sources is becoming increasingly reliable and genetic technologies are helping to better explore the roles of specific genotypes in specific disease outbreaks.

Reported knowledge gaps

This section presents an overview of analyses performed by others regarding pathogen related research needs and notes on contemporary Australian and New Zealand research.

Research gaps

Kay et al (2007) report the conclusion from a review of diffuse pollution controls in the United Kingdom that it is an 'emerging policy imperative to understand and predict catchment microbial fluxes' – and that it was 'the challenge of the 21st Century'. They noted a lack of sound deterministic models for pathogens in catchments, which were able to predict the impact of remedial programs on pathogen fluxes.

Harter (2009) noted that there was considerable research on the transport of microorganisms at the small (laboratory) scale – but 'transport dynamics of zoonotic pathogens at the field, farm and catchment scale are still poorly understood'.

The ecology of waterborne pathogens (their movement, viability and 'survivability' from host-source to tap) was identified as a research need by Newman et al (2003), along with knowledge of the prevalence of *Cryptosporidium* in cattle herds.

Population dynamics for pathogens were cited as the most urgent research need regarding water bodies by Hipsey et al (2008). They observed that:

- While there is evidence of growth of coliforms there is little data on growth rates and the influence of nutrients and temperatures.
- Similarly, there is insufficient data on grazing and predation of enteric organisms and its spatial and temporal variability.
- Mortality rates are reasonably well qualified (especially for coliforms) apart from the responses of oocysts and phages to salinity and pH.
- Inactivation rates due to sunlight under different oxygen and pH levels would also benefit from additional data.

When reviewing models of pathogens in catchments from Australia and overseas, Haydon (2006) noted that while numerous models exist, few present a sound statistical treatment integrating hydrology and a spatial analysis of factors influencing the survival of pathogens. Risk assessments of dairy catchments and their performance as 'barriers' in protecting public water supplies will require predictions of pathogen numbers (temporally and spatially) in response to different landuses and management; as well as run-off, temperature etc (Haydon, 2006).

In a very structured review, Ferguson et al (2003) reported that few models dealt with the processes that link the generation of pathogens (sources) with hydrological processes of a watershed. They also noted that great care was required in extrapolating information from one scale to another and that knowledge was required at the laboratory scale, field or plot scale, sub-watershed and total watershed (catchment) scale. Buffer strip attachment processes and the prevalence of pathogens in different wild, feral and domestic species were noted as significant knowledge gaps.

After considering the size of knowledge gaps, the benefits of closing them, and the cost of doing so, Ferguson et al (2003) concluded that research should focus on quantifying general relationships to provide data for models that could be tailored to individual circumstances. Their research priorities were:

- Inactivation kinetics of pathogens in soil and faecal matrixes,
- Description of the particle sizes in which pathogens are transported,

- Characterization of pathogen properties and watershed specific features that affect the fundamental processes driving terrestrial transport and attenuation,
- Inactivation and sedimentation of pathogens during their initial introduction to the aquatic environment.

Ravva et al (2006) noted that pathogens ingested with vegetables (or fodder) from fields irrigated with dairy effluent may be revived or resuscitated either on (or in) vegetation or once ingested. It is therefore crucial to determine the factors affecting pathogen survival in manure (effluent) lagoons to minimize the risks of contamination from recycled dairy effluent. Pathogens generally only re-grow in the presence of a carbon source.

Newman et al (2003) saw a need for more knowledge about calves and the age at which they build resistance to pathogens such as *Cryptosporidium parvum*. Understanding of infection levels – or 'carriage levels' - in stock of different ages and management, and ways to contain them at low levels was considered a research need by Adcock (2001).

Further study is needed of *E. coli* transport behaviour under different field conditions (soils and aquifers), media (dung, water etc), and growth conditions according to Bolster et al (2009).

Journeaux (2006) recommends further research on:

- The cost-effectiveness and social implications of all mitigation options,
- Alternatives to permanent fencing of streams and improved understanding of factors that influence the attraction of cattle to water,
- Further evaluation of the entrapment of microbes in riparian buffer strips across different soils, slopes and vegetation,
- Improved understanding of the attachment of microbes to particles,
- Evaluation of the effectiveness of constructed wetlands, vegetated drains, and farm ponds in attenuating microbes, and
- The processes at play within watercourses affecting pathogen survival and movement.

Understanding, and tracing, the processes by which pathogens may move in water and infect stock and humans is another priority identified by Journeaux (2005).

Kay et al (2007) identified numerous research topics, including:

- An emphasis on interactions at the sub-square kilometre scale (where uncertainties and contradictions in data are most problematic) to improve models (that often rely on export coefficients set for larger areas).
- Basic, field validated, data on microbial kinetics, especially the survival of faecal indicators and pathogens in fresh water and sediments.
- An emphasis on understanding processes during high flow events when the majority of pathogen transport occurs.
- Understanding the partitioning of different pathogens with different sedimentary fractions, and how particle size and mineralogy influence attachment, transport and survival in freshwater streams.
- Understanding how effective farm scale interventions may be in reducing catchment fluxes of pathogens, and their costs.

Wrigley (1992) nominated a list of effluent related topics as possible areas for further research including:

- Potential animal and human health hazards of milking shed waste.
- Earthworms and waste decomposition.
- Groundwater pollution under intensive dairy farming.
- Efficacy of wetlands in treating milking shed wastewater.

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There appears to be little research into the effectiveness of alternative dairy effluent treatment systems in controlling different pathogens. The same is no doubt true in regard to their impact on any endocrine disruptors from veterinary treatments.

The tracing of pathogen strains that cause infection and re-infection of cattle would help explain the persistence of some pathogens in the landscape; and information linking production with the quality of cattle drinking water could also be important (Journeaux, 2006).

The specific sources of waterborne pathogens and their significance in disease outbreaks – e.g. using genetic markers - along with information on the prevalence of diseases in wildlife were other information gaps noted by Newman et al (2003). With many zoonotic species it is only some genotypes that pose a severe risk to human health. Identifying them, and the extent to which they reside in different animals, could help focus risk mitigation programs. At present, it is not possible to identify virulent strains of *Cryptosporidium* from non-virulent ones, without recourse to molecular (genetic, or DNA-based) techniques.

A conference early in the life of the Cooperative Research Center for Water Quality and Treatment (1998) concluded that it would be useful to establish a national *Cryptosporidium* oocyst 'bank', to describe each of the oocysts, including cultural and genetic characterizations. It was noted that better methods were required to genotype different strains of *Cryptosporidium* in order to better understand their viability and pathogenicity.

Contemporary Australian research

Policy tools and models

SA Water invested in 'Catchments as Assets' to understand the economic value of catchments to water supply and Melbourne Water has undertaken similar work associated with establishing values for environmental services.

The University of Melbourne (Department of Veterinary Science) use a two step molecular diagnostic approach to screen large samples for specific forms of *Cryptosporidium*. A polymerase chain reaction (PCR) technique is used to screen for genetic variation, before a single strand conformation polymorphism (SSCP) technique is applied to selected samples. The unit is working with a NSW partner to develop an automated robotic diagnostic system for the rapid identification of eight distinct pathogens known to cause diarrhoea in humans, including *Cryptosporidium*, *Giardia* and *Campylobacter*. It has good prospect for tailored application in dairy investigations.

Field data – generation, survival and transport rates

Sydney Catchment Authority has undertaken work looking at *Cryptosporidium* persistence in dairy effluent ponds and infectivity rates in different animals. They have previously been involved in upgrading dairy effluent management systems and are now emphasizing education and training, e.g. developing best practice management guidelines for broadacre farmers in conjunction with Industry Innovation NSW (formerly DPI), supporting the roll-out of the Farm Nutrient Loss Index and developing dairy farm effluent operational plans with Dairy NSW.

SA Water has been working with dairy farmers in part of the Mt Lofty Ranges (Upper Torrens Land Management Program) to reduce pathogen loads by changing farm management (e.g. buffer strips). Dairy farms with calves were identified by modeling as sources of high concentrations of pathogens and hence were a target for change. No monitoring or analysis of impacts has occurred, although there is a downstream monitoring site at Gumeracha.

Few Melbourne Water catchments contain farmed land, so their focus is on natural areas. Research is underway on means to better identify *Cryptosporidium* from native animals and deer. A PhD (Cinque) is nearly completed on the effectiveness of management (e.g. buffers) on water quality in the Tarago catchment (which does contain some dairying).

The University of Melbourne (veterinary science) is monitoring three catchments using PCR (DNA) detection techniques to identify virulent genotypes of *Cryptosporidium* and *Giardia*. The four year ARC Linkage grant project is in conjunction with Melbourne Water, and mainly involves feral and native animals. However, they have an application in with the Gardiner Foundation to undertake a wide-spread molecular study, screening Victorian dairy cows for virulent pathogens – in response to concerns expressed from the Department of Health. The proposal seeks funds to develop a high-throughput tool and dairy-screening techniques as well as funds for the sampling and analysis. Nolan et al (2009) have applied the techniques honed by this group of researchers to assess *Cryptosporidium* levels in calves in Victoria.

ACTEW (the water provider in the ACT) are investigating the water quality impacts (including pathogens) of best management practices (e.g. buffers and contour/swales) on cattle farms in the ACT, through a project with Ecowise. Similar work has also been undertaken by the UNSW Water Research Centre with the Sydney Catchment Authority, other industries (e.g. feedlots via MLA) - and groups in the US and Europe.

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SA Water have developed DNA characterizations for *Cryptosporidium* isolates and are interested in source tracking for contamination, but have not secured funding to undertake such work. Streams are regularly monitored for *Cryptosporidium* and *Giardia*.

The rainfall driven dispersion of *Cryptosporidium* oocysts from cow pats, and their biological-thermal inactivation, has been studied by the Water Research Centre, University of New South Wales. The data may be used in risk assessments and for pathogen budgets. Projects like this can be undertaken through post-graduate studies as Australian Research Council projects. The Centre also has considerable expertise in Quantitative Risk Assessments.

Water Quality Research Australia (established out of the former CRC for Water Quality and Treatment) aims to develop projects around verifying the outcomes of treatment processes, the use of storm-water for potable supplies and better understanding the dynamics of catchments – the pathogens being generated and the natural barriers within catchments. Dairying will be a landuse of interest and WQRA has a preference for partnership based research. A literature review is currently underway of the 'grey literature' within water utilities etc for information and knowledge about pathogens in catchments.

WQRA has industry members (water managers such as Melbourne Water and the Sydney Catchment Authority) and research members (many universities, including UNSW) who are research providers; see http://www.wqra.com.au/WQRA_members.htm. They have an education program and are willing to assist in putting proposals together in the form of ARC post graduate applications (e.g. for the November round).

Meat & Livestock Australia (MLA) have engaged the UNSW Water Research Centre to determine what contaminants (chemicals and pathogens) are in feedlot manure at all stages from fresh deposition to compost. The work is feeding into a 'Quantitative Risk Assessment' and the development of best management principles for feedlot operators (e.g. ensuring only composted manure is provided for horticultural applications). Dust transmission has been identified as an occupational health and safety issue for employees. Reports from this work will begin emerging in mid-2011.

Work through UTAS has investigated naturally occurring pathogens (*Vibrio sp*) in oysters but not dairy related pathogens. The Australian Seafood CRC has recently invested in a project to differentiate between *Norovirus* (a common pathogen in oysters) from cattle and human sources. It will test cattle faeces for human forms of *Norovirus* – and further investigate suggestions that a standard testing technique may be detecting bovine specific forms as human forms.

Ongoing New Zealand research by the National Institute of Water and Atmospheric Research (NIWA) for Dairy NZ, is indicating that ruminant associated pathogenic *Campylobacter* tend to contaminate streams during flood events; but nowhere near so much during low flows (when avian strains dominate). There is also work underway to develop a comprehensive *Campylobacter* model that links environmental generation and transport with human health aspects of occurrence and exposure, to record current knowledge and identify risks. Researchers are also contributing to a forthcoming text on animal wastes, water quality and human health for the WHO and US EPA.

WA researchers have experience in assessing the impacts of changed farm management and modeling catchments and aquatic systems (including pathogens). A pathogen module could be added to dynamic nutrient modeling that is currently underway (e.g. through a PhD student). Monitoring is already in place in catchments feeding into oyster growing areas and a pathogen component could also be added there.

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The potential for virus persistence in bio-films formed inside water distribution pipes is being studied by the Water Research Centre, University of New South Wales. The Centre also has a strong body of field and laboratory effort dealing with bio-solids in general and with chemicals in waterways, such as endocrine disruptors from livestock sources.

Management innovations

Though not Australian, there is research underway in the United States to develop genetically modified vaccines for calves against *E. coli* 0157:H7 (Wood, 2009). Calves vaccinated with a modified form of the bacteria produce proteins that trigger an immune response and bacteria counts in manure drop (and are undetectable in some cases) within days.

Key points

- There is very good capacity within Australia to undertake research into, and model, the links between waterborne dairy pathogens and human health. There are some strengths in virtually every State – although there are fewer examples of all aspects being brought together in single projects.

- There are a number of animal health issues that have been identified, or are receiving, research attention. They include;
  - new vaccines for stock,
  - the persistence of pathogens on farms and their infection of stock (especially calves),
  - the effect of different effluent treatments on pathogens, and
  - molecular screening of dairy cows for virulent pathogens, by the University of Melbourne.

- There is considerable interest in improving, testing and applying cheaper and faster detection techniques based on genetics, e.g. the University of Melbourne, Melbourne Water and SA Water.

- There has been work to gain better data on some parameters regarding dairy pathogens (e.g. University of NSW and Sydney Catchment Authority) – but the dairy industry may not have ready access to the results.

- Work has been undertaken, is underway, or is planned to address risk management issues. Examples include;
  - Quantitative Risk Assessment for feedlots by the University of NSW for MLA,
  - The effectiveness of riparian buffers by Melbourne Water,
  - Development of a model covering the generation, transport and human health impacts of *Campylobacter* in New Zealand,
  - Development of best practice guidelines for farmers by Sydney Catchment Authority, and
  - Differentiating between *Norovirus* (in oysters) from cattle and humans, via the Australian Seafood CRC.

- Water Quality Research Australia is a vehicle for collaboration between much of the research capacity in Australia. It, like many others, has experience in developing and managing PhDs and attracting funding through Australian Research Council grants. WQRA is undertaking a major literature review in preparation for a program of research into better understanding catchment dynamics.

Conclusions

This section summarizes the information presented in this report, notes some 'best management' principles for dairy farms, and presents the research priorities recommended for the Australian dairy industry.

Understanding

Reflecting again on the key "understanding dairy catchments' questions it is apparent that most processes that may link dairy farms, pathogens and public health issues are generally understood – but there is a lack of data on fluxes, few detailed species specific knowledge bases, and considerable difficulty in establishing evidence of direct contamination resulting in observed infection. The general basics are well established but the detailed specifics and proofs are not.

Impacts and causes

It is difficult and costly to isolate specific sources of human disease. Although feasible to determine if pathogens are from human or animal sources, identifying the species from which they originated has been problematic. If technology permits tracing back to individual source species, farms or cows the immense data collection and computational load required may itself make the task impractical.

It is apparent that dairy cows excrete large numbers of pathogens, that pathogens from dairy sources are found in surface waters, and that pathogens of the general type found in dairy excretions do impact upon public health – e.g. bacteria like *Campylobacter* and parasitic protozoa such as *Giardia* and *Cryptosporidium*. It is also apparent that those pathogens may come from numerous other sources (and pathways other than via water). Some pathogens have strains that are virulent only to humans or select species of animals.

The uncertainty that exists makes it difficult to ever prove causal links between dairy farms and individual health issues – and to disprove accusations of their probable existence. However, the identification of specific genotypes that are a risk to humans will help to focus investigations and could help clarify if specific species of animal are involved as sources. Capacity in this regard is increasing rapidly.

The use of faecal coliforms (such as *E. coli*) as indicators of water quality and contamination is understandable, but the use of a general indicator (that may not be representative of dairy zoonoses) is unlikely to help unravel questions about the possible impact of dairy pathogens on human health. Advances in molecular techniques are rapidly providing new tools to move beyond generic approaches to ones focusing on individual host species and genotypes of pathogens.

Sources and pathways

The level of waterborne pathogens in the environment is driven by run-off, e.g. from rain, irrigation or liquid effluent. Grazing or spreading effluent prior to run-off occurring increases pathogen loads; and wetlands appear to concentrate pathogens (from waterborne sources and by attracting grazing cows who defecate there).

Different pathogen groups exhibit different sizes and shapes which affect their mobility. Different pathogens will travel via alternative preferred pathways and at different rates in different circumstances. Pathogens may move attached to particles or as microbes in solution.

Sub-surface flows may be significant pathways in some circumstances – especially in soils with extensive macro-pores.

Grassed filter strips have highly variable effect in mitigating pathogen fluxes. Slope, the speed and depth of overland flow, the duration of flow, and initial loads may contribute to the observed variation in the performance of filter strips. It is also worth noting that healthy pastures are in effect large 'filter strips' – where edge effects may be important. Dams, constructed wetlands and effluent ponds may also be 'sinks' and mitigate losses. However, all these filters and sinks may, in some circumstances, also act as sources of pathogens.

A significant percentage of pathogens entering streams are likely to settle into sediments – from which they may be reactivated and re-suspended in the water column. In some conditions it appears that bacterial populations may grow in sediments. Cows are stimulated to defecate more in streams adding direct pathogen loads as well as stirring up sediments.

Other in-stream processes are largely un-quantified.

Generating dairy pathogens

There is great diversity in the genotypes of pathogens and in the rates at which the microbes are generated from cattle (including significant differences with different age groups – calves being more susceptible to infection by some diseases and to shedding pathogens in greater numbers).

Excreted pathogens have exponential die-off, but there is great diversity between (and within) different groups. Micro-environments may be part of the reason for the observed diversity and long survival of some pathogens. Additionally, bacteria may grow and reproduce in some moist or aquatic environments.

Effluent ponds, irrigated effluent, spread manure, and soils with high organic matter levels are some special 'dairy environments' where micro-climates (and other biological factors such as predation) may have uncertain influence on the mortality of pathogens. There have been recent studies of *Cryptosporidium* survival in cow pats and dairy effluent ponds but this review has not located the data.

Managing dairy pathogens

Environmental protection regulations cover the management of dairy effluent, one of the potential sources of pathogens in waterways. Regulators are often skeptical of how well the dairy industry performs in terms of effluent management, although dairy farmers appear to have a more positive view of their operations. Riparian management programs (including fencing to exclude stock) may be available in many catchments in which dairying occurs. However, there are few risk-based programs aiming to engage farmers that focus on dairy farm management to reduce pathogen loads to streams – with exceptions such as reported for the Sydney and Myponga catchments.

Few programs directed at farmers have much emphasis on pathogens. Effluent management may cover nutrient (and water) recycling, and stock health initiatives may cover calf management to reduce infection, but neither tend to make much mention of public health issues via waterborne zoonoses.

Management

There are some management principles that could be relatively to adopt in many situations that would reduce infection rates in stock and reduce the risk of waterborne pathogens being 'exported' from dairy farms into waterways.

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Farm management guidelines

Management to reduce the risk of human health issues arising from waterborne dairy pathogens include:

Minimising the infection (and pathogen shedding) rates of stock – especially calves; e.g.

- Not allowing calves in paddocks that have been grazed by infected stock or irrigated with effluent.
- Not allowing stock to drink from streams that may be contaminated from upstream sources.
- Applying waste-water thinly (or incorporating it in the soil) and withholding stock from paddocks treated with recycled effluent to allow for pathogen die-off.

Reducing run-off that may carry dairy pathogens; e.g.

- Not grazing high-risk paddocks, or applying liquid effluent, prior to anticipated heavy rain or irrigation. (NB - or adopting high water use efficiency irrigation methods with zero run-off).
- Managing effluent that is due for recycling to minimize pathogen survival.
- Not grazing calves in paddocks with high risk of run-off to nearby streams.
- Maintaining well grassed pastures and riparian areas, and slowing the flow-rate of surface waters.
- If practical, holding water run-off, from paddocks that have been recently grazed or treated with recycled effluent, in drains to allow for pathogen die-off.

Avoiding direct contamination; e.g.

- Precluding stock from streams with riparian fencing and improved crossings.
- Fencing shallow wetlands or wet-soil areas and precluding stock in wet seasons.
- Ensuring effluent is not discharged or recycled in a manner that permits direct flow to watercourses.

Most of these matters require little new knowledge – apart from methods to minimize the survival of pathogens in effluent and identifying high-risk paddocks; and perhaps better understanding stock infection causes and management.

A challenge will be getting farmers engaged with the topic. Wedding pathogen management with production priorities such as profit through efficient production and optimal stock health may be a useful generic response. Specific programs are likely to be required (on a 'needs basis') in high-risk catchment areas.

Some of the physical environments in which dairying occurs may provide logistical problems in adopting the guidelines above – entire farms may be 'high risk' situations. Innovative management responses (e.g. new approaches to effluent management) may have to be developed through applied research.

Industry initiatives

A 'multi-barrier', risk-management approach to water quality and public health is conducive with the dairy industry being more involved in the public health aspects of catchment management.

Industry should be urging (and assisting) public health researchers to better define specific causes of health issues and any direct links with specific pathogens and dairy farm sources via waterborne pathways.

Industry should also be working with catchment/pathogen risk modelers to ensure their products can better estimate the benefits (and costs) of changed on-farm management and by helping to generate more accurate data on pathogen generation (and inactivation) rates for various environmental and stock management settings.

There may also be an opportunity to promote exchanges between nutrient research within dairy farms and catchments with pathogen research at plot to catchment scales.

Knowledge gaps; R&D priorities for Australian dairy

Policy tools and models

The dairy industry has had little interaction with the development of catchment pathogen risk models. Closer ties would enable industry to ensure models were constructed to meet their needs as well as the needs of other stakeholders (e.g. regarding the benefits, and costs, of changed management). It would also enable industry to review the models in use and ensure they fairly reflect situations on dairy farms.

For models to be of use to dairy farmers they will need to provide for:

- Different rainfall events – especially storms – and transport pathways,
- Different age classes of stock,
- Different pathogen types – with different infection, deposition, decay and transport rates, and
- Improvements arising from costed changes in management – e.g. riparian fencing.

A farm scale tool to help identify high risk areas and practices (akin to the Farm Nutrient Loss Index) could also be of assistance.

Industry should also seek to be involved in any research with potential to provide direct evidence of specific links between dairy farming, water-way contamination and human health impacts. Identifying genotypes that pose most risk and their hosts would also be advantageous.

If dairying is implicated in oyster contamination, research would be needed to determine the pathogens accumulating within the oysters and to then determine their source and the transport mechanisms at play in order to better appreciate any options for remedial changes in farm management.

Field data – generation, survival and transport rates

A key role for dairy could be helping with the generation of data to assist the performance of models the industry chose to support. Haydon (2007) noted that generation rates were of particular importance to the outcomes from models.

Knowledge of deposition rates for different pathogens – and patterns of manure distribution around farms (e.g. areas near water or preferred grazing sites) – would be of particular use to models. Such information may already be available from dairy nutrient research. Industry should ensure that rates data is available for different age classes of cows; and it would be useful to also better understand typical rates of infection for cows with different pathogens. Much more accurate estimates would then be possible of pathogen generation rates on dairy farms.

Studies of mortality (survival/inactivation) of different pathogens would also be if interest; along with better knowledge of transport pathways and rates relevant to different microbes and different environments. Within those topics, priority may be given to inactivation within dung and the soil (including the effects of invertebrates such as dung beetles and earthworms), the management of effluent (in ponds, slurries and alternative processes), and – possibly – constructed wetlands. These studies would need to include appropriate field scales.

In summary, data is needed on:

- Infection rates for different pathogens and different age classes of stock.

- Dung deposition patterns and deposition (generation) rates for different pathogens and stock age classes.
- Pathogen concentrations in effluent from alternative treatment methods.
- Decay, or inactivation, rates – and recommended with-holding periods - for different pathogens in different micro and macro environments.
- The influence of ecological factors such as dung beetles and earthworms on decay rates.

Studies on transport processes should focus on:

- Partitioning between alternative transport processes, especially in high-flow events.
- The effectiveness of vegetated filter strips and constructed wetlands, especially in high-flow events.

The situations in which mitigation options are effective require clarification as do the likely benefits to come from changes in management such as stock exclusion or with-holding and the management of wet areas.

Management innovations

Whenever possible, data mentioned above should be collected and reported in the context of farm management and the interactions with other production topics – e.g. animal health. Similarly, it would be useful for industry application to take a holistic approach involving all the practical, environmental issues that may confront farmers in the future – e.g. greenhouse emissions, nutrient exports, pathogens and farm-sourced chemicals – when considering aspects such as effluent management and recycling.

There appears to be little information available on alternative treatments for dairy effluent and the implications for pathogen survival. Issues such as greenhouse emissions and energy generation are stimulating renewed interest in effluent management, including Probiotic Low Energy Aeration. It would be useful to inject pathogen management (and perhaps endocrine disruptors) as a standard component to such investigations, seeking ways to reduce pathogens and other contaminants in effluent. Studies could consider liquids and bio-solids, including bio-drying or composting. Special attention should be given to solutions for high-risk locations; such as all land having high hydrological connectivity with surface waters, or high frequencies of run-off events.

Key Points

- From a risk assessment perspective, the potential impact on public health from a zoonotic disease outbreak is high – but (as evidenced by history) the likelihood of that occurring is low.

- Dairying could be implicated in a public health problem, but proof of involvement (or non-involvement) would be much more difficult. However, advances in gene technology are rapidly changing our capacity in that regard.

- In general terms, standard best practices to minimize catchment risks from dairy pathogens are well known and they would fit well with animal health, effluent and riparian management guidelines. However, there are two caveats.
  - Firstly, in high risk situations (such as strong hydrological connectivity between farm and catchment) there are few practical options currently available (particularly for effluent management).
  - Secondly, there is poor detailed knowledge of pathways and fluxes – better knowledge could show that 'best practice' is not required or is ineffective.

- There are animal health issues, such as Johne's Disease, mastitis, and reducing levels of infection in stock, that could be beneficial to explore in a catchment and farm productivity context.

Recommendations

It is recommended that:

- Research and best practice development regarding animal health, effluent management, riparian management and nutrients also consider the implications for pathogen survival and transport to waterways.

- 'Best practice' to reduce the risk of pathogen loads to waterways be defined and promoted to farmers, especially those in high risk situations.

- A multi-barrier quantitative risk assessment (as described in this report) be initiated to unravel issues and management options if dairying is at risk of being implicated in major public health issues in any specific catchment.

- If dairy supports any collaborative, catchment based research into waterborne pathogens, it focus on;
  - filling data gaps regarding fluxes,
  - better understanding pathways and the impact of management interventions, and
  - models that factors in the cost of management change.

- Any industry specific farm research should focus on innovative management options for high risk sites (especially alternative effluent management systems for locations with high hydrological connectivity to waterways); or the influence of ecological factors (such as dung beetles and earthworms) on decay rates.

The research components referred to above may be suitable for investigation by a PhD student, in collaboration with experts (and programs) already active in these fields of investigation.

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