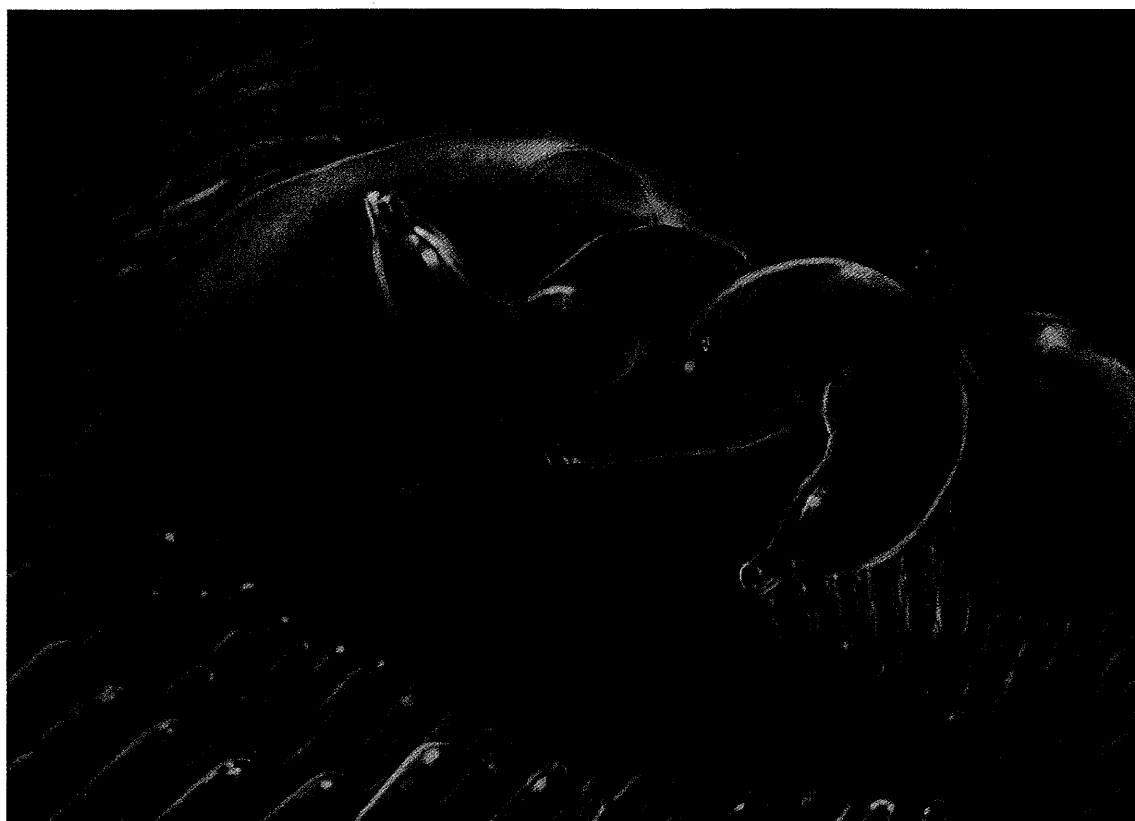


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# *Cryptosporidium in Water* A Consensus Conference

October 1998  
Melbourne Australia



Cooperative Research Centre  
for Water Quality  
and Treatment



Water Services Association  
of Australia



Australian Water and  
Wastewater Association

*Occasional Paper 3*

# ***Cryptosporidium in Water***

## **A Consensus Conference**

**5th October 1998  
Melbourne, Australia**

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**Cooperative Research Centre  
for Water Quality and  
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Excystation of *Cryptosporidium* oocyst. © Russell Kightley Media, GPO Box 3021, Canberra, ACT 2601.

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## INTRODUCTION

The *Cryptosporidium in Water* conference was convened to provide Australian water industry and public health professionals with an overview of current scientific knowledge on this protozoan parasite, and an opportunity to discuss and debate the needs for future research, public health strategy, and risk assessment and management for water supplies.

The conference on 5 October 1998 brought together more than 280 delegates from diverse backgrounds in water supply and management, parasitology, general microbiology, epidemiology and public health.

The conference was organised in three themes, each with its own objective:

Parasitology and Genetic Typing	Introduce genetic typing to assist in locating the source of the parasite.
Epidemiology	Improve epidemiological surveillance, outbreak management and public health response.
Risk Assessment and Management	Understand and manage the health risks implied by <i>Cryptosporidium</i> monitoring results.

During the opening session, five prominent speakers presented an overview of the "state of the art" on *Cryptosporidium* research. The conference then split into three parallel workshops for more specialised presentations on each of the themes. The conference closed with a plenary session where the discussions on each theme were summarised by expert reporters, and questions were invited from the audience.

Next day, following on from the general conference, small groups of experts continued discussions on each theme, with the aim of arriving at consensus positions on research needs, public health strategy and risk management principles. The deliberations of these groups and their outcomes are summarised in these Proceedings.

### Organising Committee

John Langford	Water Services Association of Australia
Martha Sinclair	Monash University / CRCWQT
Martyn Kirk	Department of Human Services, Victoria / CRCWQT
Daniel Deere	South East Water Limited
Joe Owzinsky	Australian Water and Wastewater Association
Kit Fairley	Monash University / CRCWQT
Bob Dorrat	Water Services Association of Australia

### Workshop Reporters

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Martyn Kirk	Department of Human Services, Victoria / CRCWQT
Peter Nadebaum	Egis Consulting / CRCWQT

### Proceedings Editor

Martha Sinclair	Monash University / CRCWQT
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## **The Conference Sponsors**

### **The Cooperative Research Centre for Water Quality and Treatment**

The CRC for Water Quality and Treatment has been a major focus of water quality research in Australia since its formation in 1995 under the Commonwealth Government Cooperative Research Centres Program. The mission of the Centre is to help the Australian water industry produce high quality water at an affordable price, through a comprehensive program of research, commercialisation, education and training.

The 19 participants in the CRCWQT are representative of the cross-section of the Australian water industry including: private and public water industry managers, water research scientists, public health professionals, university researchers, water treatment technologists and chemical suppliers, and environmental consultants. A unique feature of the CRCWQT is the involvement of public health experts in water quality research to ensure that developments in drinking water quality and treatment are accompanied by appropriate evaluation of their public health impacts.

URL <http://www.med.monash.edu.au/epidemiology/crc/>

### **Water Services Association of Australia**

The Water Services Association of Australia is a relatively new industry association meeting the needs of the Australian urban water industry. WSAA was incorporated in 1995 and has 19 full Members that collectively provide 12.5 million Australians with water supply and sewerage services. In representing its Members WSAA seeks to be respected and sought after for its strategic contribution on national issues for the provision of water services, focussing on: customer service; public health; environmental management; and business performance.

The goals and strategies for WSAA's activities and research in the field of drinking water and public health have evolved after considerable discussion with Members and public health professionals. The principal philosophy underpinning WSAA's work on drinking water quality is that public health outcomes are paramount. Public health outcomes should also be achieved in cost effective ways, balancing expenditures on improving drinking water for health reasons against other public health initiatives.

URL <http://www.wsaa.asn.au/>

### **Australian Water and Wastewater Association**

Australian Water and Wastewater Association The Australian Water and Wastewater Association is an independent national association of individuals and organisations interested in all aspects of the water industry. AWWA has a membership of over 3,500 individuals and more than 500 organisations drawn from all sectors of the water industry.

AWWA provides information, networking, meetings, publications, education and advocacy through branches in each state and territory of Australia. The organisation is also affiliated with national and international bodies in the water and environmental fields. Every year, AWWA stages a major, national conference and an associated trade exhibition, Ozwater-Ozwater, as well as several, smaller, specialist conferences. A Bookshop operates from the Sydney headquarters, where there is also a specialist water library.

URL <http://www.awwa.asn.au/>

### **Additional Sponsorship**

The organisers thank the Department of Human Services, Victoria for their generous

## CONFERENCE PROGRAM

- 8.00am                   Arrival and registration
- 8.45am                   Opening by Professor Richard Larkins  
*Chair, National Health and Medical Research Council*
- 9.00am                   Dr Peter O'Donoghue  
*The Cryptosporidium "Crisis"*
- Dr William MacKenzie  
*Epidemiology of Cryptosporidium in the US*
- 10.20am                 Coffee break
- 10.40am                 Dr David Casemore  
*Epidemiology of Cryptosporidiosis in the UK*
- Dr Jeremy McAnulty  
*Epidemiology of Cryptosporidiosis in Australia:  
"Where does Sydney fit in ?"*
- Dr Peter Nadebaum  
*Risk Assessment Overview*
- 12..40pm               Lunch
- 1.30pm                   Parallel workshops
- Parasitology and Genetic Typing*  
                                  Detection  
                                  Production of Parasites  
                                  Genetic Typing
- Epidemiology*  
                                  Surveillance  
                                  Analytical Studies  
                                  Human Infection
- Risk Assessment and Management*  
                                  Catchment Issues  
                                  Treatment Correction  
                                  Distribution System, Consumption and Dose-response
- 4.25pm                   Coffee break
- 5.00pm                   Plenary session - summaries by workshop reporters
- 6.00pm                   Close

## OPENING ADDRESS

**Professor Richard Larkins**

*Chair, National Health and Medical Research Council*

I believe that going into the next millenium, the public health aspects of the environment are going to be one of the major areas of importance to medical research, and certainly the National Health and Medical Research Council regards this as very much a priority area. If there was any doubt about this, the recent events in Sydney have focussed our minds on how little we really understand about the potential threats posed by changes in our environment and within our public services.

In many ways Australia has taken the lead in the question of the quality of our public services, particularly with respect to water supply. The establishment of the CRC for Water Quality and Treatment in 1995 is a world first in trying to bring the public health aspects of water together with the public services approach to the supply of water. It therefore places Australia in a very strong position to establish consensus guidelines with respect to the quality that it expects in the water supply.

I think on the particular question of *Cryptosporidium*, the sorts of issues that come out of the incidents in Sydney, and that will obviously be a central theme for todays conference, relate to what the expectations should be with respect to the quality of the water.

Should we be aiming for zero tolerance with respect to *Cryptosporidium*?

Should we be looking to international benchmarks?

Should we be trying to use epidemiological approaches to establish what the safe levels of *Cryptosporidium* are?

Or should we be monitoring trends and if there is a change in trend or levels within the water supply should we alert the public at that stage?

How do we educate the public about the significance of a finding?

I think if you asked people in the street the only acceptable level for parasites in the water would be zero - they would be very much opting for the first alternative. There are also questions of cost-effectiveness. How much would it actually cost to remove all the *Cryptosporidium* and what are the opportunity costs entailed in that? How many hospital beds would have to be closed? or what antenatal services would have to be restricted?

With the advances in our basic understanding of science and the genetics of parasites, we are now in a situation of being able to track the epidemiology of the contamination of water much more accurately and when there are changes, as in the Sydney circumstance, theoretically at least able to track their source. The NHMRC has over the years been very interested in the question of water quality and has national guidelines with respect to drinking water quality. Currently, the Australian Drinking Water Guidelines are under review and the new guidelines will be published in the next year or so. So this is therefore a symposium of very great interest to the NHMRC.

I wish you very well with your conference. Along with other members of NHMRC, I will look forward to the outcome of the meeting with great interest. I think it is a highly significant meeting because of bringing together the public health and public service aspects of water supply, so I wish you very well for the rest of the day and hope it is highly constructive, and I am very happy to declare the meeting open.

## THE *CRYPTOSPORIDIUM* "CRISIS"!

**Dr Peter O'Donoghue**

*Department of Parasitology, The University of Queensland, Brisbane, QLD, Australia.*

Water provision and public health authorities in many countries are faced with a mounting dilemma. New technologies are being adopted by water microbiology laboratories and by medical and veterinary pathology laboratories which has increased the frequency of detection of the protozoan parasite *Cryptosporidium* in public water supplies and in patients with diarrhoea. The dilemma is to establish a causal relationship and determine the degree to which contaminated water may contribute to clinical disease outbreaks. This is not an easy question to answer.

*Cryptosporidium* is a spore-forming apicomplexan parasite similar to other enteric coccidia commonly found in wild and domestic animals. All infections are transmitted by contamination of the environment with resistant oocysts excreted by infected hosts. The parasite infects a wide range of vertebrate hosts and has been associated with clinical disease primarily involving watery diarrhoea in mammals, diarrhoea and respiratory signs in birds and gastritis in reptiles and possibly fish. Recent surveys have indicated that many infections may remain asymptomatic while comparatively few may produce clinical signs. At present, there is no effective treatment for infections. Fortunately, most infections are self-limiting and resolve spontaneously although chronic infections may develop in high risk patient groups, especially neonates and immuno-compromised individuals (those with congenital or acquired immuno-deficiencies or undergoing immuno-suppressive chemotherapy).

Different parasite species occur in different host groups but they cannot be distinguished simply on the basis of oocyst morphology. Infections have been shown to be transmissible between hosts belonging to the same vertebrate class (mammal-to-mammal, bird-to-bird) but not between different vertebrate classes (mammal-to-bird, bird-to-mammal, etc). Only one mammalian species, *Cryptosporidium parvum*, has been associated with disease in humans and most epidemiological studies have implicated direct human-to-human transmission and to a lesser extent animal-to-human (zoonotic) transmission.

The parasite has recently gained worldwide notoriety as a water-borne pathogen following its detection in public water supplies and swimming pools following focal outbreaks of disease. This encouraged many agencies to begin monitoring raw and treated water sources for contamination by the parasite. Prophetically, oocysts are being detected at alarming rates in urban and rural water sources. Nonetheless, medical and veterinary laboratories continue to diagnose infections in humans and animals only sporadically and outbreaks of disease are infrequent. Water contamination therefore does not correlate well with disease occurrence (contamination being more common than disease).

This produces the dilemma faced by health authorities when oocysts are found in water supplies. Are they a cause of public health concern? Where did the parasites come from? Are they alive or dead? Are they killed by water disinfection procedures? Are they removed by filtration? Are they infective for humans or only for animals? Are they virulent or pathogenic in their hosts?

Researchers have only just begun to address these questions as the resources and techniques become available. Despite promising advances made in several areas of research, many problems remain in the areas of epidemiological surveillance, outbreak management, parasite characterization and risk assessment. It is fervently hoped that sensible codes of practice will be adopted by industry and government authorities pending comprehensive studies on parasite

## EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN THE US

**Dr Willam R MacKenzie**

*Division of Parasitic Diseases, Centers for Disease Control and Prevention, US.*

In 1976, *Cryptosporidium* was first discovered as an etiologic agent of gastrointestinal illness in immunocompromised persons. It is now recognized as an important cause of illness in persons with normal immune systems. *Cryptosporidiosis* can be transmitted through direct contact with human or animal feces, eating contaminated food, or drinking contaminated water. Because *Cryptosporidium* is highly chlorine resistant inadequately filtered drinking water and swimming pools may be common vehicles for infection.

*Cryptosporidiosis* is characterized by diarrhea that is typically profuse and watery, abdominal cramping, fatigue, muscle aches, headache, and fever. In children, vomiting and low grade fever are common. In immunocompetent persons the illness on average lasts 3 to 5 days, but may last several weeks in some persons and recurrence of symptoms after initial recovery is common. In immunocompromised persons, *cryptosporidiosis* may be unrelenting and severe depending on the level of immune function. Symptoms typically begin 6-7 days after exposure (range 1-14 days). The dose needed to cause primary human infection with *Cryptosporidium* is likely to be less than 30 oocysts. However, persons with antibodies to *Cryptosporidium* require higher doses to become infected and experience milder symptoms. To date, there is no generally accepted antimicrobial treatment for *Cryptosporidiosis*.

Laboratory surveys of stools for *Cryptosporidium* have shown positivity rates of 0.4% to 6% which is comparable for isolation rates of *Salmonella* and *Shigella* in the U.S. In a limited number of study populations examined by CDC it appears that >50% of participants have antibody evidence of prior infection with *Cryptosporidium*. These findings suggest that exposure to infection with *Cryptosporidium* is relatively common.

In the United States, outbreaks of *cryptosporidiosis* have been primarily attributable to consuming contaminated drinking or recreational water. Since 1984, there have been nine *Cryptosporidium* outbreaks attributed to drinking water in the U.S. Both surface water and ground water sources have been implicated. In all but one outbreak, the drinking waters implicated have met existing water quality standards. Recognition of outbreaks is frequently delayed because: 1) Persons with gastrointestinal illness frequently do not seek health care, 2) *Cryptosporidium* is often not considered in the differential diagnosis of gastrointestinal illness, except in HIV positive persons, 3) most laboratories do not routinely test for *Cryptosporidium* (only 5% of U.S. labs routinely test), 4) there is a lack of awareness by physicians that special laboratory testing is required, and 5) the prolonged time between exposure and reporting of cases to the health department (14-24 days in the best of circumstances).

*Cryptosporidium* surveillance data reveals a bimodal distribution of cases with greater numbers of reported cases among young children and persons 30 - 40 years of age. For young children peak exposures likely occur mid-to-late summer and early Fall. These data suggest that recreational water is an important vehicle in young children during the warmer seasons. Investigations of outbreaks associated with recreational water support this hypothesis. The high resistance of *Cryptosporidium* to chlorination, its low infectious dose, the profuse/prolonged shedding of oocysts by infected persons, and the presence of incontinent children, combine to make recreational water an efficient vehicle for infection.

## **EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN THE UK**

**Dr David P Casemore**

*Public Health Laboratory Service Cryptosporidium Reference Unit, Rhyl, Wales, UK.*

Human cryptosporidiosis emerged in the 1980's as a cause of acute gastro-enteritis, especially in children. Evidence from early studies in the UK led, intuitively, to the view that there were two cycles of infection, zoonotic and urban, with the latter predominating. The importance of water as a route of transmission also emerged in the 1980s. A notable feature was commonly a marked increase in adults among early primary cases and the amplification of such outbreaks by secondary propagation, especially amongst children. Such outbreaks are thus apparently community outbreaks initiated by water. The role of water in sporadic (endemic) infection is unclear but may be considerable.

A picture has emerged of the complex dynamics, which includes several key variables, including immuno-prevalence in the exposed population. One consequence is that those areas supplied from sources perceived to be safe may tend to experience a greater impact (attack rates) from a contamination event than those supplied from poorer quality sources. Surface-derived sources are likely to lead to exposure to isolates derived from a variety of sources, both human and animal.

The application of molecular methods, in collaborative studies, has enabled these areas to be explored in increasing detail. Preliminary findings, which have revealed striking support for the dynamics described, will be presented.

## **EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN AUSTRALIA: WHERE DOES SYDNEY FIT IN?**

**Dr Jeremy McAnulty**

*Department of Health, Sydney, NSW, Australia.*

Several studies have examined risk factors for cryptosporidiosis in Australia. In the 1980s, laboratory studies identified cryptosporidiosis as an important cause of diarrhoea in children and among persons with AIDS. In the early 1990s, outbreaks of cryptosporidiosis were reported in child care settings, and a South Australian study suggested that consumption of spring or tap water was a possible risk factor for illness. In the mid 1990s, a large outbreak was linked to a contaminated swimming pools in Sydney, and in 1997, several more large outbreaks on the east coast were traced to multiple contaminated swimming pools.

These findings prompted initiatives including mandatory notification of cryptosporidiosis in some states and -- increasingly -- environmental testing of water. The health implications of positive environmental testing remain unclear.

The detection of *Cryptosporidium* oocysts and *Giardia* cysts in routine testing of Sydney drinking water in July 1998 (and subsequent detections in August and September) provided an opportunity to examine the relationship between environmental findings and human health effects.

In response to these findings, and the subsequent boil water alert, NSW Health initiated enhanced surveillance for diarrhoea in general, and for cryptosporidiosis and giardiasis in particular, through sentinel agencies including laboratories, general practitioners, emergency departments, pharmacies and nursing homes.

Preliminary results indicate that apart from expected day-to-day variations in reports of diarrhoea, there was a sharp increase in stool specimens submitted to laboratories and an associated mild increase in expected reports of giardiasis in the week following the boil water alert. At the same time there has been no significant increase and very few reports of cryptosporidiosis in Sydney residents (0-2 per week). Apart from background fluctuations, sentinel sites reported no increases in the number of cases of diarrhoea.

Cryptosporidiosis remains an emerging disease in Australia. Further epidemiological research is necessary to improve our understanding of risk factors for cryptosporidiosis in Australia, and of the implications of positive environmental tests for human health.

## **RISK ASSESSMENT OVERVIEW**

**Dr Peter Nadebaum**

*CMPS&F Pty Limited, Melbourne, VIC, Australia, and  
CRC for Water Quality and Treatment.*

The management of water quality by water authorities is an area critical to their operations. Over the past few years there has been a shift to corporatisation and a greater focus on achieving a return on the investment that has been made in the water supply system. Water authorities are becoming subject to greater regulation and scrutiny by their customers and the media, and some authorities are required to undertake independent audits of their operations and performance.

Water authorities are adopting a higher level of quality assurance in their operations, and some have achieved certification under accepted quality standards, such as ISO 9000 (Quality Assurance) and ISO 14000 (Environmental Management Systems and Auditing). Some have adopted a risk management framework similar to that outlined in AS/NZ 4360 (Risk Management) with a risk focus on various critical parts of their water supply operations, such as public health.

The key elements of a risk management approach are:

- water quality management policy: including the levels of service with respect to water quality; are these to be minimum standards or best practicably achievable?
- planning: including establishing the water quality objectives consistent with its policy, legal and other requirements (such as duty of care under the Trade Practices Act),
- identification of problem areas, assessment and prioritisation on a risk basis, leading to
- management and corrective action, and formulation of water quality improvement strategies.

One of the most difficult areas for water authorities to address is the issue of what is an acceptable level of water quality. There has been considerable advances in our ability to detect a wide range of constituents at very low levels, and the water industry is experiencing a similar problem that the environmental field found a decade ago, where improved analytical methods became able to detect a variety of highly toxic substances such as dioxins and furans at very low levels. In the case of the water industry, a limited range of microbiological pathogens are now able to be detected. It is likely that an increasing number will be able to be detected in the future, extending to other substances such as MX and the furanones (disinfection byproducts).

Health Authorities are considering the issues and what guideline values should apply. There are two approaches that can be adopted: setting guidelines based on **observed** effects involving percentages of the population per year (which correspond to tens of thousands of people in a large city), or on **predicted** effects (which can be much lower and affect only a few of the population). In environmental regulation and some areas of health regulation (such as protection against cancer) the basis has been precautionary and based on prediction, even though the ability to predict effects is highly imperfect. It is quite possible that a precautionary approach will be adopted in Australia, although this is not at all certain.

At the heart of this is the ability to understand and predict the risk that is involved, so that informed decisions can be made that reflect the requirements that apply in Australia, and in

possible to determine whether there is a real problem which must be addressed, or not. In this way, a risk-based management strategy becomes possible.

A key aspect of this workshop is to explore these issues, and to determine what can be agreed on. To simplify the problem we have focused on two pathogens: *Cryptosporidium* and *Campylobacter*. A project is underway in the CRC for Water Quality and Treatment which is exploring the following issues:

- what incidence of infection can be expected to result from the concentrations of these pathogens that are typically measured in water supplies?
- can we estimate the level of infection and, if so, how do we do this and what assumptions should be made in this estimation?
- how should pathogen sampling and analysis results be interpreted, and what is the level of uncertainty in such results?
- On what basis should water authorities manage their water supplies?

These issues and the findings of our preliminary reports on the assessment of risk associated with *Cryptosporidium* and *Campylobacter* will be discussed in the following workshop, and will be reported on later in these Proceedings.

## **PROGRESS IN DETECTION OF CRYPTOSPORIDIUM IN WATER**

**Dr Colin Fricker**

*Head of Microbiology for Thames Water, UK.*

In recent years there has been considerable interest in the development of methods for the detection of *Cryptosporidium* in water. Recognising that there are three basic steps to the detection procedure - concentration, separation and detection - workers have tended to address one or other of these steps. This has resulted in a plethora of techniques for each stage. Whilst some developments have focused on molecular procedures which detect an internal molecule such as the DNA or 18S rRNA, most concentration and separation techniques have adopted a holistic approach resulting in preparations which can be examined using one of several detection methodologies. Such approaches are to be commended as they generally allow more than one detection technique to be used and may impart further information such as the species or viability of the detected organisms to be determined.

Initial concentration techniques are of fundamental importance and many devices have recently become commercially available resulting in an often confusing choice of procedure for laboratories involved in the difficult task of detecting *Cryptosporidium* in water. Separation technology has largely depended on either flow cytometry or immunomagnetic separation, both of which are now widely used. Each of these techniques has benefits and drawbacks and it is often necessary to choose the separation technique based on the quality of sample to be analysed. The range of detection methodologies offers the widest choice. Methods range from simple epifluorescence microscopy to procedures combining tissue culture infectivity and the polymerase chain reaction. No single protocol has become universally adopted and this is largely because of the range of equipment and technical skills required for the different methods. In general, holistic approaches are to be favoured since they offer the widest range of possibilities with regard to detection and subsequent characterisation.

This presentation will describe some of the range of methodologies which are available together with their perceived benefits and drawbacks. Furthermore, procedures for comparing the efficiency of different methods will be described, demonstrating that much of the information found in the scientific literature is misleading and in some cases blatantly incorrect.

**EFFECTS OF THE CLEANING PROTOCOLS ON THE VIABILITY  
ASSESSMENT OF COMMERCIALLY AVAILABLE  
*CRYPTOSPORIDIUM PARVUM***

**Mr John Watkins** (*Alcontrol Laboratories, Bradford UK*) and Mark Smith  
(*Drinking Water Inspectorate, London*)

Studies of *Cryptosporidium parvum*, whether looking at infectivity, environmental survival or the effects of disinfectants, rely mostly on availability of oocysts on a commercial basis. These oocysts are produced by animal infection and the faecal material may then be cleaned by a variety of protocols including differential centrifugation or flotation using sucrose, caesium chloride or sodium chloride with sulphuric acid as a flocculating agent. In addition preservatives such as potassium dichromate may be added. The cleaning protocols may have an effect on the surface chemistry of the oocyst and may alter its survival and performance in subsequent studies. In addition, there are a number of commercial 'strains' available. There are, therefore, a number of confusing factors which exist when trying to compare results obtained from studies using oocysts. Many researchers fail to identify the source and age of oocysts or cleaning protocols used when conducting such studies.

The presentation will examine the effect of cleaning faecal material from four commercially available 'strains' of oocysts on viability assessment. In addition, SDS-PAGE typing has shown significant differences between 'strains'.

## **PROPAGATION AND MAINTENANCE OF *CRYPTOSPORIDIUM PARVUM* IN THE LABORATORY**

**Professor Saul Tzipori**

*Head, Division of Infectious Diseases, Tufts University School of Veterinary Medicine,  
Massachusetts, US.*

The inability to continuously propagate *C. parvum in vitro* remains the most important obstacle to performing the kind of studies possible with so many other pathogens of major interest. This limitation is reflected in the lack of access to stages of the life cycle other than sporozoites and the oocysts, and the need to use animals to generate oocysts for laboratory investigations. Furthermore, oocysts generated in animals have a limited viability span. For instance, infectivity is markedly diminished within 6-8 weeks for *in vitro* and *in vivo* infectivity studies. They may however still be useful for animal propagation, and as a source of antigen for serology, biochemistry and genetic work. As presently there are no methods which allow an indefinite storage of infectious material, isolates have to be continuously passaged through animals, usually calves. The lack of recognized and characterized molecular, antigenic, or virulence markers makes it impossible to conduct meaningful comparative studies among isolates obtained from different hosts, geographic locations, or propagated in different host species. This makes the use of standard isolates over time for comparative studies, impossible. A limited cell culture propagated parasite, largely confined to the asexual stages, is available but provides only a limited scope.

Oocysts and sporozoites are the only stages of the life-cycle which can be produced in large quantities in experimentally infected calves. Oocysts are readily purified and concentrated from calf feces. Oocysts can also be obtained from experimentally infected small ruminants immediately after birth. Some investigators use rodents to generate small quantities of oocysts. Another major source of material is from people with AIDS, chronically infected with *C. parvum*. However these sources tend to provide small and variable quantities of material which limit certain studies on genetic variation, virulence and host susceptibility.

## **BIOCHEMICAL APPROACHES FOR THE IDENTIFICATION OF PARASITES AND THEIR POTENTIAL FOR STUDYING CRYPTOSPORIDIUM**

**Neil B. Chilton** (*Department of Veterinary Science, The University of Melbourne, Melbourne, VIC, Australia*) and **Ross H. Andrews** (*Department of Microbiology and Immunology, The University of Adelaide, Adelaide, SA, Australia*).

It is essential that we have effective and reliable methods to identify and characterise, with confidence, parasites of medical importance in order to implement effective strategies aimed at their detection, prevention, treatment and/or control. It is also important to establish whether the animal hosts represent a 'reservoir' for those parasites infecting humans, that is, to determine unequivocally whether the parasite species in animals represents the same or different species as those infecting humans. However, it has always been problematical to distinguish between closely-related species of protozoan parasite using traditional methods (eg. morphology). Such is the case for *Cryptosporidium*, where the exact number of species present within the genus is debatable. Therefore, other techniques need to be employed to effectively identify and characterise these pathogens.

Multilocus enzyme electrophoresis has been used widely as a valuable alternative/adjunct to the identification of metazoan organisms, both non-parasitic and parasitic. It is an extremely powerful technique when used in an appropriate manner and is very cost-effective compared to some of the more modern DNA technologies. This biochemical technique has been refined and used successfully in the characterisation and identification of a wide range of protozoan parasites. We demonstrate the value and effectiveness of multilocus enzyme electrophoresis in providing answers to questions relating to the identification and characterisation of protozoan parasites by highlighting previous studies on *Leishmania* and *Giardia*. We also discuss the applicability of the technique for the identification and characterisation of *Cryptosporidium* and its potential for establishing diagnostic genetic markers and for examining genetic variation.

**CHROMOSOME SEPARATION TECHNIQUES  
FOR CHARACTERISATION OF GENETIC VARIATION:  
ONE GENE, BIG PROBLEM**

**Peter Upcroft and Jacqueline A. Upcroft**

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*Giardia duodenalis*, the anaerobic, amitochondrial, flagellated protozoan parasite is a well known agent of waterborne outbreaks of diarrhoeal disease although the most common route of transmission in Australia is faecal/oral. Parasites isolated from humans can be grouped into at least two different demes, varieties or even species by techniques such as electrophoretic karyotyping, rDNA unit sequence, hybridization patterns with gene specific probes and isoenzyme analysis. However, these groups can not be presently distinguished from one another on the basis of pathogenicity, virulence, zoonotic potential or host range. Moreover, genes from some isolates of *G. duodenalis* are sufficiently diverse that they do not hybridise to other isolates. Thus a single gene probe from one group may not detect parasites of the other group(s).

On the other hand, a single gene such as rDNA may detect all parasites with no discrimination regarding virulence, viability or zoonotic potential. Axenic culture of *Giardia* trophozoites from biopsy, stool and environmental samples has enabled detailed study of the *Giardia* genome.

These studies have revealed a very plastic genome. Chromosomes are polyploid with considerable variation in copy number as well as carrying aneuploid accessory chromosomes. From our chromosome mapping and sequencing studies we have shown that gene gain, loss and copy number are variable among isolates and an entire chromosome in one deme is not present in the other. These are the background conditions essential for understanding virulence traits in *Giardia* and similarly in other organisms such as *Cryptosporidium*.

Using these genomic data we have followed waves of human infection by different *Giardia* demes and determined that a highly pathogenic bird *Giardia* isolate was indeed a "human" isolate. This latter information has serious implications for environmental *Giardia* cyst contamination since prior to our studies it was believed that birds did not carry *Giardia* which was infectious to man. However, there are no genetic markers for pathogenicity, virulence and host range and thus pathogenic *Giardia* cysts in the environment cannot be discriminated nor can cyst origins be identified. In addition cyst viability assays can be difficult. More biological information is required to determine what levels of *Giardia* contamination are acceptable in the environment.

**THE MOLECULAR EPIDEMIOLOGY OF CRYPTOSPORIDIAL INFECTIONS****Una M Morgan and RC Andrew Thompson**

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Sensitive methods for detecting and characterising *Cryptosporidium* in clinical and environmental samples are urgently required for the predictive epidemiology of infections with this organism. Available diagnostics are of limited value due to insensitivity, poor reproducibility, problems with interpretation and cost. We have developed a range of PCR-based DNA techniques and have applied these directly to isolates of *Cryptosporidium* from humans, pets, livestock and native animals. In collaboration with colleagues in North America, Africa, Asia and Europe, we have established a comprehensive reference collection of over 250 isolates of *Cryptosporidium* from numerous host species. Several genotypes, some of which may represent new species, have been identified and appear to be maintained in different transmission cycles<sup>1,2,3</sup>. Results to date from our laboratory indicate that at least two of these genotypes are infective to humans<sup>1,2,3</sup>. One of these appears to only infect humans whereas the other is maintained in animal reservoirs including cattle, sheep, pigs and mice. The availability of such molecular epidemiological tools will be of value in cryptosporidial outbreaks where determining the source of infection will limit transmission, particularly to those most at risk. For example, in a recent comparative study of clinical specimens, we showed that microscopy exhibited a sensitivity of only 83.7% and specificity of 98.9% compared to PCR which was easier to interpret and differentiated between the major genotypes of *Cryptosporidium* known to cause disease in humans<sup>4</sup>. Approximately 80% of human cases were associated with infection by the 'human' genotype (Genotype 1) whereas the remaining cases were infected with the 'cattle' genotype (Genotype 2).

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## GENOMIC METHODS FOR DETECTION AND TYPING OF *CRYPTOSPORIDIUM* FROM THE ENVIRONMENT: A REALISTIC OPTION?

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Environmental *Cryptosporidium* detection methods that are to be beneficial in understanding health risk need to answer key questions such as: is it the pathogenic species, is it viable or infectious, and how many organisms are present? In addition methods need to be reliable, convenient to perform and cost effective for routine analysis. Currently there is no one approach that can demonstrably fulfill all these criteria. Physical capture of oocysts by various antibody-dependant separation and identification devices, such as flow cytometry, immunomagnetic separation and epifluorescence microscopy, are the most accepted means for detecting *Cryptosporidium* in the environment. However these approaches have significant limitations imposed on them because of the assumption that all oocyst surface epitopes in all sample types will share the same antibody affinity. Furthermore, the endpoint of all antibody based detection techniques requires human recognition of characteristic oocyst morphology and morphometry to confirm a positive result. Methods that allow direct detection of parasite-specific genomic sequences, a permanent marker of an organism's identity, overcome the limitations imposed by potentially unpredictable epitope/antibody interactions and subjective human observations.

PCR is one molecular genetic approach that has been reported by a handful of laboratories to detect *Cryptosporidium* in water and the environment but this technology is also not without problems. Inhibition of the PCR by even trace amounts of co-concentrated contaminants has been a significant issue and has hampered the widespread use of this technology to environmental monitoring. In our laboratory we have largely overcome PCR inhibition and have achieved high detection sensitivity of *C. parvum* with a method based on direct detection of *C. parvum hsp70* mRNA by reverse transcription (RT)-PCR. Paramagnetic beads facilitated convenient and efficient isolation of sufficiently pure template mRNA. The technique has also been amenable to simultaneous detection of *Giardia* spp. To demonstrate the efficiency of this procedure we have undertaken some comparative analysis of a variety of water samples by RT-PCR and IFA microscopy. Results of 54 surface water samples analysed to date indicate that only a small proportion of *Cryptosporidium* in the environment are *C. parvum*. In an investigation of 30 swimming pools during an outbreak of cryptosporidiosis, *C. parvum* was detected in 30% of samples by RT-PCR compared with 10% by IFA microscopy. In addition, by sequencing the PCR products obtained from some positive swimming pool samples it was possible to detect small regions of nucleotide sequence polymorphism within the *hsp70* amplicon. These differences have been sufficient to distinguish *C. parvum* isolates obtained from different sources.

In our experience PCR is proving to be a cost effective, flexible and powerful tool for environmental parasite detection, offering high detection sensitivity, pathogen specificity, viability determination, sub-species typing and objective assessments in a rapid/batch sample processing format. But there are several issues that need to be addressed to enable more widespread acceptance of PCR based methodology. These include further research to achieve consensus regarding suitable genetic loci, continued improvements to nucleic acid purification methods and standardizing methodologies. Further investigation of quantitative PCR methods should also be undertaken. Finally, interlaboratory comparisons of proposed methods need to be promoted as a means of establishing reliable protocols for widespread use

**THE STUDY OF NATURAL AND LABORATORY ISOLATES OF  
*CRYPTOSPORIDIUM PARVUM*  
USING HIGH-RESOLUTION GENETIC FINGERPRINTING**

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The origin of *Cryptosporidium parvum* oocysts in surface water is difficult to determine. Molecular tools for characterizing *C. parvum* can provide some information on the host origin of waterborne oocysts. Polymerase chain reaction (PCR) combined with restriction fragment length polymorphism (RFLP) has been used in several laboratories to differentiate between *C. parvum* isolates. Based on the application of multiple and unlinked PCR-RFLP markers, two genotypes, designated H (1) and C (2), have been described. These genotypes are distributed worldwide. These markers failed to detect recombinant genotypes, indicating a lack of genetic exchange between H and C populations. Currently, the main limitations of the PCR-RFLP method for typing waterborne *C. parvum* oocysts are: (1) PCR amplification can be inhibited by waterborne solutes; (2) *C. parvum* oocysts of genotype C can originate from humans or from calves; (3) mixed genotypes may remain undetected.

In order to improve the resolution of the genetic fingerprinting method, the variability of a non-coding genetic locus was investigated. We focused on the intron located in the  $\alpha$ -tubulin gene of *C. parvum*, the only intron in *C. parvum* described to date. Confirming the expected high degree of polymorphism, 18% of the nucleotides within the intron were polymorphic as compared to 6% polymorphism in the adjacent coding region (exon 2). In the samples analysed, which included oocysts recovered from people with AIDS, from acute human infections and from animals, at least 8 sequences (alleles) were identified. This finding contrasts with the published RFLP markers which typically identify two genotypes only. The comparison of multiple sequences from the  $\alpha$ -tubulin intron confirmed the existence of two main genetic subgroups, which corresponded to the H and C genotypes. A third genotype with considerable divergence from the H and C groups was identified in an isolate originating from an AIDS patient. Within each subgroup extensive sequence variation was found. Genotypic analysis of an isolate serially passaged in calves showed changes in the  $\alpha$ -tubulin RFLP profile, suggesting that the makeup of *C. parvum* populations can change.

Conclusions: Although the analysis of currently available polymorphisms can yield valuable information on the genetic makeup and transmission routes of *C. parvum*, the approach is limited by its technical complexity, by the fact that the same genotype can infect different host species and by an apparent lack of stability of certain markers.

## HIGH RESOLUTION ANALYSIS OF GENETIC VARIATION BY MUTATION SCANNING

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Being able to accurately identify a parasite at any stage of its development is central to the diagnosis of infection, and has important implications for disease control and investigating transmission patterns. However, the identification of parasites to the species or strain level is frequently not possible using morphological features, in particular for *Cryptosporidium*.

DNA technology can provide alternatives for parasite identification, and polymerase chain reaction (PCR) techniques have found broad applicability because their sensitivity permits the analysis of DNA from minute amounts of parasite material. For instance, PCR-linked restriction fragment length polymorphism and direct cycle sequencing methods have been employed to define genetic markers for the species or strain specific identification of parasites.

However, these approaches do not necessarily accurately resolve sequence variation because they rely on the size-separation of DNA molecules. Also, they can be laborious and time-consuming to perform when large numbers of samples are to be examined. Such limitations may be overcome by employing high-resolution mutation scanning methods which separate DNA molecules in a sequence-dependent manner. Methods such as denaturing gradient gel electrophoresis (DGGE), single strand conformation polymorphism (SSCP) and their modifications are widely used in a range of areas of biomedical research for analytical and diagnostic purposes, and are finding increased application to parasite genes because of their exquisite resolving capacity.

The aim of this paper is to demonstrate (using selected examples) the usefulness of mutation scanning techniques for the high-resolution display of sequence variation in parasite DNA for the purpose of species and/or strain identification and to indicate their excellent potential for the genetic characterisation of species and strains of *Cryptosporidium*.

**HIGH RESOLUTION ANALYSIS OF GENETIC VARIATION II:  
MICROSATELLITE MARKERS**

**Dee Carter**

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Microsatellites are short sequence motifs repeated tandemly, and are found throughout the genomes of all eukaryotes. These sequences have a high mutation rate, with repeat units added or lost during replication. Different organisms may therefore possess a different complement of microsatellites, which can be exploited in strain typing and epidemiology studies. In this talk I will review the application of microsatellites to other lower eukaryotic organisms, and discuss their potential in the differentiation of individuals and clones of *Cryptosporidium*

**FINGERPRINTING *CRYPTOSPORIDIUM*:  
PROBLEMS, REASONS FOR CAUTION, AND A NEW POLYMORPHIC MARKER.**

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The water industry needs methods that can differentiate between *Cryptosporidium* species and between isolates of *C. parvum*. Some reports have indicated that random amplification of polymorphic DNA (RAPD) is useful for differentiating species and discriminating between isolates of *Cryptosporidium* from different sources. However, in our evaluations of RAPDs using previously published and new arbitrary primers it was determined that the technique did not demonstrate sufficient reproducibility to be used on a routine basis. In addition, using the same primers and identical extracted DNA (supplied by the original authors) we obtained different RAPD profiles compared to those in a published report. Consequently, we concluded that specific PCR targeting polymorphic markers was the most appropriate method.

There have been several reports of polymorphic markers that differentiate between isolates of *C. parvum* depending on the animal host of origin. In parallel with evaluations of previously published polymorphic markers we developed primers targeting a new marker. These primers span an area of the beta-tubulin gene which contains an intron and amplify a 625 bp fragment. This region contains the only intron so far discovered in *C. parvum*. These primers are specific for *C. parvum* and do not amplify DNA from *C. muris* or *C. baileyi*. Bovine isolates of *C. parvum* were differentiated from human isolates by restriction digestion of the beta-tubulin amplicon with *Hae*III and sequence differences. The bovine and human isolates differed at nine sequence positions over the length of the amplicon and the human derived sequences had a two base deletion compared to bovine isolates. This represents a 1.8% sequence divergence between isolates which compares to sequence divergence of 0.7 to 1.4% for other published *C. parvum* polymorphic markers. Four of the base substitutions occurred within the intron and the seven substitutions that occurred in the coding region were all silent with respect to amino acids. The base differences were consistent in bovine and human isolates from diverse geographic origins. The sequencing information demonstrates that the beta-tubulin sequence deposited in GenBank contains an erroneous insert of two amino acids suggesting a typographical duplication. Analysis of many of the sequences related to previous reports reveal that, while the overall conclusions of the work do not change, there are inconsistencies in the published information. Examples include differences in the sequence of a single isolate reported by different groups, differences between text based sequences and database sequences, incorrect insertions and deletions, and typographical errors in published sequences. While most of these problems are minor they need to be recognized and removed from the information database.

Our results confirm that genetic differences exist between human and non-human isolates of *C. parvum* and make available an additional polymorphic marker. However, analysis of current information highlights the necessity for close scrutiny of database sequence information and the importance of thorough evaluations and validation of genetic typing schemes before drawing conclusions.

## SURVEILLANCE OF CRYPTOSPORIDIOSIS IN AUSTRALIA

**Dr Mark Veitch**

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Routine surveillance of human cryptosporidiosis is justified by the recognition of *Cryptosporidium parvum* as a relatively common cause of infectious gastroenteritis, the severity of disease in immunocompromised persons, the threat of enormous costs related to preventive measures, the need to evaluate control activities, and the unparalleled public interest in this protozoon.

As of September 1998, all Australian States and Territories routinely collect (or have ready access to) data on laboratory diagnoses of human cryptosporidiosis. These processes are supported by existing legislation, proposed legislation and agreements with diagnostic pathology services. Cryptosporidiosis is not currently included in the National Notifiable Diseases Surveillance System.

Surveillance of cryptosporidiosis as an AIDS-defining illness in HIV-infected persons provides additional data relating to a specific high risk population, albeit with delay between illness and collation of data.

Clusters of cryptosporidiosis may also be detected by States and Territories through additional (but non-uniform) requirements to report cases of gastroenteritis linked by epidemiological features such as temporal or geographic clustering, or when water or food is suspected as the source for the illness.

The access of State and Territory health departments or disease control authorities to reports of the detection of *Cryptosporidium* in water is defined by various specific or general legislative requirements, and non-legislative agreements.

Advancing techniques for detecting *Cryptosporidium* in the environment, and public disclosure of test results, place public health authorities in a difficult situation. Emergency and definitive responses must be informed by ongoing surveillance for human disease, and insights from systematically collected data on the distribution of *Cryptosporidium* in the environment.

Public health intelligence from routine surveillance may be enhanced by studies of the burden of sporadic cryptosporidiosis (including the multipliers between notified cases and estimated total infections, and the socioeconomic effects of illness); a national approach to systematic recording of clusters of enteric illness (including microbiological and epidemiological features); and typing of isolates of *Cryptosporidium parvum*.

## **THE SURVEILLANCE OF CRYPTOSPORIDIOSIS IN NEW ZEALAND**

**Dr Michael Baker, Dr Phillip Weinstein and Ms Nina Russell**

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Cryptosporidiosis and giardiasis were made notifiable diseases in New Zealand in June 1996. Surveillance is based on notification to local public health units. These data are in turn collated and analysed at the national level by ESR, on behalf of the Ministry of Health.

This surveillance system has supported local disease control measures. A notable example was the identification of an outbreak of cryptosporidiosis linked to a swimming pool in the Wellington region. The subsequent investigation identified deficiencies in the design and operation of the pool that had contributed to the outbreak. Action was taken to control the outbreak and reduce the risk of such events occurring in future.

At the national level, these surveillance data have helped to define the epidemiology of cryptosporidiosis in New Zealand. A total of 357 cases of cryptosporidiosis were notified in 1997, a rate of 9.9 / 100,000. Of these cases, 4.9% were hospitalised. Incidence was seasonal, with highest rates in Spring. There were also marked geographic variations in incidence, with higher rates generally in the more rural health districts. The age distribution was bimodal, with the highest rate in children (68.9 / 100,000 for those aged 1-4 years) and a second smaller peak in young adults (7.5 / 100,000 for those aged 20-29 years). Rates in Maori and Pacific Islands people were lower than in Europeans. A case-control study is underway to identify potential sources for sporadic cryptosporidiosis to help develop control strategies.

## **CASE-CONTROL STUDIES OF SPORADIC CRYPTOSPORIDIOSIS IN AUSTRALIA**

**Dr Brent Robertson**

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The protozoal pathogen *Cryptosporidium parvum* is a relatively common cause of sporadic human gastroenteritis, and is transmitted by the faecal-oral route. Persons may be infected by direct contact with infected persons or animals, or indirectly via contaminated water, food or the environment. Large outbreaks of cryptosporidiosis have been attributed to water-borne transmission, despite modern water treatment facilities. However, the relative contribution of various risk factors for apparent sporadic cryptosporidiosis remains unknown.

A case-control study of risk factors for sporadic cryptosporidiosis in the general population is currently underway in Melbourne and will soon commence in Adelaide. Cases defined by symptoms and oocysts in a stool specimen are identified, then interviewed using a computer assisted telephone questionnaire. Asymptomatic controls matched for sex and age are also interviewed. Data analysis will involve logistic regression to evaluate the relative contribution of the different risk factors.

Due to the importance of drinking water as a risk factor we have completed a preliminary validation and reliability study. This has assessed the validity of telephone estimates versus diary recordings of water volume consumption, as well as the reliability of telephone estimates of "usual" water volume consumption. Preliminary data analysis has involved the generation of intraclass correlation coefficients (ICC's). For plain unboiled tap water the ICC's for validity were 0.66 and 0.71 for participants 0-11 years and 12 years and above respectively. The ICC's for reliability were 0.75 and 0.74 for participants 0-11 years and 12 years and above respectively. These results indicate that the questions enquiring into water as a risk factor for the case-control study have a satisfactory level of both validity and reliability.

## **CRYPTOSPORIDIOSIS: A CASE-CONTROL STUDY IN NEW ZEALAND**

**Ms Nina Russell, Dr Philip Weinstein and Dr Alistair Woodward**

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*Cryptosporidium*, an emerging microbial infection, is rapidly becoming recognised as one of the most important enteric pathogens world-wide. Modes of transmission include; directly from animal reservoirs, person to person and the waterborne route. There is considerable evidence from international studies that water may be the most important vehicle for transmission of *Cryptosporidium* as the oocysts are chlorine resistant and environmentally hardy, surviving viably for up to six months in the water.

This is a cause for concern for water consumers in New Zealand, given that many sources of our drinking water supplies are from surface catchments and as such are potentially susceptible to contamination from *Cryptosporidium* oocysts.

The objective of this study is to identify, for the first time, the disease burden and the importance of the various modes of transmission of *Cryptosporidium* in New Zealand. Also, to determine what the main risk factors for endemic versus outbreak cryptosporidiosis are, given that interventions for the two differ. Findings will provide a basis for making decisions about the need, nature and targeting of *Cryptosporidium* control programmes, thereby reducing the incidence of the disease.

The study is a nation-wide case-control study of laboratory confirmed cases of cryptosporidiosis and age and sex matched controls, obtained by random digit dialling. Using a structured questionnaire, participants are being asked by telephone about exposure to possible risk factors in the two weeks preceding their illness/interview. These risk factors include those most commonly cited in the literature as resulting in zoonotic, waterborne and person to person infection. The number and percentage of cases and controls exposed will be recorded for each risk factor. Then the probability of exposure to selected risk factors will be compared between cases and controls.

## **CRYPTOSPORIDIUM PARVUM VOLUNTEER STUDIES: INFECTIVITY, ILLNESS AND IMMUNITY**

**Assoc. Prof. Cynthia L. Chappell**, Pablo C. Okhuysen, M.D., Herbert L. DuPont, M.D.  
(*University of Texas Houston, School of Public Health and Medical School*), Charles R.  
Sterling, Ph.D., (*University of Arizona*) and Walter Jakubowski (*EPA*)

*University of Texas Houston, School of Public Health and Medical School, US*

*Cryptosporidium parvum* is a protozoan parasite that causes diarrheal disease in both immunocompetent and immunocompromised individuals. Until recently, information concerning this pathogen in humans has come from outbreak situations, cases studies and infections in travelers. In 1993, a study of *Cryptosporidium* infectivity and natural history of the infection in healthy adult volunteers was instituted at the University of Texas Health Science Center in Houston. These studies have generated information that has been used in risk assessment models for waterborne transmission.

Volunteers between the ages of 18 and 55 are enrolled only after they have undergone a complete history and physical examination and a battery of tests to ensure that they are in excellent health. Importantly, all volunteers are proven HIV-negative with normal T-cell subsets and no immunodeficiencies. After challenge, volunteers are monitored daily for the first 14 days and 3 times per week for an additional 4 weeks. Active surveillance of volunteers' households and/or other close contacts for diarrheal illness is maintained throughout the study period. When diarrhea occurs in the subjects or their contacts, a complete enteric workup is performed. No secondary transmissions have been documented to date.

All *C. parvum* isolates are used within six weeks of calf production and are tested for viability by excystation rate and mouse infectivity. All of the isolates used in these studies belong to the genotype 2 (animal) subgroup of *Cryptosporidium parvum* as defined by multilocus analysis. Fecal oocyst excretion is detected using the direct immunofluorescence assay (Merifluor kit, Meridian Diagnostics).

To date, three geographically-diverse, genotype 2 isolates have been studied for their infectivity in volunteers who had no evidence by ELISA of previous exposure. Challenge doses ranging from 10 to 1 million oocysts have been given, and infectivity has been documented by the presence of fecal oocysts and/or a diarrheal illness characteristic of cryptosporidiosis. The dose necessary to cause infection in 50% of volunteers ( $ID_{50}$ ) varied with isolate and ranged from approximately 10 to 1500 oocysts. Interestingly, the isolate with the lowest  $ID_{50}$  was also the most virulent when assessed for illness attack rate (86% vs 50-55%). However, the onset, duration and severity of illness did not differ among infected individuals.

The group receiving one of the isolates (Iowa isolate) was rechallenged with the same isolate one year later. The results revealed an illness attack rate that was similar to primary challenge; however, the severity of illness and the number of volunteers shedding oocysts were significantly reduced. Protective immunity was studied in 17 volunteers who had high levels of serum IgG before challenge. In this group, both infection and illness were correlated with dosage levels exceeding 5000 oocysts. Indeed, the  $ID_{50}$  was significantly increased to approximately 1800 oocysts, a 20-fold increase over the  $ID_{50}$  in serologically-negative individuals. Subjects receiving the lower dosage levels, such as those that might be associated with waterborne exposure, were protected from infection and illness. Of those that

## SEROLOGICAL EVIDENCE OF ENDEMIC WATERBORNE CRYPTOSPORIDIUM INFECTIONS

**Dr Floyd J. Frost**, Tim Muller (*The Southwest Center for Managed Care Research, Albuquerque, New Mexico*), Rebecca L. Calderon (*USEPA, N.C.*), Steve A. Hubbs (*Louisville Water Co., Kentucky*), William Lockwood (*American Red Cross, Louisville, Kentucky*) and Gunther F. Craun (*Gunther F. Craun and Associates, Staunton, Virginia*).

The health effects of waterborne *Cryptosporidium* oocysts, commonly detected in surface-derived drinking water, are uncertain. In order to estimate infection rates, baseline serological responses and seroconversions to 15/17-kDa and 27-kDa *Cryptosporidium* antigen groups were compared between filtered surface vs. ground water consumers. A Western blot assay was used to measure baseline and 9-month serological responses of blood donors consuming either surface (N=462) or ground (N=503) water.

A higher fraction of surface compared to ground water consumers had detectable baseline serological responses (46% vs 26%,  $p < 0.00001$  for IgG-15/17; 54% vs 39%,  $p < 0.00001$  for IgG-27). Surface water consumers with a detectable baseline response had a more intense 27-kDa response than ground water consumers ( $p < 0.0001$ ).

Nine months later, a higher fraction of surface water consumers converted from no baseline to a positive serological response (33% [N=132] vs. 11% [N=216],  $p < 0.00001$  for IgG 15/17; 34% [N=111] vs 25% [N=181],  $p = 0.11$  for IgG-27). A higher fraction of follow-up donors with a detectable baseline response increased their response intensity (31% [N=108] vs 5% [N=76],  $p < 0.002$  for IgG-15/17; 24% [N=109] vs 9% [N=100] for IgG-27,  $p < 0.003$ ). Multivariate adjustment for risk factors collected at baseline and follow-up did not alter these comparisons. Few participants reported cryptosporidiosis-like illness.

This study suggests that surface-derived drinking water consumers may be at higher risk of serological response to *Cryptosporidium* infection but that cryptosporidiosis-like symptoms were uncommon.

## DETERMINING OOCYST CONCENTRATION IN WATER

**Dr Daniel Deere**

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Estimation of the pathogen concentration in a water body is the first step in quantitative assessment of the risk of drinking that water. A means of achieving this is to determine:

- the numbers and species of carriers of human infectious pathogens in the catchment;
- the faecal inputs to water courses;
- the rates of die-off of these pathogens on surfaces and in surface waters.

The more commonly used approach is to measure pathogen densities in the water body. The reported monitoring results cannot be directly interpreted as actual pathogen densities in water. This is because there are a number of factors that lead to errors:

- **Overestimation:** In general, detection methods do not assay infectivity, and often do not indicate species, strain or viability. Pathogens detected have unknown infectivity to humans and may have lower infectivity than the strains used in feeding trials;
- **Underestimation:** Most detection methods are less than 100% efficient at recovering pathogens from the water, some recover no more than a few percent. In addition, pathogens detected may have higher infectivity than the strains used in feeding trials;
- **Variability:** Inherent variation in numbers of pathogens detected in different samples can be great. Even in a perfectly mixed system, pathogens will be at least Poisson distributed leading to inescapable variation between numbers of pathogens recovered in different samples of up to several fold. In practice, pathogens are probably much more heterogeneously dispersed than Poisson, leading to even more variation. Pathogen densities in water bodies will also vary widely with time and place;
- **Uncertainty:** All of the above variables (infectivity of detected pathogens compared to those used in feeding trials, recovery efficiency of detection method, sampling error, time/place variability) are subject to uncertainty in natural systems. As a result, the small number of tests that are typically used for pathogen monitoring give rise to very wide confidence intervals in the estimate of pathogen density, often exceeding an order of magnitude.

For determining pathogen densities as inputs to microbial risk assessment, frequency distributions, best guess or worst case values can be used for each of these variables and this gives a frequency distribution, best guess or worst case value, respectively, for the pathogen density. The worst case risk estimate may at first sight seem to be the most protective and is certainly the most simple to perform. However, risk overestimation is undesirable where risks are being traded off (ie disinfection:disinfection by-product) or where costs involved in risk mitigation are proportional to the degree of risk. If action is required based on a worst case estimate of the pathogen density in the water, more accurate estimation is required. The uncertainty in an estimate of pathogen densities based on monitoring results decreases with:

- increased numbers of samples to indicate time, place and sample to sample variability;
- increased quality control and quality assurance of monitoring methods to indicate recovery efficiency of the method and its variation;
- increased detail in the output of monitoring methods (ie species, strain, viability and infectivity information).

## **ASSUMPTIONS ABOUT THE ORIGIN AND INFECTIVITY OF CRYPTOSPORIDIAN OOCYSTS IN PROTECTED CATCHMENTS**

**Dr David Adams**

*Animal and Plant Health Branch, Bureau of Resource Sciences, Canberra, ACT, Australia*

Risk assessment is a powerful methodology for evaluating risk in areas as disparate as the natural environment, the built environment and finance. The use of risk assessment for managing the risk to human health of food and water-borne pathogens is a relatively recent development and difficulties with terminology and definitions have not been universally resolved. Accordingly, the current treatment uses and seeks to promote the terms and definitions proposed by the Codex Alimentarius Commission and restated by the Australia and New Zealand Food Authority.

The potency of the risk assessment process is that has a dimension that goes beyond providing a simple estimate of risk in support of decision making. This extra dimension is the key to the present task of addressing some major unknowns about the ecology of cryptosporidia. Risk assessment sets up a framework for the highly systematic organisation of information and knowledge that characterises the nature and magnitude of the risk (the first dimension) and describes the degree of uncertainty associated with that information and knowledge (the extra dimension). As a result, risk assessment can pinpoint where information and understanding is deficient and where more research is required. Risk assessment is a rolling process that makes use of evolving knowledge. It cannot be used as a surrogate for scientific research.

Uncertainty dominates answers to questions about the origin and infectivity of cryptosporidian oocysts in protected water catchments. Virtually all mammals could be incriminated as reservoirs. Which species might be important? What is the state of knowledge about the host/parasite relationship that occurs between the cryptosporidia and these mammalian hosts? What is the distribution, abundance and behaviour of possibly important mammalian species? What can be said about the persistence of cryptosporidian oocysts in the environment? A pictorial model of the natural history of the cryptosporidia is used as a prop for discussing where scientific research can clarify some important unknowns. What are the technical impediments to pursuing these unknowns and how can they be addressed?

## OOCYSTS FROM CATCHMENT TO TAP: FACTS AND ASSUMPTIONS

**Prof Nicholas J. Ashbolt**

*Centre for Water and Waste Technology & CRC for Water Quality and Treatment, University of New South Wales, Sydney, NSW, Australia.*

*Cryptosporidium parvum* oocysts are excreted in the faeces of infected animals and humans. Hence, all catchments are impacted to some degree, with greater impact arising from various events and land uses, such as heavy rainfall, animal production and human activity. The factors which therefore result in the occurrence of oocysts of public health significance in drinking water supply systems are: oocyst source (species), treatment efficacies and time to reach consumers (infectivity). The most commonly acknowledged transport mechanism for oocyst-containing faeces to reach raw waters involves heavy rainfall events in poorly protected catchments, allowing relatively fresh and infectious oocysts to reach high numbers. Failure of the treatment barrier is also associated with cryptosporidiosis.

Hence, critical factors required to estimate the health risk from *Cryptosporidium* are: (1) the magnitude and variation in oocyst numbers in raw waters, (2) their likely hydraulic retention times and movement in storage and distribution, (3) probability of treatment barrier breakthroughs, (4) their species and (5) infectivity. The first fact to realize is that we have little data on all of these parameters. Internationally we therefore assume all oocysts counted are *C. parvum* and infectious to humans. The second fact is that estimated numbers are rarely adjusted for method recoveries, but worst still, sampling frequencies generally used are inappropriate to identify the magnitude or duration of events in oocyst numbers. Lastly, only one dose-response curve is used to extrapolate from oocyst consumed to infection likelihood, taking no account of the variability in virulence of different strains.

Hence, it is recommended that risks should be estimated with two conditions in mind, the "normal" situation, possibly leading to endemic disease; with the second accounting for events, which may lead onto epidemic disease. It is therefore inappropriate to average whole data sets which include event data. Surrogates for oocysts in individual catchments may be possible, but in general there has been no suitable surrogate identified to date. In contrast, surrogates for treatment performance are available (particle counts, aerobic spores). Thus, with good hydrologic models, the possible age (and viability) of oocysts could be estimated as they leave the treatment plant. The remaining unknown is the level of "treatment" in distribution systems which results in apparent losses or clumpiness of occurrence (via sediments and biofilms).

**REMOVAL BY TREATMENT**

**Dr Jerry Ongerth**

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Abstract not available at time of printing

## DRINKING WATER DISINFECTION: THE *CRYPTOSPORIDIUM* PROBLEM

**Professor Gordon R. Finch**

*Department of Civil & Environmental Engineering, University of Alberta,  
Edmonton, Canada.*

Waterborne disease has plagued humankind since before recorded history. However, the introduction of simple water supply technologies such as wells, filters, and chlorine disinfection have made classic waterborne diseases such as typhoid fever, cholera, and dysentery unusual in the industrialized world. However, with increased standards of living and emergence of diseases such as AIDS, the desire to improve the level of public health is great. Furthermore, the purveyors of water to homes must be aware that there is an expectation of a quality product delivered to the point of use. The organoleptic properties should be neutral and the water should be free of microbial pathogens. Also, long-term consumption of such water should not increase the lifetime risk of cancer or other illnesses.

Unfortunately, encysted waterborne parasites such as *Giardia lamblia* and *Cryptosporidium parvum* have presented a challenge to water suppliers. The United States Environmental Protection Agency has adopted an approach to defining the engineering design criteria whereby the microbial risk reduction needs are matched to the engineering design requirements. Therefore, if the dose-response of the microbe is understood in the host, and if the number of microbes that is challenging the treatment scheme is known, then the engineered barriers can be designed to reduce the risk of extra cases of waterborne disease to a defined level. Engineers depend upon the public health sector to define the acceptable level of risk. Given the allowable risk, an appropriate water treatment scheme can be defined.

Processes for controlling *Cryptosporidium* include physical and chemical removal processes such as coagulation, dissolved air flotation, sand filtration, and membranes. Chemical disinfection is an important barrier that does not remove the parasites, but rather alters them chemically so that they cannot reproduce and cause disease.

The use of chemical disinfectants such as chlorine in drinking water has been a great public health step dating back to the latter half of the 19<sup>th</sup> century. During the 20<sup>th</sup> century drinking water disinfection has been dependent upon chlorine somewhere in the treatment scheme. Some places have used alternatives to chlorine such as ozone as the primary disinfectant. *Cryptosporidium* has a great deal of resistance to conventional chlorine disinfection practice leading to a search for more effective ways to control this parasite. Currently, the use of ozone and chlorine dioxide as the primary chemical disinfectants provides the most promise for use in conventional water treatment. The use of these processes as the primary disinfectant may enhance the action of free chlorine or monochloramine when applied sequentially, such as occurs in treatment facilities.

Choosing the correct chemical disinfectant combination may enable the designer to control 0.5 to 1 log-units with relative ease and may achieve up to 3 or 4 log-units of control of *Cryptosporidium* under some conditions. Factors that will influence the degree of control will include pH, temperature, organic content of the water, and other site specific features such as the reactor vessel type, mixing and so on.

The multiple barrier approach to control *Cryptosporidium* involves processes for physical and chemical removal as well as chemical inactivation. The use of multiple barriers including

## **DOSE RESPONSE MODELLING**

**Dr Peter Teunis**

*RIVM-IMA, Bilthoven, Netherlands.*

Quantitative risk assessment for pathogenic micro-organisms depends on exposure analysis (the probability of ingesting a certain number of organisms) and dose-response analysis: the probability that certain health effects occur after exposure.

After ingestion of contaminated water, one or more of the swallowed pathogens may succeed in reaching a site within the intestinal tract that is suited for growth and colonisation. When this results in a high number of pathogens living in the gut, we speak of infection.

For microbial risk assessment, a hit-theory model is employed. Basic assumption is that, before reaching a favourable site for colonisation, each organism has to pass a number of barriers of the host defense system. With a few reasonable assumptions, a mathematical relation can be developed: the Beta Poisson model. This model has been used for statistical analysis of experimental data, and the results have been successfully applied for quantitative risk assessment. Experimental data on infection of human volunteers by *Cryptosporidium* are well suited for demonstrating the use of this model.

Estimation of the infection risk is important for public health purposes, not in the least because infection is considered a necessary condition for illness, and because excretion of pathogens includes potential transmission, but the final interest often lies in the probability of becoming ill. The opportunities for dose-response analysis for illness (gastro-enteritis) are discussed, and a tentative model is presented. Analysis of experimental data with this model will be discussed. Finally, directions for future research will be addressed, with their opportunities and difficulties.

## OPENING SESSION

*Summary by Professor Robert Douglas, NCEPH, Australian National University.*

After the official opening of the Conference by Professor Richard Larkins, Chair of the National Health and Medical Research Council, the Executive Director of Water Services Association of Australia, Dr John Langford, chaired the opening Plenary session. In their opening remarks, both Professor Larkins and Dr Langford drew attention to the need for consensus on public health questions posed by *Cryptosporidium* and its capacity to cause major disruption to public water systems. The recent Sydney episode is forcing a reappraisal of strategies. Do we seek a zero tolerance of this organism in water supplies and what are the costs and benefits of differing approaches? Now that technology is permitting a more accurate tracking of the epidemiology of this organism, there is a need for both regulatory and service agencies to review procedures. Professor Larkins indicated that the NHMRC is involved in a review of the Australian Drinking Water Guidelines and that the considerations of this consensus conference would be a very important input to that review.

**Peter O'Donoghue** from the University of Queensland set the scene for the scientific discussion by pointing out that knowledge on *Cryptosporidium* is still emerging and the community are receiving conflicting opinions on the nature and seriousness of the hazards. He suggested it was time for a "reality check" on what we now know of the medical, veterinary and environmental problems caused by this organism. Whereas the human disease had only been detected in the late 1970s and oocysts were now being discovered in swimming pools and water supplies, one of the remarkable features of the recent Sydney episode was that it was still not clear that there had been any excess in human infections. The problem had become more complex because of the threat of litigation at a time when scientific understanding of the behaviour of the organism is still far from complete. Dr O'Donoghue described the growing recognition of the pathogenicity of this organism in man, and the fact that it is now being identified with increasing frequency in the faeces of humans.

The life cycle of coccidial organisms such as *Cryptosporidium* means that there can be massive contamination of the environment by relatively stable oocysts. It is now clear that infection in humans is often asymptomatic; that there is no specific therapy for the condition that is of proven efficacy; and that transmission can occur both from human to human and from other mammals to humans. It is not clear whether infectious doses may be lower in immunocompromised people, and it is possible that even one oocyst may sometimes be sufficient to establish infection. There are multiple species besides *Cryptosporidium parvum*; oocysts from multiple species are being transmitted into the environment all the time; recognition of different species of oocysts is vital, although the diagnostic precision of laboratory methods is still questionable and the molecular 'magic bullet' for precise detection of, and discrimination between, species is not yet available.

Many human outbreaks have occurred as a result of water contamination and a number of outbreaks have been associated with contamination of foodstuffs and beverages. For water supplies, contamination can occur either from human or agricultural waste, and the dairy industry and contact with calves in particular are often implicated. Passive carriage by water birds may occur, although the significance of this route is not clear. The focus is on watershed management, control of contamination of surface waters, the need to better understand decontamination procedures, and issues surrounding disinfection. A substantial industry is developing around the issues raised by environmental management of *Cryptosporidium*.

Turning to the issue of water treatment, Dr O'Donoghue pointed out that while coagulation and sand filtration are effective in greatly diminishing the concentration of oocysts in water, some get through. Further, conventional methods of disinfection using chlorine require long contact times, and although ozone shows considerable promise as a disinfectant, it is currently too expensive for routine use. What is not clear is whether following chlorination, the oocysts are inactivated or made less virulent. There is a need for improved methods to estimate the efficacy of treatment processes.

If it is true that current technologies are inadequate, the question needs to be asked, 'Why are we not getting huge outbreaks of infection?' Australia is not alone in its uncertainties in this field. It is not yet possible to come up with health-based guidelines of tolerable contamination levels and much of the current international activity is based on the need to improve data collection rather than to set standards. In its 1996 Australian Drinking Water Guidelines, the NHMRC indicated that regular testing was not appropriate, but responsible agencies who attempted to monitor activity in their treatment systems were faced with the uncertainties of the science.

What constitutes a background level and at what point does infection of the community become possible? Is there evidence of correlation between contamination and disease? Now that disease notification is occurring in most Australian states, the possibilities of improved epidemiologic surveillance are in sight, but we need considerably more information on morphology, immunochemistry, genetics, viability and pathogenicity of these organisms if the water treatment industry is to make rational decisions. There is fault with all of the available methods and although there has been rapid development in recent years, it is clear that occurrence does not equate to pathogenicity; that different vertebrate classes are susceptible to differing *Cryptosporidium* species, and that despite its rapid evolution, the genetic and molecular technology needed to understand the risk associated with specific isolates of oocysts is not yet available to the water industry.

Dr O'Donoghue emphasised that there are still numerous practical problems in the study of this organism, particularly the lack of an effective *in vitro* culture system, and the inability to isolate genetically homogenous strains. Added to these problems there are many uncertainties about its epidemiology, and therefore risk assessment is still very imprecise in nature for this organism. In his view, we do not yet have the basic biological understanding needed to effectively deal with these organisms.

**William MacKenzie** from the Centers for Disease Control and Prevention in the USA, who played a key role as epidemiologist investigating the Milwaukee epidemic, reviewed a number of epidemiologic issues. Since 1984, there have been nine documented drinking water outbreaks in the United States. Most of these were chlorinated supplies and mostly these have occurred in association with treatment plants which were meeting normal operating standards of the time. The epidemics include water derived from ground and surface sources. In only one instance has the contamination been also associated with coliform contamination. The Milwaukee epidemic, in which 403,000 people were estimated to have become ill, was associated with contamination in one of two water treatment plants; oocysts were found in ice samples collected several days after the contamination had occurred; and studies of victims' stools identified *Cryptosporidium parvum* as the only pathogen present. The epidemiology was tracked by both laboratory confirmed cases (oocysts detected in stools) and clinically diagnosed cases (people with watery diarrhoea, identified by telephone survey). The intensity of symptoms was variable and often included abdominal cramping but vomiting was less frequent. For laboratory diagnosed cases the duration of

days). Most clinically diagnosed cases experienced relatively minor symptoms, and the most severe cases occurred in those who were immunocompromised.

The frequency of symptoms may vary with age; - fever and vomiting appear to occur more commonly in children than in adults. Dr MacKenzie suggested that the feeding studies are revealing a very complex picture and that immunity likely alters susceptibility and severity of infection. Secondary transmission rates in outbreaks have varied from 5% to 40%, and are probably related to personal hygiene (secondary transmission being higher among children than adults). To date over 100 therapeutic agents have been tried for the treatment of *Cryptosporidium* infection, but none are consistently efficacious. Two agents, paramomycin and clarithromycin show some promise on preliminary evaluation, the latter as a preventive agent. Supportive therapy with fluid replacement is needed in severe cases. The advent of improved antiviral drugs for HIV treatment has been associated with a decline in the rates of chronic *Cryptosporidium* infection in the HIV+ population.

Dr MacKenzie pointed out that in systematic studies in the US, 96% of 63 source waters contained evidence of *Cryptosporidium* and that 54% had at least one finished water sample testing positive. The amounts of contamination are usually small, but we do not know whether organisms which pass the treatment plant are pathogenic and/or viable. Laboratory studies of oocyst excretion indicate that 2% of all stool specimens reaching pathology laboratories in the US contain oocysts; seroprevalence surveys suggest that antibody persists for a number of years, and that 70-90% of adults have antibody to *Cryptosporidium*, strongly suggesting prior exposure.

Turning to a review of surveillance, Dr MacKenzie pointed out that during the Milwaukee outbreak, prior to recognition that *Cryptosporidium* was the cause, only 6% of people with diarrhoea saw a doctor, only 6% of those who saw a doctor had a stool specimen collected, and most specimens were not tested for *Cryptosporidium*. Therefore the existing disease surveillance system was therefore highly insensitive. It generally takes up to 14 days from the time individuals become ill to the time that the epidemic is noted by the health department and in some epidemics it is only possible to relate drinking water as the etiological agent on retrospective case control analysis. There is also the problem of doctors in the community having low index of suspicion for this condition and being unaware that a specific request for *Cryptosporidium* testing is required by most laboratories.

Dr Mackenzie reviewed CDC experience of this condition and indicated that CDC is notified of about 2,000 cases annually, which is about eight cases per 100,000 of population, that there are two age peaks, and that peak exposure times are mid- to late-summer and early autumn. It appears that children are more heavily represented in the summer peaks, but that adults have endemic disease across the seasons. He pointed out that there have been 12 outbreaks related to recreational water in the United States, and that recreational water in the hot season provides a highly efficient transmission mechanism as children in nappies can excrete huge loads of oocysts into swimming pools. He concluded by saying that this is an emerging pathogen on which we still have sparse data and that the sort of discussions which this conference planned could move the science forward.

In the discussion that followed these two presentations, the speakers emphasised that it is not yet clear how best to conduct surveillance for this organism; that swimming pool dissemination probably accounts for the biggest proportion of the problem; and that engineering out all risk to the community from this source is particularly difficult.

pathogen in animals, it had become increasingly clear that the problem not uncommonly affected humans. A study of 60,000 stool specimens across the United Kingdom had revealed that the organism was present in 2%. It was more common in children and the peaks of disease often lagged slightly behind peaks of lambing and calving. He pointed out that many catchment areas in Britain are now inhabited and farms are increasingly opened to recreational activity. A growing number of cases have been shown to be associated with contact with livestock. He also pointed to the British habit of eating uncooked sausages while cooking, the fact that rodents and mice excrete large numbers of *Cryptosporidial* oocysts, and that swimming pools are increasingly associated with infection. He also suggested that infected children who vomit may transmit the infection to carers and that on the basis of British national surveillance data, there are well documented examples of outbreaks due to person-to-person spread, animal contact, swimming pool outbreaks and drinking water outbreaks. He indicated that the epidemiology suggests there is a high seroprevalence of positivity amongst people in the United Kingdom, that 93% of the organisms isolated from humans are "human" genotypes, suggesting that the significant contribution may be from human sewage or faeces; that many of the human strains do not infect animals, and that he now has some uncertainty whether this is or is not a true zoonosis.

In the questions that followed this paper, the issue of ozonization was again discussed and it was agreed that it was relatively effective for treating backwash waters.

**Jeremy McAnulty** of NSW Health, described the experience in Australia and particularly the recent Sydney episode. He summarised the findings of the 1990 case control study in Adelaide of 51 matched cases, which failed to clearly implicate mains water. There have been several outbreaks in childcare centres, and in 1995 and 1998 substantial numbers of swimming pool outbreaks. There has been little evidence that municipal drinking water was a cause of any outbreaks, but a great deal of pressure has come on water operators to ensure that drinking and recreational water are clear of these organisms.

Dr McAnulty emphasised that we do not really understand what the presence of *Cryptosporidium* in a catchment means and then summarised events in Sydney. The city draws most of its water from the Warragamba Dam via three filtration plants. While there are no current recommendations on water testing for *Cryptosporidium* from the NHMRC, the Sydney water authority had commenced routinely testing water for *Cryptosporidium* during 1998. The tests have usually proved negative, but on 22 July, a routine test in metropolitan Sydney revealed levels of *Cryptosporidium* and *Giardia* in the mains water and high counts on repeat testing. No reason for this contamination was found, though there was suspicion of some localised negative pressure events sucking in contaminated material and a 'boil water' alert was issued for a small area of the inner city.

Levels dropped rapidly but then, on 29 July, reasonably high levels in water were discovered coming from the Prospect plant. A 'boil water' alert was issued to the three million people who were served by the Prospect plant. Further monitoring showed clearing of the contamination from the distribution system and by 4 August the alert was lifted for the entire city. Throughout August there were record heavy rains in the catchment area and the Warragamba Dam filled from 58% to 100% capacity. Raw water reaching all three water treatment plants had high counts of both *Giardia* and *Cryptosporidium*, the protozoa were also detected in treated water and a new 'boil water' alert was issued on Aug 25. Progressive lifting of the 'boil water' notice began on 1 September as testing showed clearing of the contamination from the distribution system. However by 5 September, there were further high counts at all three plants and a two-week 'boil water' alert was issued. Following a week

The question clearly being asked was, 'Did anyone get sick?' Active surveillance was instituted across Sydney, making use of GP surveillance, nursing homes and pharmacy sales. Laboratory surveillance was upgraded, and although there was a marked increase in the number of stool specimens examined and some increase in *Giardia* reports, notifications of *Cryptosporidial* infection experienced one of the quietest months on record. Nor was there any convincing change in the frequency of consultations for diarrhoea recorded by the GP surveillance system. Two community surveys were conducted by telephone. On both surveys there was no difference between the affected water supply area and a control area with respect to diarrhoea attributable to *Cryptosporidium*. There was a decline in compliance with the 'boil water' alert on the second occasion. Dr McAnulty concluded that we are still trying to understand what the risks of *Cryptosporidium* are, that the evidence of risks in Sydney at this stage are not very convincing, but that our environmental testing is not a good feature of our current surveillance, and that it is important to develop guidelines for the management of such incidents in the future.

**Peter Nadebaum** of CMPS&F spoke on the issues of risk assessment and risk management. He defined this task as the structured process of assessing where the risk occurs, what is its magnitude and how it can best be managed. He highlighted the fact that few Australian water agencies have yet fully embraced quality management systems, though risk assessment of chemicals is widely used and engineers have been trained in risk assessment for many years. In the water industry, it has been more common to utilise performance targets such as physical and microbiological parameters, rather than risk assessment to control water quality.

However, it has become increasingly possible to identify micro-organisms and to link them to disease, and we are becoming a more litigious society with trade practice acts requiring due diligence of water utilities, in addition to licensing requirements relating to specific water quality parameters. Dr Nadebaum described research being conducted in the CRC for Water Quality and Treatment, extending methodology from the field of environmental risk assessment through to the water supply area.

Using real data obtained from local water authorities, his work has endeavoured to identify the absolute risks of contamination. In this theoretical model, *Cryptosporidium* has emerged as the greatest risk determinant of gastrointestinal illness, but he emphasised that predicting the risk precisely using current knowledge was not possible. He felt that it was unclear what an appropriate target would be in terms of *Cryptosporidium* in the source water; that levels suggested in other countries may be too low for Australian purposes, and that we have a very inadequate knowledge base on which to make risk predictions. He underlined the fact that the water industry has huge capital decisions to make around the issue of water treatment, and that there is a need for it to be able to pass the due diligence test. He saw the way forward as a combination of a precautionary approach, quality management, and the development in each agency of a systematic risk review beginning from catchment and going through to the tap.

**John Langford**, in summing up the discussion in the symposium, emphasised the importance of the epidemiological work already being supported by the Australian water industry through the CRC for Water Quality and Treatment. The Water Quality Study ongoing in Melbourne, using a randomised controlled trial of filters, will determine whether microorganisms in drinking water are making a significant contribution to gastroenteritis in the community. Case control studies on *Cryptosporidial* infection in Melbourne and Adelaide will identify the major risk factors for infection. He noted the uncertainties around whether *Cryptosporidial* oocyst counts were representative of dead or live parasites and the view that, for now, risk assessment involved a general quality management approach that did not necessarily require

## PARASITOLOGY AND GENETIC TYPING

Summary by Professor Alan M Johnson, School of Microbiology, University of Technology, Sydney.

### Day 1 - Afternoon Workshop Session

The afternoon session on Parasitology and Genetic Typing comprised three presentations on the detection and production of parasites, and seven presentations on a variety of techniques for genetic typing.

**Colin Fricker;** described four parasite concentration methods (cartridge filtration, flocculation, membrane filtration, vortex flow filtration). These were of low recovery (18%-33%) and even Envirocheck capsules only gave recoveries of 25%-40%. The interpretation of recovery data was considered "poor", and the "controversy" over "viability" was raised.

**John Watkins;** discussed four cleaning methods and dichromate storage experiments on four commercial *Cryptosporidium* strains (Moredun Institute, PHLS New Castle, Arizona Harley Moon, Iowa Harley Moon) - they were extremely inefficient at recovering oocysts. Western blotting experiments were carried out using the Cellabs monoclonal antibody to cyst wall protein, and three common bands at 230 kDa, 90 kDa and 80 kDa, were found to be common among all strains. However, after preservation in dichromate, none of these bands could be found. Problems with DAPI staining were raised (DAPI negative oocysts can occur during cleaning, DAPI negative oocysts are a natural phenomenon in faeces), the different commercial strains show 1D SDS PAGE antigenic differences, but disinfection studies do not always take these factors into account when interpretations are made.

**Saul Tzipori;** believed that neonatal calves were the best source of parasites, although human type I isolates could be grown in piglets. *In vitro* cultivation is limited to asexual stages of the parasite, but it can be used for oocyst viability/infectivity. Had little confidence in dye staining systems, but also accepted that *in vitro* cultivation was not perfect. Raised the inability to correlate antigenic attributes and genetic attributes with virulence and host specificity and stated in his abstract "The lack of recognized and characterized molecular, antigenic, or virulence markers makes it impossible to conduct meaningful comparative studies among isolates obtained from different hosts, geographic locations, or propagated in different host species." He confirmed that some isolates do not grow in some hosts, and said that there was a lack of clearly defined methods of speciation.

**Neil Chilton;** described his experience with isoenzyme electrophoresis and how the technique had aided the identification of lineages in *Giardia intestinalis*. Reviewed two papers which used isoenzyme electrophoresis to analyse *Cryptosporidium*, but pointed out that where usually 20 or more loci should ideally be used to compare taxa, in the only two studies reported, Ogunkolade *et al.* (1993) had used only 2 loci and in the other study by Awad el Karem (1995), only 4 loci were compared, but even then 2 of these were found to be the same. Recommended using a combination of techniques for genetic analyses.

**Jacqui and Peter Upcroft;** described their experience with ribosomal DNA genomic rearrangements in *Giardia*. Chromosomal analysis by Pulse Field Gel Electrophoresis found that each *Giardia* isolate has a unique karyotype. Potential problems with some gene probes were highlighted.

**Una Morgan;** suggested that good *in vitro* or *in vivo* culture models were needed. Has

livestock. Isoenzyme electrophoresis, Random Amplified Polymorphic DNA, PCR Restriction Fragment Length Polymorphism, ribosomal DNA sequence analysis and protein coding genes can be used for genetic typing. What has been reported is what has been found with the tools in her laboratory, what other tools will find in other laboratories was not known. Raised the question of whether viability testing was necessary, and suggested that Ficoll gradient purification was an acceptable method of purification.

**Timothy Stinear;** compared antibody capture with direct polymerase chain reaction identification. Physical capture of oocysts by various antibody-dependent methods such as flow cytometry, immunofluorescence and immunomagnetic separation have severe limitations because monoclonal antibodies are not pathogen specific. They give a quantitative result, but how reliable are the data? He discussed the advantages and disadvantages of polymerase chain reaction and compared the results of immunofluorescence with reverse transcriptase-polymerase chain reaction. Used a 590 base pair *Cryptosporidium parvum* heat shock protein 70 to analyse polymorphisms among three isolates. Summarised that more work is needed to identify suitable target sequences and to improve nucleic acid purification methods. We need to achieve consensus among laboratories and conduct interlaboratory comparison of proposed methods to generate reliable protocols.

**Giovanni Widmer;** suggested that polymerase chain reaction-restriction fragment length polymorphism analysis has limitations when compared with complete nucleotide sequencing. Genotyping using multiple polymerase chain reaction-restriction fragment length polymorphism markers showed two lineages, a human and a calf. Sequence analysis of the beta tubulin intron showed two major lineages based around the human and calf classifications, with another isolate belonging to a third lineage, but the genotype based on the beta tubulin intron "changed" after passage through a calf. Also compared the RNA signal of beta tubulin messenger RNA with mouse infectivity and found good correlation.

**Robin Gasser;** compared random amplified polymorphic DNA, restriction fragment length polymorphism and sequencing with mutation detection methods in worms. Suggested that single strand conformation polymorphism and denaturing gradient gel electrophoresis may offer advantages for determining genetic diversity in *Cryptosporidium*.

**Dee Carter;** described microsatellites that are one to six DNA bases in a sequence repeated at least five times. They are present in all eukaryotic genomes and can be used to differentiate individuals and clones, analyse population structure and recombination, map a genome and identify virulence markers.

**Paul Rochelle;** said that random amplified polymorphic DNA profiles had been used to differentiate among strains but it has its disadvantages. It depends on the extraction procedure of the DNA and is very difficult to reproduce. Two genotypes have been found using a range of polymorphic markers. Based on beta tubulin, TRAP and COWP markers, there is no geographic variation around the world. DNA sequences deposited in databases are not reviewed and can be wrong - eg 18S ribosomal RNA and COWP, so close scrutiny of sequences is necessary.

From these presentations, Alan Johnson gave a summary overview on Monday evening, and suggested that the first aim of the research and development on *Cryptosporidium* should be the production of:-

*"Stable markers that can be used in a test to accurately, quickly, cheaply and reproducibly identify and quantify Cryptosporidia that are viable and therefore potentially infectious to*

This aim was more simplistic (but therefore much more achievable) than many of the ideas and specific scientific "needs" that general conference participants and some participants in the Epidemiology and Risk Management Workshops suggested were definitely required to prevent another Sydney water "crisis" during Monday's discussions. However, the achievement of this aim would then allow further studies to develop rigorous genetic typing, and generate knowledge on the viability of *Cryptosporidium* and how it could be accurately determined. In addition, trying to provide the numerous specific scientific "needs" raised during Monday's discussions would be an enormous task which would be very expensive, extremely difficult to coordinate and plan, and therefore very likely to be not achievable.

## Day 2 - Closed Workshop Session

On Tuesday 6<sup>th</sup> October, a group of scientists with expertise in the biology of *Cryptosporidium* and/or expertise in the molecular analysis of related parasites met to discuss the issues raised from the previous day in the context of six specific objectives.

### Objective 1    *Leader* Prof Saul Tzipori

To review techniques for isolation, propagation and preservation of *Cryptosporidium* for molecular biological research, including approaches to the *in vivo* and *in vitro* propagation of the parasite(s).

### Objective 2    *Leader* Dr Peter O'Donoghue

To reach a conclusion on the feasibility of establishing a reference collection of *Cryptosporidium*

### Objective 3    *Leader* Dr David Casemore

To identify the key methodologies for genetic characterisation of *Cryptosporidium* species and strains including biochemical and DNA methods

### Objective 4    *Leader* Dr Giovanni Widmer

To review the methodologies and DNA target regions that have been used thus far for *Cryptosporidium*

### Objective 5    *Leader* Dr Una Morgan

To reach a conclusion on the feasibility, and likely timetable for genetic typing of *Cryptosporidium* to improve the strength of public health surveillance and incident management systems

### Objective 6    *Leader* Prof Alan Johnson

To develop the outline of a research strategy for genetic typing providing the conclusion in point 5 above is positive

The Leaders of the discussions on these objectives used the above aim to focus their discussions. With this guidance, it was felt that Objective 5, "To reach a conclusion on the feasibility, and likely timetable for genetic typing of *Cryptosporidium* to improve the strength of public health surveillance and incident management systems", was possible. The achievement of the above aim would allow relatively easy progression to definitive genetic typing for public health surveillance and incident management systems.

## Outline of a Research Strategy

All previous studies have been hampered by the lack of sufficient numbers of oocysts from which to prepare material for exchange among different laboratories that would allow parallel comparisons with different tests.

1. Therefore, the most important and urgent need is to establish an oocyst bank(s) of 100 to 200 different isolates from as large a variety of hosts as possible (previous studies have used isolates of humans and calves to represent mammals, and these taxa are clearly too few on which to base broad generalisations with respect to genotypes infecting humans). Given the non-labile nature of some isolates it appeared reasonable to establish an oocyst bank in North America, one in Australia and one in the United Kingdom so that isolates would not have to be shipped between continents for storage. The three oocyst banks would follow similar purification, culture and parasite preparation methods.

2. In order to make optimum use of the parasites in the oocyst bank(s) it is essential that a detailed biological history is made of all isolates. This should include but not be restricted to:-

- i) host identity
  - species,
  - age,
  - sex
- ii) geographical location
- iii) clinical patient/animal summary
- iv) biological characteristics
  - morphometrics
  - antigenicity
  - excystation characteristics
- v) handling since isolation
  - number of animal (which?) passages
  - number of in vitro manipulations

3. Because there are often few parasites in some animals hosts, it will be necessary to develop culture techniques to multiply these for establishment in the oocyst bank. Therefore, in parallel with the establishment of the oocyst bank, culture methods will be developed. *In vitro* culture has not been successful, so efforts should be directed at establishing *in vivo* techniques such as growth in gamma interferon knockout mice. These mice are now well established in many major research institutes and have been used recently to culture *Cryptosporidium parvum*.

4. With a large supply of well documented and characterised oocysts grown *in vivo*, it will be necessary to optimise standard techniques for purification of the oocysts and for extraction of nucleic acids and proteins from them. This will ensure that specimens obtained from all of the three oocyst banks have been treated identically and the results obtained on them are therefore scientifically comparable.

*It was anticipated that to reach this stage of the research plan might take 2 to 3 years, depending on the human and financial resources available.*

5. Having established the biological material that will allow in depth genetic typing to improve the strength of public health surveillance and incident management systems, it will be a relatively straightforward program to apply these techniques to the cryptosporidia

enzyme electrophoresis, mutation scanning methods, DNA and RNA gene sequencing, reverse transcriptase polymerase chain reaction, and random amplified polymorphic DNA polymerase chain reaction are very straightforward, common, and have been used to great affect in establishing the genetic typing of a number of closely related coccidian parasites such as *Toxoplasma*, *Neospora*, and *Eimeria*. It is therefore anticipated that within a year of reaching section 4. above, an enormous amount of accurate, definitive knowledge on the differentiation and diagnosis of *Cryptosporidium* species from a range of hosts and environments, will be obtained.

The knowledge gained from the successful completion of this research plan will aid public health measures in public swimming pool contaminations and make the "crisis" in the Sydney water supply, that now, according to the *Sydney Morning Herald* of 31 October 1998, appears to have been largely the result of faulty and/or poor scientific testing for the parasite, a "crisis" never to be repeated in Australia or elsewhere.

## EPIDEMIOLOGY

*Summary by Professor Robert Douglas, NCEPH, Australian National University.*

### **Day 1 - Afternoon Workshop Session**

The afternoon session on epidemiology included four papers on surveillance, four on analytic studies and two on what we understand of human infection.

**Bill MacKenzie** pointed out in his discussion of surveillance that in Milwaukee, increased turbidity was the signal that something was going wrong in the system. Several days elapsed before cases occurred. Efforts to monitor the rates of the disease through the sale of diarrhoeal agents is complex and involves many artifacts. He suggested that we need to evaluate a range of surveillance procedures, both for their sensitivity and specificity and also for their practicality. Water quality parameters are not sensitive or specific, but provide a practical approach, especially if turbidity is used. Absenteeism has the same problem of lack of sensitivity and specificity. Nursing home surveillance offers a quite good approach to expanded surveillance when there is suspicion that something is going on - it is not very timely, but is as good a model as he knows. He was impressed with the potential of the Australian ASPREN GP surveillance system, and emphasised that stool specimens are specific but not very sensitive and not very timely.

**Mark Veitch** from The University of Melbourne presented an analysis of reported cases of *Cryptosporidium* in recent years. Of the 744 cases in Victoria reported to a surveillance scheme in 1995 (rate 16.5/100000 pa), the median age was 5 years. In children aged less than 5 years, males predominated 1.5 to 1, whereas among cases aged 20 to 39 years, there was an excess of women. Seventy percent of the cases were notified between January and April; during Autumn *Cryptosporidium* exceeded *Salmonella* and *Giardia* notifications. The estimated minimum annual incidence for people with AIDS was 6%. In March 1998 the Victorian Department of Human Services instituted a voluntary notification system for pathology laboratories. The median delay between reported onset and receipt of notification was 13 days. 200 cases were notified from February to September. As yet there is no national register of *Cryptosporidium* infections, but the National Communicable Disease Network is working to improve consistency of reporting practices between states. There is good evidence that this infection is widespread in the community and predominantly affects children, particularly through swimming pools. It is recognised that most cases do not come to the attention of medical practitioners (this is true for gastroenteritis in general), and there is a need for a national system of validated multiplier values that will permit estimates of likely community incidence, based on the notification system.

**Michael Baker**, from ESR NZ, spoke of the New Zealand experience and indicated that the rate of disease was rather similar to that seen in Australia. Recreational water was the only identified source of outbreaks, and in April 1998 there had been a major swimming pool outbreak where the subsequent investigation identified deficiencies in design and operation of the pool that had contributed to the outbreak. In 1997, there were 357 laboratory diagnosed cases notified, giving a rate of 9.9 per 100,000; 4.9% of these were hospitalised; incidence was seasonal, with the highest rates in Spring.

**David Casemore**, from the UK, described the role of 50 laboratories in surveillance in that country. He said that although *Cryptosporidium* is not notifiable, it is possible, when laboratories recognise an increased number of isolates, to undertake a trawl for various kinds of information. There has been some standardisation of reports around the country. Most of

**Bill McKenzie** spoke about outbreak investigations. He said that it was becoming increasingly clear that there are no formulae for undertaking these investigations and that the investigative team needs to determine what questions need to be answered and what resources are available, and that lines of communication and responsibility need to be established early. He said that genotyping of strains could be very valuable and that it was always important to estimate the 'at risk' population and the level of infection in that population. He illustrated the way CDC has worked in the investigation of some epidemics coming in to add value to local investigations already underway, assisting to improve the sensitivity of surveillance.

**David Casemore**, speaking of outbreak investigations in the UK, indicated that case control studies are often a waste of time once the issue has become public, as there is potential ascertainment bias in determining exposure. There are increased rates of case ascertainment as people will often send specimens in the hope of suing the water company. Specimen increases of this kind may give a spurious impression of the outbreak. There is value in determining the dose responsiveness of exposure to volumes of water drunk. He indicated that making it a criminal offence for water companies to allow contamination of their water with *Cryptosporidium* seriously interferes with the efficacy of the investigation, and with the effective introduction of preventive actions.

**Brent Robertson** of Monash University, Melbourne, described a case control study of cryptosporidiosis which is going on in Melbourne and Adelaide. The aim is to better understand the risk factors for infection in sporadic cases. Cases are defined by detection of oocysts in a stool specimen, and are age and gender matched with asymptomatic controls. A range of risk factors are under consideration and responses are sought from both cases and controls by a computer-assisted telephone interview. Serology is being considered on a proportion of cases and controls to ensure that there is no serious misclassification of controls.

**Nina Russell** presented preliminary data from a nation-wide examination of endemic cases of *Cryptosporidium* in New Zealand. As in the Melbourne study, cases are laboratory diagnosed and interviewed by computer assisted telephone interviews, with two controls per case, matched on age and sex. They have excluded swimming pool cases *post hoc*. Most of the cases have been children under 4-years-of-age. Risk factors have included rural bathing in run-off water, and contact with farm animals - quite commonly contact with calves. Raw milk has been implicated and there are differences in rural and urban cases. Dr Russell emphasised the need to examine environmental sources as well as explore the quality of water itself.

In the discussion that followed these papers, there was agreement that although work is now proving to be productive in the use of serology, the immune response is still incompletely understood.

There is a move in the United Kingdom towards a water treatment standard for *Cryptosporidium*, but there is not a health basis for such a standard at this point. The view was expressed that greater value would come from tightening up of filtration processes in treatment plants, than from large expenditures on uninterpretable oocyst counts.

**Cynthia Chappell**, from the University of Texas in Houston, presented the results of careful feeding studies in human volunteers which her research group has been conducting since 1993. The purpose is to describe the natural history of *Cryptosporidium* infections in healthy subjects, to identify infectious dose, and to describe the immune response. Volunteers come from the campus of the University, must be HIV negative and have normal immunoglobulins

collection of stool specimens, recording of symptoms in a health diary and physical examinations.

The investigators have used three different strains of *Cryptosporidium parvum* of Genotype 2 (the type transmitted between other mammals and humans), and evaluated the impact of dose, both in those with and those without pre-existing antibody. To date, 107 human subjects have participated in these studies. It has been found that illness in infected volunteers ranges from 1 to 10 days, with a mean of 6 days; most have cleared oocysts in 14 days; and the longest carriage period was 38 days. Of the three geographically diverse isolates which have been studied in this way, using challenge doses ranging from 10 to 1 million oocysts, the dose necessary to cause infection in 50% of volunteers (ID50) varied with isolate and ranged from approximately 10 to 1,500 oocysts. Interestingly, the isolate with the lowest ID50 was also the most virulent when assessed for illness attack rate (86% of infected people became ill versus 50-55% for other isolates). However, the onset, duration and severity of illness did not differ among individuals infected with the different strains. The severity of illness also did not vary with the number of oocysts used to establish the infection.

In a small group of individuals who had been infected with the "Iowa isolate", and rechallenged with 500 oocysts of the same isolate one year later, the results revealed an illness attack rate that was similar to that of the primary challenge event. However, the severity of illness and the number of volunteers shedding oocysts was significantly reduced. Protective immunity was also studied in 17 volunteers who had high levels of serum IgG before challenge. In this group, both infection and illness were correlated with dosage levels exceeding 5,000 cysts and the ID50 was increased by 20-fold over the ID50 in serologically negative individuals.

Future work will include similar studies with Genotype 1 isolates (transmitted from human to human), however in order to avoid the risk of transmitting unrecognised human viruses it will first be necessary to passage the isolates through an animal host. While genotype I isolates do not readily infect most animals, a suitable animal model has been identified for this purpose.

**Floyd Frost** of New Mexico presented serological evidence of endemic water-borne *Cryptosporidium* infections using baseline serological responses and seroconversions to two *Cryptosporidial* antigens measured in blood donors in two cities. A Western blot assay was used to measure baseline and 9-month serological responses of people who were consuming either surface or groundwater. Baseline antibody responses were higher in those who consumed surface water, and surface water consumers with a detectable baseline response had a more intense response than groundwater consumers. Few participants reported *Cryptosporidial*-like illness, although many appeared to show seroconversion during the follow up.

## Day 2 - Closed Workshop Session

On Tuesday 6 October, a group of public health and epidemiology experts met to consider major issues in research relating to *Cryptosporidium* and the elements of rational public health response strategies. This meeting resulted in the formulation of a Consensus statement, agreed on by all members of the group.

### Research Questions

What are the major questions in cryptosporidiosis research?

What is the current state of knowledge on each of these questions?

What remains unanswered in the fields of surveillance and epidemiological study design?

How can we answer the remaining questions - what research is needed?

Is there opportunity for international collaboration?

How might a "typing" system for *Cryptosporidium* isolates impact on this research?

### Public health triggers

What are the current criteria for triggering a public health investigation in the US, UK, NZ and Australia?

What are the current criteria for public health interventions (eg boil water notices)?

What are the reasons for differences?

Can the Australian representatives develop a consensus approach with a common set of criteria?

### **Points raised in discussion leading to formulation of consensus statement**

There was general agreement in the group that we still remain very ignorant of the immunity issue, the factors which determine illness in those who are infected, and that there is some urgency for specific immune assays that, ideally, should be able to be carried out on saliva rather than blood.

A second issue was the genotyping of specimens of *Cryptosporidium parvum* and the need for laboratory markers of their virulence.

A third item of considerable importance to epidemiologists was the way laboratories handled stool specimens and the haphazard likelihood of identifying *Cryptosporidium*, and being able to assess the relevance of such identification.

A fourth important issue was a better understanding of the spectrum of disease in a community of infected individuals with varying levels of immunity.

Another problem which bedevils epidemiological understanding was the uncertainty of origin of *Cryptosporidial* oocysts which were being found increasingly in environmentally contaminated areas.

A sixth question which the group agreed needs to be resolved, is a better understanding of the role of specific risk factors in endemic local areas. It was recognised that this may vary from region to region and from sporadic to epidemic illness.

It was agreed that when oocysts are detected in water bodies, current technology does not enable epidemiologists to determine what this means. We need measures of virulence; we

ensure that the community gets the benefit of the best opinion possible on the meaning of this information.

It was agreed that epidemiologists and water agencies have different interests here. Epidemiologists are most concerned about the protection of community health in an environment in which understanding of the risk is still very imprecise. Water managers are naturally concerned at their duty of care and the need to comply with provision contracts, and maintaining community satisfaction and confidence.

It was agreed that *Cryptosporidium* counts in finished water are impossible to interpret from a public health risk perspective. We do not know whether such organisms are viable, virulent and what the infecting dose might be, and to what extent oocysts are damaged by water treatment and disinfection.

There was considerable discussion around the delineation of disease burden in the community. It was recognised that without an ongoing national notification system of known sensitivity and specificity, estimates of disease burden would remain thoroughly conjectural.

There was further agreement that the primary notification source of *Cryptosporidial* activity will need to be the laboratory, and that there is a need for standardisation, both in the criteria whereby doctors request stool examination, and the processes and mechanisms which laboratories apply when requested. Basic information reaching the public health unit should include: time, place, person and age.

There was recognition also that geographic information systems might be used to monitor *Cryptosporidial* activity around the nation and that simple epidemiologic criteria could be used to trigger the need for enhanced surveillance in a community or region.

As soon as routine and/or enhanced surveillance reveals an increased incidence of cases over baseline, a full-scale outbreak investigation is required. Such an outbreak should involve careful brainstorming by health and water authorities, planning for the collection of serum samples, and the possibility of long-term storage of samples for future studies.

There was general agreement that we know too little at present about the role of infection in immunisation and the relationship between immunity, strain virulence and the manifestations of infection. Outbreaks were opportunities to learn more about these matters.

The group spent considerable time discussing the issues of prevention and control. Given the importance of swimming pools as a source of infection and 'kiddies' pools in particular, there is clearly a need for engineers to ensure that adult and 'kiddie' pool filters are separate; that routine maintenance is adequate, and that faecal "incidents" are rapidly handled. There is also a clear need for wide community education on this matter. Children with diarrhoea should not be allowed to swim in the pool and overnight disinfection of filters should be capable of handling large doses of oocysts.

Another issue of vital importance is the management of human sewage and animal waste contamination in water catchment areas. The recent Sydney incident may well have come about because of huge run-off from insufficiently protected catchments.

The issue of managing diarrhoea in day care centres is an important preventive activity. Standard protocols already exist and have recently been shown to be vital to changing

Finally, there was a clear recognition within the group of the importance of media in informing rather than creating anxiety in the community about a problem on which we are still imperfectly prepared.

The group discussed the application of criteria for public health investigation in the various countries represented. In the United States, utilities monitor the activity of turbidity and coliforms, and when finished water quality indicators are suspiciously elevated or when *Cryptosporidium* is isolated in finished water, enhanced disease surveillance goes into action. The trigger for a full scale public health investigation is increased cases, not an increase in isolation of *Cryptosporidium* from water.

In New Zealand, where *Cryptosporidium* has been notifiable for two years, the criterion would be increase in number of cases as the organisms are not routinely tested in treated waters.

In the United Kingdom, the decision to embark on a public health investigation depends on close working relationships and liaison between public health authorities and the water industry. *Cryptosporidial* counts are only one element of the data that are considered.

There was discussion about the effect of the recent Sydney episode on activities in other Australian states. In Queensland, an interim protocol has been developed whereby if water quality is in doubt an expert panel is called. The inclination in Sydney is to work in a similar way in future if *Cryptosporidial* counts go above 100 per 100 litres.

It was agreed in discussions that 'boil water' alerts are themselves risky exercises. They carry health risks in their own right and if used often, will rapidly destroy public confidence and water system credibility. In the case of the Sydney experience recently, there have been three major 'boil water' alerts, despite the fact that there has been little or no evidence of excess *Cryptosporidial* disease.

The issue of 'boil water' alerts and immunocompromised individuals was discussed. Some doctors advise people with CD4 counts less than 200 per microlitre to use boiled water. No water supply can guarantee an innocuous pathogen dose to individuals with grossly impaired immune function.

The group agreed that it is almost impossible to interpret the meaning of *Cryptosporidium* counts in treated water. The interpretation of such findings requires an expert analysis in the light of a range of other data about the history of the water supply, treatment processes and source water data. It provides no guidance to public health action. While there was a recognition that water agencies might wish to continue to monitor treated water, the view was that this was not a helpful aid to public health decision making and action.

## Consensus Statement

Discussions over the last two days have identified a number of areas of research priority, together with the need to develop rational public health policies despite the current limitations in our understanding. The group agreed that the **primary aims of public health** in relation to *Cryptosporidium* in water supplies are:

- to control disease
  - only to intervene when we need to
- and
- to use public funds as efficiently as possible.

The research priorities agreed by the group highlighted the current inadequate understanding of the natural history of the disease in humans, the nature and role of the immune response and the determinants of disease.

It was agreed that in this current state of uncertainty, there is a need to invest in **well-targeted research**:

- that will help us to understand the natural history of the disease and its immunology;
- that explores the factors which enhance transmission of the infection in man and the risk factors which predispose to that infection;
- that explores the effect of prior serological experience on infection outcomes.

It was agreed that considerable effort should go into developing **methods for routine phenotyping or genotyping of strains** isolated from humans and from water. Such techniques would represent a significant advance in epidemiology by allowing us to trace the origins of individual strains, and determine the relative importance of drinking water relative to other routes of transmission. There is also a great need for improved **tests to determine the viability and pathogenicity** of oocysts isolated from the environment so that we can better assess the degree of health risk posed by oocysts detected in drinking water.

There is also a need for continuing efforts to develop **effective methods of treatment** of people with known infection. The availability of such treatment would markedly reduce the risks associated with infection in immunocompromised individuals, and would significantly change the public health perspective on this organism.

There is clearly a need for **better community education** to improve the understanding of the disease, and permit the effective protection of the community without promoting panic. It is important that we make efforts to explain our current limited knowledge on these issues, and the difficulties in predicting health risks from water testing results. We must also endeavour to use scarce public health resources as efficiently as possible.

We believe there is a need to develop a **national best practice protocol for surveillance** that is based on standard advice to laboratories on which stool specimens to examine, which methods to use, and the minimum notification data that they should pass on to public health agencies when they identify the organism. Doctors around Australia should be advised by public health agencies on which cases to request stools for, and the most common risk factors as they are currently understood in this condition. At present there are considerable variations in testing practices and reporting procedures between different states and territories which makes it difficult to establish a comprehensive picture. There is also a need for a validated system of multiplier values to relate the number of laboratory defined cases to the number of cases in the community.

surveillance. We believe that increased water turbidity events and the presence of contamination with other pathogens may be appropriate triggers for enhanced surveillance for *Cryptosporidial* infection. Enhanced surveillance should include a systematic approach to rates of diarrhoeal illness in nursing homes and other institutions, a follow-up of individual cases of cryptosporidiosis, active contact with laboratories, and possible activation of other sentinel systems, including school absenteeism, general practice systems, oncology units, etc. Geographic Information Systems mapping of such data may be useful as an adjunct to identify and track outbreaks. The National Communicable Diseases Network is seen as the appropriate forum to progress these improvements in surveillance mechanisms for *Cryptosporidium*.

On the issue of **investigation of suspected waterborne outbreaks**, we are not convinced that case control methodology is always the best approach to investigation. A clear specification of the relevant investigative questions requires the input of both public health and water teams and this may require brainstorming and application of cutting-edge technology. We emphasise that while outbreaks of illness are undesirable, they nevertheless represent opportunities to improve our knowledge. We need to take the opportunity to store faecal and blood specimens and expect that each outbreak will help us further to understand the natural history of the disease. Finally, there is a need for national and international collaboration in outbreak investigation and in the standardisation of questionnaires for use in such investigations.

In our review of the Sydney events, we and others have been puzzled at the huge oocyst counts reported in finished water, with no detectable evidence of increased disease despite a substantial effort in enhanced surveillance. We note that the availability of the technical capacity to detect *Cryptosporidia* in finished water makes it difficult for water authorities to avoid monitoring, but emphasise that the Sydney episode underlines our inability to interpret such findings.

This may be a case where the technology is ahead of the medical science. There are real hazards from multiple 'boil water' alerts, both in terms of injury risks to the community, loss of credibility for water and health authorities, and community outrage. We emphasise our view that, **at present, public health is not a reason for monitoring *Cryptosporidium* numbers in finished water**. Given the current state of testing technology we cannot establish the viability or infectivity of oocysts, and thus we cannot use such counts as a basis for public health action. However we recognise that such monitoring may serve a purpose for the water industry in assessing the effectiveness of water treatment processes for the removal of *Cryptosporidium* oocysts, and in identifying environmental factors which lead to elevations in oocyst numbers.

We believe that a better approach to the monitoring of treated water is careful investigation of all turbidity events. Changes in turbidity in treated water require collaborative discussions with health personnel, a review of water treatment procedures, and may require introduction of enhanced surveillance if raw water *Cryptosporidium* counts are raised. Where *Cryptosporidium* counts on treated water are elevated, we cannot assume that they necessarily constitute a significant public health hazard without extensive accompanying data and advice from a range of experts. Thus the detection of such events would not automatically trigger a boil water alert, but would trigger investigations by water and health officials to determine the causes and consider the need for such an alert to be issued.

The available evidence on natural history indicated that several oocysts are needed to infect

oocyst. Few if any water authorities can guarantee complete freedom from such a risk in their finished water in their current state of technology.

Finally, we have agreed that *Cryptosporidium* constitutes a substantial **public health hazard in swimming pools**, and that this risk will continue while parents take young children to pools in the summer. The only approaches available are public education to minimise contamination of pools, improved engineering of pool filters, and the value of hyperchlorination overnight of affected filters and pools. Pool closures are as undesirable for the recreational water industry as boil water alerts are for the drinking water industry, and may create similar public responses. While there is some scope for reducing the problem by better design and operation of pools, it is clear that the major need is for better public understanding of the issue.

There was further discussion of the **consensus public health strategy** for drinking water in the final Plenary session, with the following points being agreed:

- relevant health and water industry personnel should have frequent routine contact so that rapid and effective consultation can take place whenever unusual water quality events occur.
- a stepwise response protocol should be established depending on the degree of health concern associated with different circumstances.
- it is important that the response protocol agreed between health and water authorities is subject to public comment during its development.
- similarly, the final response protocol must be made available to the public and the media. The protocol should set out the circumstances which would trigger a response, the investigative and corrective measures to be implemented for various levels of response, and the time period required to carry them out. Placing this information in the public domain in advance of any water quality events helps to address industry concerns over 'duty of care' with respect to the time taken for confirmatory testing and investigations.
- it is preferable that one person in each state or territory is responsible for dealing with the media during the investigation of water quality events. The media should be kept informed of the progress of investigations being undertaken to ascertain the degree of health risk to the community.

## RISK ASSESSMENT AND MANAGEMENT

*Summary by Dr Peter Nadebaum, Egis Consulting, Melbourne, Dr Daniel Deere, South East Water Limited, Melbourne and Mr Martyn Kirk, Dept of Human Services, Melbourne.*

### Day 1 - Afternoon Workshop Session

The afternoon session on risk assessment included six papers, all of which related to minimising and estimating the risk of human *Cryptosporidium* infections from drinking water. In particular, the presenters focussed on the validity of the many assumptions used in risk assessment.

**Nick Ashbolt** from the University of New South Wales reviewed the process of risk assessment for *Cryptosporidium* from catchment to tap. Risk assessment relies upon an understanding of *Cryptosporidium* density in water, and the likelihood of infection and ultimately illness. A considerable amount of work has been conducted on the magnitude and variation of *Cryptosporidium* in raw or source waters, but this has not addressed the issue of events in the catchments. Nick suggested that a large number of samples were required to get reliable estimates of *Cryptosporidium* density in treated water, due to the large number of negative samples. There are a variety of mammals in Australian catchments which can excrete *Cryptosporidium*. Currently, there are several problems with risk estimation, including: a lack of environmental data for hydrograph peaks, lack of standardisation of analytical methods and reporting, inappropriate water sampling, and the use of only one dose response curve. Nick also discussed the problems of determining viability of environmental *Cryptosporidium* isolates, and the inadequacies of the mouse model for estimating human infections.

**David Adams** from the Bureau of Resource Sciences suggested that the Codex Alimentarius risk assessment framework, developed for the food industry, is a good model for *Cryptosporidium* in water. This framework incorporates a scientific assessment of the risks, the managerial processes required to manage the risks, and the communication of this information. The whole process needs extensive consultation with all stakeholders. David indicated that there is some data on the prevalence of *Cryptosporidium* and *Giardia* carriage amongst Australian animals, but there is a paucity of data on the fate of these organisms. Potentially all mammals could be reservoirs for *C. parvum*, but it is unclear which ones are important. The host-parasite relationship for *Cryptosporidium* is poorly understood, which makes it a difficult problem for the water industry.

**Jerry Ongert** of the University of New South Wales discussed the removal of *Cryptosporidium* using water treatment. He focussed on physical treatment including granular media, diatomaceous earth and membrane filtration. It is useful to think of *Cryptosporidium* as discrete particles with similar properties to other organic particles such as algae. Jerry discussed factors affecting filter performance, including: water quality, type of flocculation, filter design, and filter operation. He emphasised the need to test the removal efficiencies of filtration systems, and that these methods should be statistically sound.

**Gordon Finch** of the University of Alberta, discussed the treatment of water containing *Cryptosporidium*, particularly disinfection. Risk assessment indicates how much we need to remove to meet certain requirements. Water managers need to decide what level of control we would like to exert, based on the nature of the water source. Engineering design can then facilitate the required level of *Cryptosporidium* removal. With properly optimised treatment, 4 logs of *Cryptosporidium* removal can be obtained, which can be enhanced with the use of dissolved air flotation. Assessing the disinfection potential of different chemical agents

monochloramine are also effective, but require much longer contact times and higher concentrations. Gordon emphasised the importance of estimating treatment requirements properly, to minimise unnecessary costs.

**Daniel Deere** from South East Water discussed the role of monitoring for *Cryptosporidium* in drinking water in assessing risks to human health. Daniel pointed out that it may be more realistic to use monitoring results to determine sources of oocysts in catchment than the likelihood of human illness. Daniel discussed the results of a Cooperative Research Centre project where water authorities supplied real monitoring data for *Cryptosporidium*. Most available water quality data was either 0 or very low densities, and no typing of oocysts had been conducted. There is considerable uncertainty associated with this data, which is either random or systematic in nature. The random variation includes the natural Poisson distribution associated with the occurrence of oocysts in water supplies, while systematic variation includes errors associated with sampling and measurement. Daniel emphasised two major problems with quantitative risk assessment being the lack of method validation, and the uncertainty about infectivity of oocysts in the environment.

**Peter Teunis** from RIVM-IMA in the Netherlands presented a Beta-Poisson model for determining the health impacts of ingesting *Cryptosporidium* oocysts. This type of modelling is useful for estimating risks from low doses, where epidemiological methods are unreliable. Peter reinforced the use of a simple statistical model and presented several datasets from other human pathogens fitted to his model. The data from the DuPont *Cryptosporidium* feeding study fitted the 'one hit' model well, and their recent re-testing of the infection potential of *Cryptosporidium* in human volunteers should prove interesting to model. After a person is exposed to organisms in the environment, they may become infected, and they may then go on to develop symptoms. Peter showed data from feeding studies demonstrating that higher doses of organisms did not necessarily correlate with development of symptoms, and that this needed further investigation.

## Day 2 - Closed Workshop Session

This stream of the conference considered the requirements for risk assessment and management of *Cryptosporidium* in Australian urban water supplies. The workshop group comprised national and international experts from water authorities, laboratories, academic departments, health agencies, consulting firms and other government bodies. The group considered alternative frameworks for managing risks from the *Cryptosporidium* hazard in water supply systems as well as the assumptions to use in quantifying the risks.

This report summarises the consensus reached on the most appropriate approach for assessing and managing the risk from *Cryptosporidium*. Future research needs are indicated in each section.

## GENERAL PRINCIPLES

### Basis for Management

Water authorities should adopt a quality management approach to the management of their water supplies, such as that outlined in the ISO 9000 guidance documents. This should have Water Quality as a specific area of focus for quality assurance. This should include, for example:

- undertaking a structured risk-based analysis of the particular issues relating to water quality for specific water supply and management systems, and from this derive the most important management strategies. This could adopt, for example the principles of AS/NZS 4360:1995 (Risk Management) and Hazard Analysis and Critical Control Points (HACCP).

The overall approach for water authorities to manage the risks from *Cryptosporidium* should be based on adopting good practice and should be proactive, with the objective of avoiding future difficulties. Management should be via control of inputs (eg operational systems and barriers) rather than control via measured outputs (such as monitoring of treated water). It is essential for water authorities to have a good understanding of their supply systems, and particularly the factors which can affect the source water quality and the effectiveness of treatment. Good planning is essential if appropriate responses are to be made to water quality issues as they arise.

It is important that water authorities work together with other key stakeholders, especially the health authorities and customers, in determining management strategies. Water managers should consider all aspects of the supply system, from the catchments through to the consumers' taps. It is of concern that fragmentation is occurring in the industry, with separation of responsibilities relating to catchment management, storage, treatment, and distribution. Where this separation has occurred, management must seek to bring the relevant groups together so that systems and communications are appropriately managed.

There is a need for guidance on the appropriate response to water quality issues. Risk communication is a critical element, and the development of appropriate risk communication strategies are required.

There is a need for water authorities and health regulators to encourage and demonstrate a consistent approach to the regulation, management and communication of water quality issues relating to public health.

## **MICROBIAL PATHOGENS REQUIRING MANAGEMENT**

Although *Cryptosporidium* is an important area of focus, other microbial pathogens are likely to be linked with the presence of *Cryptosporidium* and can be similarly managed. It is important that management strategies are appropriate for the range of pathogens that may be present in particular supply systems, including those that are resistant to the methods of disinfection in common use in Australia (chlorination and chloramination). The water quality management strategy should also extend to control of the aesthetic aspects of water quality, as these can lead to a customer perception of unsafe water.

It is possible that there are other microbial pathogens present in drinking water that have not yet been identified which could be significant with respect to public health. The use of new analytical tests such as polymerase chain reaction techniques to detect pathogenic organisms will also add to our understanding of their presence and survival in water supply systems.

These factors support the adoption of a broadly based 'best practice' risk management approach, rather than an approach which is targeted only to specific pathogens.

## **RISK MANAGEMENT**

Risk management should take a holistic approach, and consider the sequence of supply systems, from the catchment to the tap. Catchments, reservoirs and treatment systems are likely to be the most important components of the supply system with respect to

### **Catchment Management**

Catchment protection and the control of contamination sources within catchments are an important components of *Cryptosporidium* management. It is essential that water authorities have a good understanding of their catchments and potential sources of contamination. This should include contamination that can occur under both normal conditions, and during events such as rainfall. Human faecal contamination is most important, and simple inspections including a sanitary survey are basic elements in understanding potential sources of contamination. Land use mapping and the use of Geographical Information Systems to provide an understanding of contamination risks and management requirements have potential to be important tools, and are seen to be a useful areas for development.

Because there is considerable use of highly protected catchments in Australia, it is important to obtain a better understanding of the contamination levels that occur in such catchments, and the necessary treatment requirements. Because there is no evidence of illness associated with these systems, it should not be assumed that the existing level of treatment is inadequate.

It would be useful if a "Catchment Index" could be developed for Australian catchments which would indicate the risk of contamination by *Cryptosporidia* (and other pathogens such as *Giardia*). This could assist in establishing the levels of treatment necessary.

In most situations it will not be possible to monitor for specific pathogens other than on an occasional basis which will not be statistically significant. Because of this, monitoring programs need to rely on indicators and surrogates, with knowledge of the variation in location and time and the conditions under which high levels of *Cryptosporidia* may occur. Some real time monitoring (eg of turbidity and flowrate) may provide early warning of risk events. There is a need for investigation and the development of guidance on appropriate monitoring and early warning strategies for different Australian catchment types.

### **Storage**

Storage in reservoirs is a first barrier, and can achieve a significant reduction in the concentrations of *Cryptosporidium* oocysts through dilution and die off. There is some data which suggest that the rate of reduction in viability may be in the order of 1 log reduction every 6 weeks in cool dark waters and 1 log reduction every week in warm sunlit waters.

It is important that the operation of reservoirs be understood from the perspective of reducing the concentrations of *Cryptosporidium* oocysts. This includes maximising retention, and avoiding stratification and short-circuiting. Some data is available for overseas systems, but information is required for Australian reservoirs. The development of appropriate monitoring and early warning strategies for reservoirs is required, similar to catchments.

### **Treatment**

Because there are practical limitations on the control of contamination within catchments, treatment is usually the point where most control can be achieved. Treatment methods include physical removal through membranes or various types of coagulation, flocculation, sedimentation and filtration, and through chemical methods such as ozonation. In Australia physical removal is generally adopted rather than chemical treatment, and there has been variability in the level of treatment able to be applied for small and large supply systems.

A key area requiring resolution is whether multiple treatment barriers (such as physical removal coupled with ozone) are necessary in the Australian situation. This is dependent on

In general, the targets that should be adopted for *Cryptosporidium* control have not been specified, and this makes it difficult for authorities to select treatment methods, to specify their reliability, and to determine whether multiple treatment barriers should be installed. There is insufficient knowledge at present to establish treatment targets for *Cryptosporidium*, although the general thinking is that viable *Cryptosporidium parvum* oocysts of genotype 1 should not be present in treated water in the volumes normally adopted for sampling purposes (10 - 100 L).

In the case of existing treatment systems, optimal operation is necessary. There is information available as to how the optimisation of existing treatment systems can be carried out in a systematic way. There is a need for dissemination of this information to the Australian water authorities and guidance on its application.

Because of the difficulty with monitoring pathogens, indicators and surrogates must be used for monitoring of treatment system performance. The performance of treatment systems should be optimised using pre and post-treatment particle counters and turbidimeters. The use of turbidimeters can be limited because of their reduced accuracy at low turbidity levels, and response to a wide particle size range. Pressure meters (membrane filters) or streaming current monitors (granular media filters) can be used for real-time process control together with finished water particle counters for confirmation of performance.

There is a need for an industry and regulator working group to be set up to agree on appropriate risk-based practices for the selection of treatment systems and the objectives for their performance and operation.

### **Distribution system**

In most of the larger Australian urban water supply systems the distribution system is less important as a *Cryptosporidium* contamination source than the catchment and storage reservoirs. This is not necessarily the case for smaller water supplies or those operating at low pressure.

Of *Cryptosporidium* oocysts that do enter the distribution system, it is possible that one or more orders of magnitude reduction in viable oocyst numbers may be achieved in the distribution system. Some oocysts are adsorbed on biofilms and, of these, some will be removed by system flushing activities, and some will release through sloughing into the finished water. Such oocysts may no longer be viable.

In general, there is currently insufficient information to predict whether a significant reduction in oocysts will occur in a distribution system. A CRCWQT project is currently investigating these relationships.

### **Monitoring finished water quality**

It is generally considered that monitoring of finished water for specific pathogens such as *Cryptosporidium parvum* is not practical for routine operation. There was no consensus on whether occasional sampling of treated water or water at customers' taps should be carried out. It was agreed that such sampling would not be practically useful in that it would not provide helpful information and could be misleading. However, some felt that there may be a need to undertake at least some testing to satisfy the requirements for due diligence and to provide a defence against negligence in the event that a disease outbreak should occur.

Because of the difficulty with direct monitoring of pathogens, reliance must be placed on

the extent of reliance on treatment), with indicators being used for routine monitoring of source and finished water quality.

Where monitoring of source waters or finished water for *Cryptosporidium* is undertaken, it is important that adequate quality control and validation of the results be undertaken. Analysis by independent laboratories of duplicate samples in which *Cryptosporidium parvum* oocysts are present is an important part of this.

There is a need for an industry and regulator working group to be set up to agree on appropriate risk-based monitoring strategies for protection of consumer health and due diligence purposes.

## **INTERPRETATION OF *CRYPTOSPORIDIUM* MONITORING RESULTS**

It is becoming more common for water authorities, particularly the larger authorities, to undertake some monitoring of both their source waters and treated water for *Cryptosporidia*. There is a need for guidance as to how these results should be interpreted, and the methods for assessment that should be applied.

Draft reports which considered the interpretation of *Cryptosporidium* monitoring results obtained by a variety of Australian water authorities had been prepared by CMPS&F under CRCWQT Project 1.1.1, and were discussed at the workshop that took place on the second day of the conference. There was good agreement with the reports. The conclusions of this work will be published separately. A summary of the workshop conclusions are as follows:

**General:** because of the limitations in the available analytical methods and their inability to measure infectivity to humans, the monitoring programs that water authorities currently undertake for *Cryptosporidium* do not provide a direct measure of the risk to human health. If conservative worst case assumptions are made, risk estimates will probably substantially overestimate the risk to human health and should be viewed in this light. It is a key regulatory issue to resolve the basis for regulation of protozoan pathogens, and how monitoring results that show the presence of *Cryptosporidia* are to be interpreted.

**Sources of pathogens and infectivity:** *Cryptosporidium* oocysts from human faeces are more likely to be infective to humans than those from other sources such as native mammals, but it is not possible to quantify this at present. There is a need for analytical methods which will determine the species and genotype of oocysts. In the absence of such information an upper estimate of the risk can be made by assuming that all detected oocysts are infective; however, this may substantially overestimate the risk.

**Dose response:** in estimating the likelihood of infection it is appropriate to use the Du Pont feeding trial dose response data with bootstrap replication to account for uncertainty and strain to strain differences. Estimation should assume that one oocyst represents the threshold for infection, as the assumption of a multiple oocyst threshold level for infection does not appear to be supported mechanistically or from other published data. The common assumption that 2 L of unboiled water will be consumed is likely to be a significant overestimate and consumption data specific to the community of interest should be used where available.

**Distribution of oocysts in water:** the distribution of oocysts probably does not follow a Poisson distribution and is more likely to be described by a negative binomial distribution.

methods and the high cost of analysis, monitoring programs often do not have the required level of quality control. There are no generally applicable corrections for method recovery.

**Viability of oocysts:** it is likely that a significant proportion of oocysts detected at the point of monitoring will not be viable. In the absence of monitoring information which is specific to viable oocysts or information which quantifies the reduction in viability that can be expected, an upper estimate of the risk can be made by assuming that detected oocysts are infective; however, this may substantially overestimate the risk.

**Removal by treatment:** the use of simple log reduction estimates can be used as an initial order of magnitude estimate of the removal of oocysts by a treatment system. However, there can be a wide variation in the performance of coagulation, flocculation and separation (such as sedimentation and filtration) with system design and water type, and it is desirable to base oocyst reduction allowances on measured indicators of performance. In general it is not practical to directly monitor the oocyst removal performance of treatment systems, and performance estimates must be based on indicators and surrogates. In addition to monitoring the inferred performance of treatment under the normal range of operating conditions, consideration should be given to the reliability of treatment (ie frequency of failure or abnormal operation).

Conventional disinfection by chlorination and chloramination is unlikely to significantly reduce oocyst numbers. Information is available to provide preliminary estimates of the reductions that will be achieved through ozonation.

**Removal in the distribution system:** there is usually insufficient information to provide estimates of the reduction in the numbers of viable oocysts that will occur on passage through a distribution system. Mass balances of particulate matter entering and leaving may provide information; however, in the absence of such information an upper estimate of the risk can be made by assuming that there is no reduction in the numbers of oocysts on passage through the distribution system.