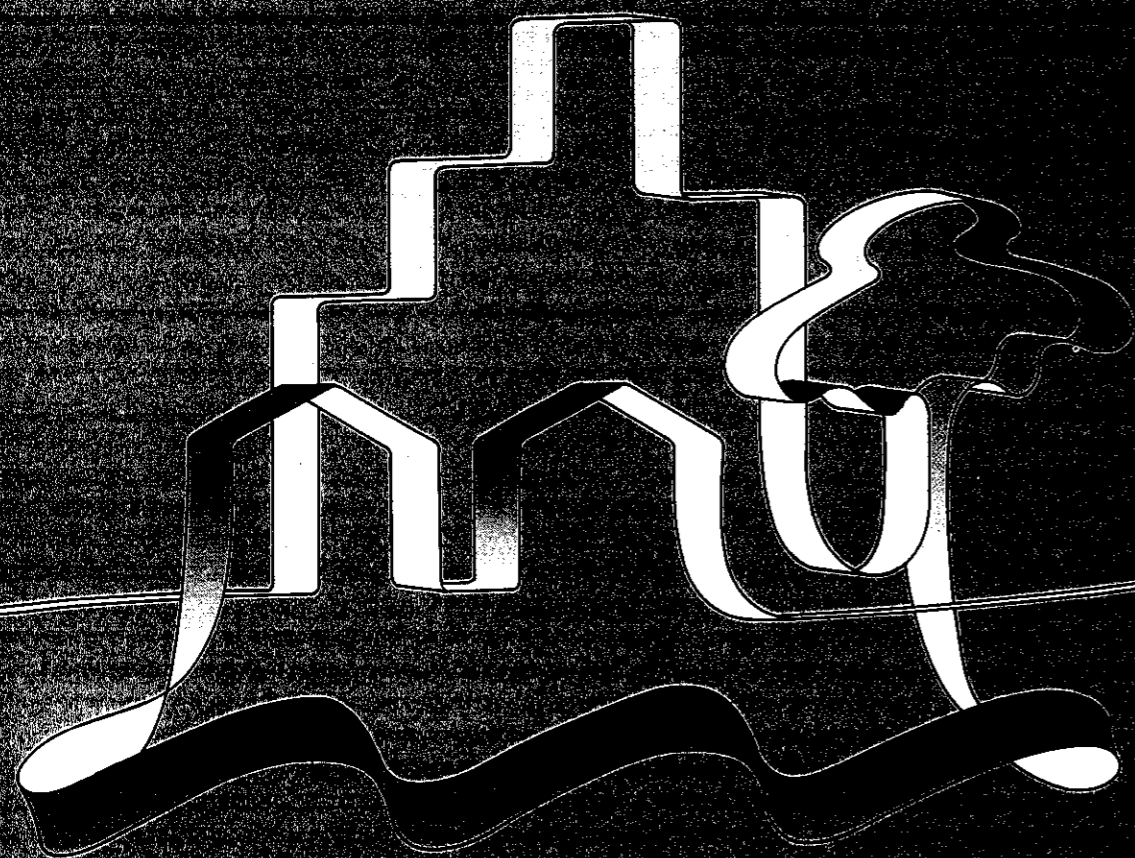




Urban Water Research Association of Australia

Survey of Australian Waters for
Cryptosporidium and *Giardia*



Research Report No. 128

Urban Water Research Association of Australia

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**Survey of Australian Waters for
Cryptosporidium and *Giardia***

A Champion
Macquarie University

**Research Report No 128
September 1998**

FOREWORD

This report is based on UWRAA Research Project No WR-27: 'Analysis of Australian Water and Sediment Samples for Protozoan Parasites using Flow Cytometry' which was commenced in July 1993. Organisational responsibility for the project was as follows:

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The project was funded by the Urban Water Research Association of Australia, Macquarie University and A W T.

ABSTRACT

A total of 191 raw water samples from 26 sites around Australia were concentrated by flocculation and analysed by flow cytometry for the occurrence of the protozoan parasites, *Cryptosporidium* and *Giardia*. *Cryptosporidium* oocysts were detected in 19.1% of samples, at numbers up to 30 oocysts per 10 litre sample. *Giardia* was detected less frequently, with 6.4% of samples being found positive, and numbers up to 20 cysts per 10 litre sample. Sites from catchments which had significant farming or recreation use had a higher probability of either parasite being detected. In addition, there were indications that sites from catchments in which land use was mainly farming had a higher incidence of *Cryptosporidium* oocysts, and those with mainly recreation use had higher incidence of *Giardia* cysts. Both parasites were found to be more prevalent at southerly latitudes, possibly due to the lower water temperature at these sites leading to increased persistence of oocysts and cysts in the environment. There was no strong correlation with the occurrence of either parasite and any water quality parameter, although turbidity (NTU) was a minor indicator of the occurrence of either parasite.

The effectiveness of the method (concentration by flocculation and analysis by flow cytometry) was tested by a quality control program which compared recoveries from several different sites, and of the effect of factors which might affect recovery rate. The method was found to be at least as good as other methods reported in the literature, with recoveries averaging close to 50% for both parasites. The major cause of losses and variations in recovery rate were shown to be due to undetermined factors during concentration step, possibly related to water quality.

1.1 BACKGROUND

The intestinal parasites *Cryptosporidium parvum* and *Giardia lamblia* are amongst the most significant causes of water borne human disease worldwide. While the two organisms are taxonomically distinct, they have marked similarities in biology, epidemiology and pathology which allows them to be considered together in the context of water borne disease. Perhaps the most relevant feature of these organisms in terms of public health risk is the occurrence of environmentally robust infective stages, the *Cryptosporidium* oocyst, and the *Giardia* cyst. Both oocysts and cysts may remain viable in water for long periods of time, are resistant to standard water disinfection protocols, and have a low infectious dose. Infection with either organism causes mild to severe diarrhoea, which may be life threatening in the very young, the elderly, or in people who are immunocompromised, such as cancer or transplant patients, those with inherited immunological disorders, or those suffering from HIV-AIDS (O'Donoghue, 1995). Cryptosporidiosis is usually self-limiting in healthy individuals, and there is evidence that infection may impart some subsequent protection. Giardiasis, tends to be much more persistent, but is treatable with chemotherapeutics such as metronidazole (Flagyl) (Markell and Voge, 1976). *Cryptosporidium* is of particular risk to immunocompromised individuals as there is currently no effective treatment against infection - cryptosporidiosis is known to be a common cause of death amongst HIV-AIDS sufferers.

Below is summary of the biology and epidemiology of each parasite, and an overview of their relevance to water quality and public health.

1.2 *Cryptosporidium*

The protozoan parasites contained within the genus *Cryptosporidium* are classified within the phylum *Apicomplexa*, class *Eucoccidiorida*, family *Cryptosporididae* (Fayer and Unger, 1986).

Cryptosporidium are small coccidian parasites, first described and named by Tyzzer in 1907. Although Tyzzer undertook much work on the genus *Cryptosporidium*, it was not considered to be of medical or economic importance until the early 1970's when it was first implicated in human disease (Current and Garcia, 1991; Nime *et al.*, 1976).

Tyzzer originally described two species, *C. muris* and *C. parvum*. Since then approximately nineteen other species of *Cryptosporidium* have been described. The classification of these species was based upon the host animal in which they were found (Current and Garcia, 1991; Fayer and Unger, 1986; O'Donoghue, 1995). *Cryptosporidium* spp. infect a wide variety of vertebrate hosts including snakes, birds, and a number of Australian marsupials such as the

brown antechinus, southern brown bandicoot, red kangaroo, koala, pademelon and echidna (O'Donoghue, 1995).

Early cross-infection studies demonstrated that *Cryptosporidium* infection was readily transmissible between species (Tzipori, 1982a and b). More recently varying degrees of heterogeneity among *Cryptosporidium* isolates from different hosts have been found using morphological, immunological and biochemical characterisation (Nina *et al.*, 1992; O'Donoghue, 1992). It appears that a degree of host specificity may occur at the vertebrate class level, ie at least one mammal, bird and reptile species (O'Donoghue, 1995).

It is currently accepted that there are six different species of *Cryptosporidium*, *C. baileyi* and *C. meleagridis* infecting birds, *C. parvum* and *C. muris* infecting mammals, *C. serpentis* infecting reptiles and *C. nasorium* infecting fish (Current and Garcia, 1991; O'Donoghue, 1995).

Although *C. parvum* is recognised as the the most common cause of human infection and therefore the species of most concern to this report, the increasing number of immunosuppressed individuals in society means that it can no longer be thought to be the only species with the potential to infect humans. (Ditrich *et al.*, 1991).

1.2.1 Human infection

The disease caused by infection with *Cryptosporidium*, cryptosporidiosis, is best known as a disease of pastoral animals (Angus *et al.*, 1982). The first human cases were diagnosed in the early 1970's in a three year old child and an immune deficient adult (Meiseal *et al.*, 1976; Nime *et al.*, 1976). It has now been documented as a disease of humans in approximately thirty countries spanning six continents (Fayer and Unger, 1987; O'Donoghue, 1995).

The main symptom of cryptosporidiosis is profuse, watery diarrhoea which may contain mucous but rarely contains blood. There is often associated weight loss. Other symptoms are abdominal pain, nausea, vomiting, and low-grade fever. Occasionally there are non-specific symptoms of anorexia, malaise, myalgia, weakness and headache (Tzipori, *et al.*, 1983; Soave, 1988). Symptoms are of varying severity from mild, transient diarrhoea to severe diarrhoea requiring hospitalisation, and may persist from three to twelve days. The disease is self-limiting in immunocompetent hosts (Fayer and Unger, 1986).

Cryptosporidiosis in immunodeficient hosts is much more serious, and infection may lead to death. The severity of the symptoms are generally greater, with voluminous diarrhoea up to 25 litres per day, severe abdominal pain and significant weight loss, which may be greater than 25% of total body weight having been reported (Flanagan and Soave, 1993). Another rare

form of the disease, respiratory cryptosporidiosis, appears to be confined to immunocompromised patients and is usually fatal. The symptoms are coughing, shortness of breath, wheezing, croup and hoarseness (Current and Garcia, 1991). As yet there is no effective drug treatment for cryptosporidiosis in humans or animals (Angus *et al.*, 1982; Soave, 1988; Current and Garcia, 1991).

1.2.2 Prevalence of cryptosporidiosis in humans

The prevalence of cryptosporidiosis among immunocompetent persons suffering from gastrointestinal complaints ranges from 0.1% and 27.1% with a mean of 4.9% in industrialised nations (56 surveys), and from 0.1% to 31.5% with a mean of 7.9% in underdeveloped countries (48 surveys) (O'Donoghue, 1995). The range for asymptomatic illness in developed countries is 0 to 2% with a mean of 0.3% (12 surveys). Surveys of underdeveloped countries show a range of 0 to 9.8% with a mean of 1.6% (20 surveys) (O'Donoghue, 1995). The higher prevalence of disease in underdeveloped countries may be related to overcrowding, poor hygiene and sanitation, contaminated water supplies and greater contact with infected animals.

Several surveys of fecal cryptosporidium have been carried out in Australia. In a study of incidence of childhood cryptosporidiosis in Western Australia, the incidence among white children was 4.1% and 9.6% amongst aboriginal children (Tzipori, 1988). Testing of fecal specimens submitted over a 2 year period in Victoria in the early 1980's revealed that 2.5% of patients were positive for cryptosporidiosis (Biggs *et al.*, 1987). In Townsville 4.6% of people studied were positive for cryptosporidiosis (Cruikshank *et al.*, 1989) with 8.6% of these being from an isolated aboriginal community. No seasonal variational or animal or environmental sources were identified. *Cryptosporidium* was the second most commonly identified intestinal pathogen at the Princess Margaret Hospital for Children in Western Australia (Assadamongkol *et al.*, 1992). It was also found to be the most common enteric pathogen reported in the general population in Western Australia (Anon, 1992).

1.2.3 Transmission of cryptosporidiosis

Cryptosporidium oocysts are transmitted via the faecal-oral route. Any mechanism which maintains this route such as direct person-to-person contact, contaminated water for drinking and recreation, or contaminated food can transmit the parasite. Originally cryptosporidiosis was thought to be transmitted directly from animals. Calves have commonly been implicated as

a source of human infection (Miron *et al.*, 1991). It is possible that companion animals such as dogs, cats and rodents may act as reservoir hosts (Current *et al.*, 1983).

The concept of zoonotic transmission of cryptosporidiosis to humans is related to the vast number of mammals capable of harbouring the parasite and the realisation that *C. parvum* readily crosses the species barrier (Current, 1989; Current and Garcia, 1991). Zoonotic transmission may be a risk to people working or living in areas where exposure to faecal contamination is likely. Transmission by zoonoses does not explain the number of large outbreaks in urban areas where the majority of people are unlikely to be exposed to animal faeces (Current and Garcia, 1991).

Many cases of direct person-to-person transmission have been documented. The transfer of cryptosporidiosis between children in child care centres and their families offers evidence of person-to-person transmission (Alpert *et al.*, 1986; Combee *et al.*, 1986). Hospital-acquired cryptosporidiosis has also been documented giving further evidence of person-to-person transmission (Current and Garcia, 1991).

The waterborne route of transmission has become an increasing concern to water utilities. The first reported waterborne outbreak of cryptosporidiosis occurred in San Antonio, Texas in 1984 (D'Antonio *et al.*, 1985). Sewage contamination of chlorinated well water was blamed for the outbreak. Approximately 13 000 people were infected in Carrolton, Georgia in 1987. Sub-optimal flocculation and filtration probably allowed the passage of oocysts into the finished water (Hayes *et al.*, 1989). In 1988 approximately 100 000 people were infected in Swindon and Oxfordshire, UK (Poulton *et al.*, 1991; Richardson *et al.*, 1991). No faults were found in the treatment works or in the parameters of water quality. It has been suggested that heavy rainfall may have resulted in an increased number of oocysts entering the treatment plant. Jackson County, Oregon, suffered a waterborne outbreak of cryptosporidiosis in 1992 which was attributed to mechanical and operational deficiencies at the water treatment plant (Leland *et al.*, 1993). In Milwaukee, Wisconsin, 1993, the most dramatic outbreak so far recorded occurred. Approximately 420 000 people were infected and at least 85 people associated with the outbreak died (Pontius, 1993). Unusually high turbidities in raw water and inefficient treatment resulted in higher than recommended turbidities in finished waters and in the passage of oocysts through the treatment plant. Table 1 shows reported outbreaks of *Cryptosporidiosis* which have been traced to contamination of water supplies.

There have been no documented outbreaks of cryptosporidiosis or giardiasis through a public water supply in Australia. An outbreak of cryptosporidiosis and giardiasis, attributed to sewage contamination of a private tank water supply, affected approximately 90 people on a

school camp in Victoria in 1992 (Lester, 1992). In January, 1995, a swimming pool associated outbreak of cryptosporidiosis occurred in Southern Sydney involving 74 cases (Anon., 1995). Since then, there have been several reported outbreaks in Australian swimming pools. Reported outbreaks of Cryptosporidiosis traced to recreational waters are shown in Table 2.

1.2.4 *Cryptosporidium* in Raw Water

The waterborne outbreaks of cryptosporidiosis and giardiasis in North America and Britain have caused increasing concern about the safety of public water supplies.

Several studies have been conducted into the occurrence of *Cryptosporidium* in raw water. A summary of recently published surveys of raw and finished water for the occurrence of *Cryptosporidium* is presented in Table 3.

Rose *et al.* (1986) tested a variety of surface waters and detected oocyst numbers ranging from 2.1 to 5,800 /L. Ongerth and Stibbs (1987) detected *Cryptosporidium* oocysts in samples from four rivers in Washington and California at concentrations ranging from 2 to 112 oocysts/L (corrected for recovery efficiencies). In a study of 257 water samples from 17 American states Rose *et al.* (1991) detected *Cryptosporidium* oocysts in 55% of surface water samples (0.2 - 4000 oocysts/100L) and 17% of drinking water samples (0.5 - 1.7 oocysts/100L). LeChevallier *et al.*, (1991a and b) found *Cryptosporidium* in 87% of raw water samples at levels between 0.1 - 484 oocysts/L and 27% of filtered water samples at levels between 0.1 - 48 oocysts /100L. The samples were source waters from 66 water treatment plants in 14 US states and 1 Canadian province. Hansen and Ongerth (1991) analysed 35 samples from two rivers. They found oocysts in the range 0.2 - 65 /L. The levels were higher with post rainfall run-off and lowest in the controlled public water supply watershed. Roach *et al.* (1993) found that of the two main towns in the Yukon one had 5% of samples contaminated with *Cryptosporidium*, whilst the other town did not suffer from any contamination.

In the UK patterns of contamination with *Cryptosporidium* have been found to be similar to those in the US. Smith *et al.* (1991) conducted a 12 month survey of *Cryptosporidium* in raw and treated water from Scotland. They found oocysts in 40.5% of raw water samples and 40.1% of treated waters. Gilmour *et al.* (1991) found all sewage effluents, 85% of recreational waters, 46% of raw potable waters and 22% of treated water samples contained cysts in the range 0.1 - 359 /L. In a study undertaken in response to an outbreak of cryptosporidiosis in Britain by Poulton *et al.* (1991) found *Cryptosporidium* in 7.8% of raw water samples with concentrations up to 88 oocysts/L.

Gornik and Exner (1991) detected *Cryptosporidium* oocysts in 78% of water samples from surface waters in western Germany. In 1992, Arvanitidou *et al.* (1992) found

Cryptosporidium oocysts in 45% of lakes sampled in northern Greece. In Kenya, Grimason *et al.* (1993) detected oocysts at levels between 12.5 -73.0 /L in raw waste water and 2.3 - 50 /L in pond effluents. The minimum retention period for the removal of oocysts in waste stabilising ponds was 37.3 days. Newman *et al.* (1993) found 22% of freshwater samples from Brazil to be positive for *Cryptosporidium*.

1.3 *Giardia*

The flagellated protozoan parasites within the genus *Giardia* are classified within the class *Zoomastigophora*, order *Diplomonadida* and family *Hexamitidae*. Members of this genus have been described in the intestinal tract of all classes of vertebrates.

Giardia was probably the first unicellular parasite ever observed, being described in 1681 by the inventor of the light microscope, Leeuwenhoek, while examining his own stools (Bareham *et al.*, 1990). The classification of *Giardia* into species is a contentious issue. More than forty species of *Giardia* have been described on the basis of their host (Thompson and Reynoldson, 1993). Cross transmission studies have shown that host specificity is not absolute (Woo, 1984; Erlandsen *et al.*, 1988). The genus was divided into three morphologically distinct groups, *G. agilis* which infects amphibians, *G. muris* which infects some mammals (mainly rodents) and possibly also some birds and *G. duodenalis* which infects mammals and also possibly some birds and reptiles (Thompson, 1994). These groups are differentiated primarily on the shape of their median bodies, an easily visible internal structure of trophozoites. *G. duodenalis* is the largest group and includes the human pathogen which may be known as *G. duodenalis*, *G. lamblia* or *G. intestinalis* (Schmidt and Roberts, 1981; Thompson *et al.*, 1994). Genetic studies have increased the debate into species names and numbers. For the purposes of this study the human pathogenic species will be referred to as *G. intestinalis*.

1.3.1 Human infection with *Giardia*

Giardia is the most commonly isolated intestinal parasite in the world (Adam, 1991). Giardiasis affects both adults and children. There are a broad range of manifestations in humans from asymptomatic carriers to chronic diarrhoea (Roberts-Thompson, 1985; Adam, 1991). The main symptoms of giardiasis are diarrhoea, abdominal distension, flatulence and malaise (Wolfe, 1984). Other symptoms such as nausea, anorexia, low-grade fever and chills may also be experienced (Farthing, 1994; Wolfe, 1984). Infection may clear spontaneously within six weeks or may continue for many months. Excretion of cysts is characteristically intermittent with as many as 20 days elapsing between positive stools (Flanagan, 1992).

Giardiasis is not always self-limiting and can progress to chronic diarrhoea. Serious weight loss can occur under those circumstances with individuals losing 10-20% of their body weight (Farthing, 1994). Complications such as nutritional insufficiency can occur in both adults and

children (Farthing, 1994; Korman *et al.*, 1990). It can have a profound effect on the growth and development of children. Asymptomatic infections have also been found to occur in both adults and children (Farthing, 1994). It is difficult to establish whether individuals acquired the infection without producing symptoms or whether they had an episode of transient diarrhoea which went unnoticed. Metronidazole (Flagyl) is the most common treatment for giardiasis (Markell and Voge, 1976).

1.3.2 Prevalence of giardiasis in humans

Giardiasis is an infection common in children in the developing world and disadvantaged groups such as Australian aboriginals (Farthing, 1994; Gracey, 1983; Meloni *et al.*, 1988). Studies in endemic areas have found that the parasite can be isolated from the stools of 10-20% of the population (Boreham and Shepherd, 1985). A random survey of the population of Mt. Isa, Queensland, showed 4.5% of people to have cysts in their stools with the highest prevalence rate of 12% in children between one and five years of age (Boreham and Phillips, 1986). In both Western Australia and Southern Australia, *Giardia* is the second most commonly reported enteric pathogen after *Campylobacter* (Anon., 1992; Cameron, 1985). The total number of cases of giardiasis notified in NSW in 1990 was 621 from a population of 4.5 million people, which gives an incidence rate of 14/100 000 per year (NSW Health Department). However, it is likely that the vast majority of cases of giardiasis are not reported.

1.3.3 Transmission of *Giardia*

Giardia is transmitted via the faecal-oral route. Person-to-person contact has been shown to be particularly important in transmission, and is well known in child-care centres (Boreham and Shepherd, 1984; Polis *et al.*, 1986). Foodborne transmission has also been reported, with foods such as strawberries, Christmas pudding and lettuce having been found to harbour the parasite (Boreham, 1987). At least three significant foodborne outbreaks have been reported (Osterholm *et al.*, 1981; Petersen *et al.*, 1988; Boreham *et al.*, 1990). In recent years it has been realised that *Giardia* can be transmitted by sexual activities (Boreham *et al.*, 1990).

Of most concern to this study is waterborne transmission which has been well documented in numerous cases in North America. *Giardia* was the most commonly implicated pathogen in waterborne disease between 1978 and 1990 in the US. The majority of outbreaks have occurred in areas lacking water treatment or using only disinfection (Craun, 1984). The first

successful detection of *Giardia* in water occurred whilst investigating an outbreak of giardiasis in New York, 1975. Approximately 5000 people were infected (Shaw *et al.*, 1977). British Columbia, Canada, has been subject to many waterborne outbreaks of giardiasis in chlorinated, unfiltered source water. Infected beavers have been implicated as a source of contamination in many of these outbreaks (Dykes *et al.*, 1980; Moorhead *et al.*, 1990; Isaac-Renton and Cordeiro, 1993).

The first reported outbreaks in filtered water systems were in Washington (Kirner *et al.*, 1978) and New Hampshire (Lippy, 1978). They were traced to failure of treatment although coliform standards had been met in both cases. A large outbreak occurred in Sweden in 1986 (Ljungstrom and Castor, 1992). The outbreak occurred when patrons at a Swedish ski resort were exposed to contaminated water following a sewage overflow into the drinking water system. Approximately 3000 people were infected.

1.3.4 Significance of *Giardia* in water

Roach *et al.* (1993) found contamination with *Giardia* in 32% of pristine water samples in Northern Canada. In North America, LeChevallier *et al.*, (1991a and b) found *Giardia* in 81% of raw water samples and 17% of filtered water samples. The samples were source waters from 66 water treatment plants in 14 US states and 1 Canadian province.

In a study of 257 water samples from 17 American states *Giardia* cysts were identified in 16% of surface water samples at an average concentration of 3 cysts/100L. Cysts were not detected in any of the 36 drinking water samples analysed (Rose *et al.*, 1991). Hibler and Hancock (1990) report finding *Giardia* in 51% of rivers and creeks and 19% of lakes. They also found *Giardia* cysts in 17% of chlorinated drinking waters, 18.5% of drinking waters treated by direct filtration and 3.4% of drinking water sources with full conventional treatment, from over 4000 samples from North American raw and treated waters.

1.4 Relevance of *Cryptosporidium* and *Giardia* to water quality

There has been a small number of studies relating the occurrence of *Cryptosporidium* and *Giardia* to the indicator organisms and other water quality factors. LeChevallier and Norton (1992) suggest that the number of cysts and oocysts detected in finished water is dependent on the number of organisms in the raw water. They found that the currently measured parameters of water quality were not accurate predictors of *Giardia* and *Cryptosporidium* in watersheds.

1.4.1 Methods for Detecting *Cryptosporidium* and *Giardia* in Water

The first successful detection of *Giardia* in water, reported by Shaw *et al.*, in 1977, involved the filtering of more than 10⁶ litres of water through a swimming pool filter filled with sand. Part of the concentrate was fed to *Giardia*-free puppies which became infected. One cyst was also recognised during microscopic examination (Shaw *et al.*, 1977). The US EPA developed a method from this using field equipment previously used for virus sampling adapted for the concentration of samples (Jakubowski and Erickson, 1979).

Due to the inability to culture the organism, and the apparently low numbers in water which may still be infectious, methods for the detection of *Cryptosporidium* and *Giardia* require the sampling of large volumes of water. This necessitates separate concentration, purification and detection/enumeration steps.

1.4.2 Concentration

The current US EPA reference method (ASTM) uses a yarn-wound filter with a 1 µm nominal porosity to filter a minimum of 100 litres of water for raw water, and often up to 1000L for treated water, depending on water quality (LeChevallier *et al.*, 1991c)). The samples are passed through the filter at a flow rate of approximately 1.5 L per minute. To liberate the filtered material from the filter, backflushing may be employed, or more commonly the fibres are cut and washed in detergent to remove particles. The washings, which may render a total volume of 4L, are concentrated by centrifugation.

There are several alternatives to the reference method. Hansen and Ongerth (1991) have described the use of a 293 mm diameter flat membrane filter to concentrate particles rather than a yarn-wound cartridge filter. This method is suitable for smaller volumes such as 10-20 L. The sample is collected and filtered through a 2 µm polycarbonate filter when back in the laboratory. The filtered material is collected in a plastic tray by scraping the membrane surface with a rubber wiper and washing with a detergent solution. As with the cartridge filtration method the collected material is further concentrated using centrifugation.

The method of Vesey *et al.* (1993a) involves the concentration of samples by flocculation. Calcium carbonate flocculation is a simple procedure suitable for small volumes such as 10-20 L. Sodium bicarbonate and calcium chloride are added to a sample of water and mixed. The pH is then raised to 10 using sodium hydroxide which causes the formation of insoluble calcium carbonate crystals which take particulates from suspension when settling out. The flocculant is left for at least four hours before the supernatant is removed by vacuum. The flocculant is then dissolved in sulphamic acid and the fluid decanted to a 1 L centrifuge pot with washings. Centrifugation is then employed to further concentrate the sample.

There is no "best" method of concentration, as to date there is not recovery data substantial enough to recommend one method over another. The method to be employed is often a function of the sample size and laboratory capabilities. It is likely that efficacy of the methods may vary depending on chemical water quality parameters such as carbonates, etc.

1.4.3 Purification

After concentrating water samples one is left with a variable volume of sample to analyse. For raw water, the volumes obtained from concentration may be in the range of less than 1 ml to more than 5 ml depending on the water quality. It is impractical to attempt to analyse such large volumes by fluorescence microscopy. There needs to be a purification step between concentration and analysis. This has most commonly been a density flotation step using Percoll, Percoll-sucrose or sucrose. Vesey *et al.*, (1991) described an alternate method employing flow cytometry as a purification step.

Density gradient centrifugation involves underlaying samples with a Percoll or sucrose and centrifuging at low speed. In principle the more dense particles sink and the less dense cysts and oocysts are retained on the density cushion and when the interface is collected a more pure suspension of cysts and oocysts is obtained. Recoveries using this method have been found to be quite variable. Thames Water have found recoveries from sucrose flotation vary between 5% to 81% (G. Vesey, personal communication). The turbidity of the water was found to have a significant effect on recovery. In raw water samples there is great potential for cysts and oocysts to adhere to larger particles of more dense material which will result in losses during density gradient centrifugation.

Flow cytometry is the only method for debris removal and enumeration which is substantially different to that of the reference method. A flow cytometer is used to sort fluorescently labelled cysts and oocysts from background material (Vesey *et al.*, 1993 a and b). Particles suspected of being *Cryptosporidium* or *Giardia* are sorted onto a target slide and examined under the microscope. This method, although it retains a microscopy step, is faster and more sensitive than microscopy alone or density gradient purification followed by microscopy (Vesey *et al.*, 1994). Larger volumes can be analysed and some oocysts appear to stain better in solution than when stained on a microscope slide. The disadvantages are the cost of machinery, the training required for the operator and the expense of commercially available monoclonal antibodies, although time and sensitivity savings will usually offset these.

1.4.4 Detection / Enumeration

There are several different commercially available antibodies for *Cryptosporidium* and *Giardia* detection. Generally these antibodies have been developed for the detection of oocysts in faecal samples. They may be monoclonal or polyclonal, and are generally labelled with fluorescein isothiocyanate (FITC). Often they are supplied mixed, and are claimed to recognise *Cryptosporidium parvum* and *Giardia intestinalis*, although it is unclear whether the antibodies actually have the specificity required to recognise these species exclusively. Staining of environmental samples poses many problems not least with non-specific binding to particles such as algae, and other auto-fluorescing particles.

The definitive identification of cysts and oocysts is largely dependent on light microscopic examination. Microscopy requires trained staff and can be very labour intensive. *Giardia* species are quite distinctive, and size and shape in comparison with a type control is generally sufficient to identify *G. intestinalis*. When looking for *Cryptosporidium parvum* oocysts the size and shape are useful but there are often many organisms in environmental samples such as algae and yeasts which can appear like oocysts. Normanski and/or phase contrast microscopy are used to confirm the presence of oocysts.

In this study, flocculation, immunostaining, and flow cytometry methods were employed, with apparently labelled particles identified by epifluorescent microscopy verified by Normanski and phase contrast light microscopy. Flocculation was selected because it is simple, does not require sophisticated apparatus, and can be easily carried out by groups at a distance from the laboratory.

2.0 Survey of Australian Waters for *Cryptosporidium* and *Giardia*.

2.1. Introduction

Post-analysis of reported outbreaks, such as those reported in section 1.2.3, have strongly suggested that the occurrence of protozoan parasites is independent of defined biological and physical indicators of water quality. This has been confirmed by several surveys of surface waters where no statistically significant relationships were found between faecal coliform count, *C. perfringens*, or indeed any other biological or physical water quality parameter, and the occurrence of protozoan parasites. Despite this, and the obvious importance of protozoan parasites to public health, there is currently no defined standards for these parasites in water in Australia. This is largely due to a lack of knowledge of the factors affecting the occurrence of these parasites in raw waters, and the efficiency of treatments to deal with the parasites if present. Also under debate are the methods for detection of these parasites, and the value of risk analysis in establishing standards. It should be noted that these problems are by no means restricted to Australia, and in fact water authorities in Australia are investigating these serious problems in a responsible manner, as evidenced by the support of this project by the Urban Water Research Association of Australia.

The first step in addressing the above problems is the determination of the background levels of parasites in surface waters in Australia. It is also important to determine how Australian data compares to that collected overseas, and to determine if there was any correlation between water quality parameters or catchment conditions which may be able to be used to determine risk. This survey was therefore designed to examine water samples from many sites around Australia in an attempt to gain data from the wide range of water qualities and environmental conditions occurring in this large and diverse country. Emphasis was placed on the major population bases, and an attempt was made to collect samples from a wide geographical range. Most sites were sampled on a monthly basis over a period of one year in an attempt to account for seasonal variations. The survey should provide a baseline for future research on the occurrence of these parasites in Australia.

The School of Biological Sciences at Macquarie University has an ongoing collaboration with AWT-EnSight to investigate and develop effective methods of detection of protozoan parasites in water. This has allowed us to use state-of-the-art detection methods in this survey. Information on the methods has been published elsewhere, and are described only briefly in this report. A quality control study carried out concurrently with the survey provides information on the sensitivity of the assays.

2.1.2 Objectives

The objectives of the project were to analyse monthly water samples from sites around Australia in order to:

- Determine the occurrence of these organisms in surface waters
- Determine the typical levels of protozoan parasites in source waters in major Australian population centres;
- Compare levels of protozoan parasites at sites with different catchment use (farming, recreation, protected);
- Compare levels of protozoan parasites at sites covering a wide range of geographical variation;
- Determine the effect of environmental factors on the occurrence of protozoan parasites
- Examine the relationship between levels of *Cryptosporidium* and *Giardia* and conventional water parameter data (turbidity, faecal coliforms, etc)

2.2 EXPERIMENTAL DESIGN AND METHODS

2.2.1 Sample collection and Analysis

191 water samples from 26 sites around Australia were analysed for *Cryptosporidium* and *Giardia*. Sites were chosen by participating authorities, and largely represented source waters for the water supplies of major population bases around Australia (with the exception of the Sydney area, which was the subject of a separate study - see AWT Report No. ES96/21, Anon. 1996), and samples from the all the major catchment basins around Australia. Generally, 2 sites were chosen by each authority, and these were designated as farming (F) and/or recreation (R), or protected (P), on the basis of catchment usage as provided by the authority. If the samples were from a water supply, the authorities were asked to sample monthly if possible, from the same site as routine sampling was carried out, and to supply other water quality and rainfall data for the samples.

All samples were of 10 litre volume, concentrated by flocculation with CaCl in accordance with previously published methods (Vesey *et al*, 1993). Briefly, the samples were collected into 10 litre plastic containers, to which was added 100ml of 1M Calcium Chloride and 100ml of 1M sodium bicarbonate. Sodium hydroxide (1M) sufficient to raise the pH to 10 was added with stirring. The solution was left undisturbed for at least 4 hours (preferably overnight) for the floc to form, then the supernatant was carefully aspirated. The floc was redissolved by adding 10% sulphamic acid, neutralised and decanted. The container was rinsed with 80% tween 20, and the rinse added to the dissolved floc. Samples were further prepared by centrifugation and filtration before analysis by immunofluorescence (flow cytometry and epi-fluorescence microscopy).

In most cases, samples were collected monthly, although several less frequently sampled sites were included. Samples were concentrated in the laboratories of the participating authorities to a stage where they were stable for transport, either at floc stage before addition of sulphamic acid, or after the redissolved floc had been neutralised and centrifuged. The samples were then shipped by overnight courier to AWT or Macquarie University labs for completion of concentration before analysis. Concentrated samples were stored as pellets for up to 30 days at 4°C before analysis.

Analysis was by immunofluorescence using commercially available monoclonal antibodies (CellLabs, Brookvale, NSW - Crypto 5X Giardia), with a purification step using flow cytometry and sorting, and verification and enumeration by epifluorescent microscopy. Flow cytometry was performed on a Coulter Elite flow cytometer with a 488nm air cooled argon ion laser, a 100µm flow cell and high voltage sort option. For details of analysis of protozoan parasites by flow cytometry see Vesey *et al*, 1994. Epifluorescent microscopy was performed on a Nikon Optophot 2 with phase contrast and Normanski (DIC) optics. Oocysts and cysts were identified from debris on the basis of fluorescence (apple green after labelling), size and shape, and other distinctive features visible under Normanski (suture line for oocysts, internal features for cysts).

2.2.2 Quality control

Recovery range was examined by sending enumerated seeds of 500 *Cryptosporidium* oocysts and 500 *Giardia* cysts to several of the participating authorities. Seeds were added to three 10 litre water samples from each of five authorities, concentrated and transported and analysed in an identical manner to the samples used in the survey. A control seed was sent and returned unused to determine any degradation with time, and an unseeded control water sample was also sent for analysis to determine background levels.

In addition, local samples were tested to determine the effect on analysis of the amount of operator experience during the concentration process, water quality (treated versus untreated), and to determine the stage at which most losses occurred (concentration or analysis).

2.2.3 Statistical Analysis

Statistical analysis to determine correlations of water quality parameters and occurrence of parasites on the entire data set was carried out using the Anova and correlation analysis. Ranking correlation with latitude was tested with the Spearman Rank statistic, and comparisons of quality control factors was carried out with correlation analysis the Students T-test. These tests were carried out with the aid of the Statview statistics package (Abacus Concepts, US). Comparison of parasite occurrence at different types of sites was determined by the Chi Squared test, with equal distribution as the null hypothesis (Sokal and Rolf, 1981).

3.0 RESULTS

3.1 Water survey

3.1.1 Parasite levels

Summary results, as numbers of samples found positive for *Cryptosporidium* oocysts and *Giardia* cysts, are presented in Table 3.

Sample analysis statistics are presented in Table 4. Pellet volumes for the samples ranged from 12ml to <0.1ml, with a mean of 0.75ml. The proportion of the pellet analysed ranged from 5% to 100%, with a mean of 59.9 %. Quality control data (see below) gives average recovery efficiency of 49.2 % (SD = 20%) for *Cryptosporidium*, and 52.7% (SD = 21.3%) for *Giardia*. These figures translate to a minimum sensitivity of 1 cyst or oocyst per 500ml, up maximum of 1 cyst or oocyst per 5 litres, with an average of 1 per 3 litres (see Quality Control section in Results and Discussion for a more detailed analysis of this data).

As the sites had vastly differing ranges of sample conditions which affected analysis and recoveries, it is impossible to place meaningful margins of error for the whole survey. Rather the figures given for occurrence or numbers of cysts or oocysts are real figures extrapolated for 10 litre samples, but not corrected for recovery.

There were 15 out of 26 sites (58%) which had at least one sample positive for *Cryptosporidium* oocysts, with the number of oocysts per positive sample ranging from 1 to 30, with a mean of 12.6. *Giardia* cysts were detected at 11 out of 26 sites (42%), and the number of cysts per positive sample ranged from 1 to 20, with a mean of 4.7.

3.1.2 Catchment type

Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts at sites categorised by major catchment usage (based on information supplied by the collecting authorities) is presented in Table 5. (The 3 treated water samples from the Forth site were excluded from this table). Out of the 25 sites, 10 were categorised as "Protected" (insignificant effects of either recreation or farming). Of the remaining sites, four sites were each categorised as having the main use as either "Farming" or "Recreation", and 11 as the use being both "Farming and Recreation". Statistical analysis of the results using the Chi squared test, assuming equal distribution across all site types as the expected result, shows that non-protected sites (all categories) had a significantly higher chance of having either parasite ($P < 0.01$). In addition, there was a strong trend for farming activity increasing incidence of *Cryptosporidium* oocysts ($P < 0.05$), and recreation activity increasing incidence of *Giardia* cysts ($P < 0.05$). These results were supported by statistical analysis using a logistical model.

There was also a significant increase in the actual number of oocysts per positive sample in sites from catchments with farming activity was evident for *Cryptosporidium* ($P < 0.01$) but there was no such trend for the numbers of *Giardia* cysts (Table 6).

3.1.3 Latitude

Numbers of positive samples from the sites arranged from northern-most to southern-most is shown Table 7. Spearman rank analysis of these data show a significant increase in the number of positive samples depending on increasing latitude. This was supported by analysis using a Poisson model.

3.1.4 Water parameters and Environmental conditions

Statistical analysis (logistical model) of occurrence of oocysts or cysts relative to other water quality parameters collected (faecal coliforms, turbidity, conductivity, pH) showed no relationship at individual sites, and only a weak prediction value for turbidity overall (data not shown). While data for many sites were collected on a monthly basis, the occurrence of drought conditions precluded any analysis of occurrence relative to seasonal or environmental factors (data not shown).

Although water quality data was collected with each of the samples, the low number of positive samples (particularly for *Giardia*), the strong effect of different sites on occurrence of cysts and oocysts, and the variation of water quality between sites made statistical analysis of the whole data set problematical. In addition to the whole data set analysis above, the four sets with the most complete and extensive data sets, and relatively high occurrences of parasites (the two Adelaide sites and the two Brisbane sites) were analysed for the effects of turbidity (NTU), coliform count (/100ml), pH and conductivity. There was a weak correlation between the occurrence of *Cryptosporidium* oocysts and coliform counts at the Mt Crosby site from Brisbane ($P = 0.05$). With this single exception, there was no significant correlation between occurrence of either parasite, and any of the above factors. There was also no significant correlation between the occurrence of *Cryptosporidium* oocysts and *Giardia* cysts.

3.1.5 Other water quality factors affecting analysis

There is a predicted relationship between the proportion of the pellet analysed and the sensitivity of the assay (see discussion below, and figure 1). As expected, there was also a clear correlation between the pellet volume and the proportion of the pellet analysed. In order to determine if the pellet volume could be predicted by water quality (and therefore enable a cost of analysis vs sensitivity evaluation), the data set was analysed in toto and site by site for correlation between turbidity and pellet volume. Surprisingly, with the exception of one site,

there was no correlation between these parameters. The exception was Mt Crosby site in the Brisbane area, where the correlation was very strong ($P < .01$).

3.2 Quality Control

The recoveries for 10 litre water samples collected at 5 different sites by 5 different authorities, spiked with 500 cysts and oocysts, and concentrated on site before being returned for analysis averaged 49.2% overall, and ranged from 9% to 76% for *Cryptosporidium*. Average recovery for *Giardia* was 49.4%, with a range from 0.9% to 86%. The results are summarised in Table 8. Average values for each sites ranged from 20% (Perth) to 64% (Brisbane) for *Cryptosporidium* and from 33% (Perth) to 66% (Darwin) for *Giardia*. Between-sample variance for both parasites was highest in samples from Adelaide and Perth (standard deviation >50% of recovery value).

In an attempt to further determine the most likely cause of variation and recovery loss, seeded samples were used to determine the amount of loss due to the analysis (flow cytometry) step compared to the concentration step, the effect of operator experience, and water quality (treated vs non-treated).

Recoveries from samples which were spiked after the concentration step were 91% ($\pm 4\%$) for oocysts, and 92% ($\pm 18\%$) for cysts. Therefore, most of the losses and variation between samples occurred during sample concentration, rather than analysis. There were no statistically significant differences in recoveries between samples concentrated by operators with high or low levels of experience, although samples concentrated by less experienced operators had higher variance. There was also no significant difference in recovery rates between raw and treated water from the same site. These results suggest that a much of the recovery variation in the study is due to as yet undetermined water quality parameters.

4. DISCUSSION

4.1 Survey

The results of the survey showed that *Cryptosporidium* and *Giardia* common in Australian raw surface waters. 19.1% of samples were positive for *Cryptosporidium*, while 6.4% were positive for *Giardia*. The average number of *Cryptosporidium* oocyst per positive sample was 12.6, and for *Giardia* cysts was 4.7. The highest levels of *Cryptosporidium* oocysts (up to 30 per 10 litres) were found at the Distillery Creek site at Launceston, Tasmania. For 16 sites where five or more samples were taken, only five sites were negative at all sampling times. Cyst levels (*Giardia*) ranged up to 20 at Distillery Creek. Of sites which were sampled five or more times, eight sites were free from cysts for all samples.

On the levels found in this study, parasite loads in Australian source waters, while low, are comparable to those found in other parts of the world. However these levels may still present a risk to public water supplies, especially at times of failure or stress of treatment facilities (Haas and Rose, 1995). These results are also very comparable with a survey of 101 raw water samples from the Sydney region in 1993 - 94 which show occurrences of up to 8%, and ranges of up to ten oocysts per 10 litres (AWT Report No. ES/9621 and Hutton, 1994 - see Table 9 for summary). The low numbers may be reflective of the fact that the survey was carried out in a period of drought in most parts of Australia.

There was a significant effect of catchment use on the occurrence of both parasites. Protected sites had a lower probability of either parasite being present. If the type of use was considered, sites from catchments which were reported as having significant farming use (most prominently cattle grazing) had higher number of samples positive for *Cryptosporidium* oocysts, and higher numbers of oocysts per positive sample. Those from catchments having significant recreation use had higher numbers of samples positive for *Giardia* cysts. Many overseas studies have found correlations with both parasites with wild animals in catchments, although most cases of public health problems have been traced to contamination from either human waste or intensive farming source. The findings of this study indicate that wild animals are not a significant source of contamination by parasites of raw waters in Australia. This supports the findings of a recent survey of the catchment of Warragamba Dam in the Sydney area, where over 200 individual scats of 19 bird, 14 native mammal, 8 feral mammal and 8 fish and reptile species were surveyed to determine their likelihood of being the source of contamination. The study revealed no occurrence of any candidate species for a source of *Cryptosporidium* contamination, although low levels of *Giardia* were detected in waterbird faecal material (AWT report no. ES 96/21).

The present survey also revealed a significant trend towards higher levels of *Cryptosporidium* oocysts in more southerly latitudes. This trend was less evident for *Giardia* cysts. This possibly relates to the lower average water temperature at southern latitudes, which has been shown to have an effect on cysts and oocysts in water. In addition, both oocysts and cysts are known to be intolerant of desiccation (Blewett, 1989) which would be more severe at most of the northerly sites (with the possible exception of Cairns and Darwin).

In addition to confirming findings of similar overseas studies, this survey also supports previous findings that coliforms are of limited use as predictors of the occurrence of either oocysts or cysts. A weak correlation was obtained between oocyst occurrence and coliforms at only one site, although the low incidence of both parasites resulted in a restricted data set for this analysis. More data at sites where occurrence are reasonably high would be needed to determine the value of coliforms (and other organisms such as *Clostridium perfringens*) as indicators of water borne parasites.

It is likely that further research may reveal seasonality in the occurrence of both parasites in water. This may be related to rainfall (as suggested in the results from Adelaide and Brisbane), increased recreation activity during summer holidays, and breeding cycles of farm animals. It is

known for example that greater than 50% of dairy calves at a major veterinary farm near Sydney will suffer high levels of *Cryptosporidium* infection within 10 days of birth, before self-curing to undetectable levels (G. Winter, A. Champion and B. Faulkner, unpublished data).

4.2 Quality control and sensitivity

4.2.1 Recovery levels

The quality control study carried out as part of this project has shown the flocculation concentration method and assay by flow cytometry to be a reliable and effective technique applicable over a wide range of water types and laboratory conditions. A study carried out concurrently with this survey comparing flow cytometry with gradient centrifugation of matched flocculated samples from Ballarat revealed that flow cytometry had a higher sensitivity, with six samples being found positive for oocysts and, and one samples found positive for both oocysts and cysts, all of which were found negative by gradient centrifugation (Faulkner *et. al.*, 1997).

Single site recovery values in experiments with controlled collection and concentration conditions for *Cryptosporidium* oocysts averaged 64.1% and 62.7% for filtered and raw waters respectively, and 78.3 and 61.3% respectively for *Giardia* cysts. These values far exceed most published recoveries using alternative methods. For example, a recent similar study carried out in the USA using the ASTM method with strictly controlled materials specified reported recoveries averaging below 20% for *Cryptosporidium* oocysts, and 50% for *Giardia* cysts, and concluded that the latter level of recovery was acceptable (Newman, 1995). Previous studies in the USA using this method averaged recoveries of 2.8% (Clancy *et. al.*, 1994), and a method comparison study of using Sydney raw water gave recoveries below 1% for the ASTM method (AWT Report No. ES 96/21). By far the largest losses in recovery in the present study was from the concentration step, with recoveries from samples spiked post-concentration averaging 91%.

4.2.2 Variability of Recovery Data

Recoveries from experiments where collection and concentration were carried out in different laboratories had a high amount of variability, although average recoveries still compared very favourably with published results of similar experiments. The source of the variation in these data remains uncertain, although it does not appear to relate to operator experience, nor is it inherent in the method, as is shown by the single site results.

4.2.3 Sensitivity

It is clear that the main factor affecting sensitivity is the proportion of the sample which could be cost effectively analysed, given the high cost of the antibody reagents and flow cytometer and microscopy operator time. The variation in pellet volume (and therefore sensitivity) from sample to sample, even in samples from the same site, was very high in some cases. While it is intuitive that there may be a correlation between turbidity and pellet size, this surprisingly did not appear to be the case in this study. It is clear that other factors are important in determining this very important quality. It would obviously be of great value to be able to predict the level of sensitivity which might be reached for a given water sample prior to concentration. It may even prove to be the case that alternative concentration methods (either different flocculation protocols or methods such as filtration) may be more appropriate for some samples. We are unaware of any previous attempt to correlate turbidity or other water quality parameters with pellet volumes, (either using filtration or flocculation), such as was the case in this study. Previous overseas studies have shown analysis of protozoan parasites in water samples to be problematical. The method employed in this study compares very favourably, especially for *Cryptosporidium* oocysts, despite having apparently high variation in recovery. The results show the method to be robust and more effective and reliable than alternative methods for a range of water types.

5. RECOMMENDATIONS

From the findings of this report it is possible to make several recommendations regarding the public health aspects of protozoan parasites in Australian waters, and for continuing research in the area:

5.1 Public Health

It is clear from this survey that both *Cryptosporidium* and *Giardia* are present in Australian raw waters, and may cause a health risk, particularly in cooler areas, and where drinking water catchments are exposed to human activities. It is recommended that water authorities are aware of the potential hazard, and develop policies to respond to the presence of oocysts and cysts in source waters. Given the lack of correlation between protozoan parasite occurrence and any other water quality parameter, it would be desirable that water authorities in these areas routinely test source waters to gain a better understanding of the factors which govern levels of contamination. These authorities should be especially cautious when it is known that treatment plants may be under stress, such as after heavy rain, or as a result of equipment failure, as it is at these times that significant outbreaks have occurred overseas.

While there is still no standard or best method for the detection of protozoan parasites in water, the flocculation and flow cytometry method described in this report has proven to be flexible,

reliable and sensitive in comparison with other published methods, and it is therefore recommended that this method be used for any routine monitoring studies.

The results of this survey also have clear implications for catchment management. It is recommended that any authority which currently manages a supply catchment in which significant human or farm animal activity is carried out undertake a survey of the water to determine the levels of *Cryptosporidium* and *Giardia*. It is also recommended that human activity of all sorts be restricted around water supply catchments, especially in areas determined by this survey to be at high contamination risk (i.e. cooler areas).

5.2 Further Research

Although the findings of this project clearly show correlation between catchment management and risk to raw drinking water sources of significant contamination by protozoan parasites, correlations with other factors are far more preliminary. Further research into the seasonal or other cyclic effects on the occurrence of protozoan parasites in susceptible waterways would be recommended. For example, seasonal effects, effects of animal numbers and age, and high rainfall on the numbers of oocysts in rural catchments, and the effect of high recreation useage in other areas needs to be investigated.

In addition, further investigation into the parameters which may affect the quality of assays for these organisms, and predictors of sensitivity is also recommended.

Summary Findings

While this study is wide ranging, it is not comprehensive enough for any particular catchment to make specific recommendations. However, there are several conclusions that can be drawn, implications of which are important for water authorities in Australia.

- Both parasites are present in Australian surface waters in most areas.
- Protection of catchments from farming and recreation activities reduces the levels of contamination by protozoan parasites.
- Catchments in southern states appear to be at higher risk of high numbers of protozoan parasites in source waters.
- The method used in this study was found to be robust and effective.
- Recoveries of protozoan parasites varied between different sites, and was apparently due to undetermined water quality parameters.

Acknowledgments

The authors would like to acknowledge the help and support of the relevant lab and administrative staff members of the participating authorities, without whom such a wide-ranging study would not have been possible. We are indebted to Dr Graham Vesey for generous help and advice throughout the project. We also acknowledge the help and cooperation of the Mr Greg Rossington for endless help and technical assistance, Mr Steve Le Moenic for help with sample preparation and analysis, Dr Julian Leslie and Ms Pam Shaw from Macquarie University School of Maths and Statistics, for assistance with statistical analyses, and numerous helpful and cooperative technical staff at AWT and Macquarie University.

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Table 1: Potable-waterborne outbreaks of cryptosporidiosis
(Adapted from Vesey, 1996)

Date	Location	Number of infections ¹	Likely source of contamination	Oocysts per 100 litres ²	Reference
1983	Cobham, Surrey, UK	16	ND	ND	Barer and Wright, 1990
1984	Bruan Station, Texas, USA	(4,200)	sewage contaminated well	ND	D'Antonio <i>et al.</i> , 1985
1985	Cobham, Surrey, UK	50	ND	ND	Barer and Wright, 1990
1986	Sheffield, UK	997 ³	run off from cattle farm	ND	Rush <i>et al.</i> , 1990
1987	Carrollton, Georgia, USA	(13,000)	run off from cattle farm and sewage	ND	Hayes <i>et al.</i> , 1989
1988	Ayrshire, UK	27	post treatment sewage contamination	0.048	Smith <i>et al.</i> , 1989
1989	Swindon, UK	516 (100,000)	run off from cattle farm	0.2-2400	Richardson <i>et al.</i> , 1991
1989	Humberside, UK	ND	ND	ND	Aston <i>et al.</i> , 1991
1990	Lock Lomond, UK	147	ND	ND	Barer and Wright, 1990
1991	Pennsylvania, USA	551	Treatment plant deficiencies	ND	Moore <i>et al.</i> , 1993
1991	Isle of Thanet	47	Treatment plant deficiencies	0	Joseph <i>et al.</i> , 1991
1991	London, UK	44	ND	ND	Maguire <i>et al.</i> , 1995
1991	Melbourne, Australia	81	Sewage contamination of a private supply	900-5,200	Lester, 1992; Vesey, 1996
1992	Jackson County, Oregon, USA	(15,000)	Treatment plant deficiencies		Leland <i>et al.</i> , 1993
1992	Bradford, UK	125	ND	0	Atherton <i>et al.</i> , 1995
1993	Milwaukee, USA	(403,000)	run off from cattle farm	13.2 ⁴	Mackenzie <i>et al.</i> , 1994
1993	England	64	ND	ND	Morgan <i>et al.</i> , 1995
1993	Kitchener-Waterloo, Ontario, Canada	146 (1400)	agricultural run off	ND	Dixon, <i>pers. com.</i>
1995	South Devon, UK	508	NA	NA	Anon 1995
1996	Collingwood, Ontario Canada	39 ¹ 118 ⁵	NA	NA	Anon 1996a <i>Cryptosporidium</i> Capsule 1 (8) 11
1996	Central Florida Day Camp	72	NA	NA	CDC 1996 MMWR 45(21)442-444
1996	Cranbrook British Columbia	29 ¹ , 114 ⁵ (1,700)	agricultural run off associated with snow melt	1.6 million	Anon, 1996b <i>Cryptosporidium</i> Capsule 1 (10) 1-3
1996	Kelowna British Columbia	160 ¹ (14,500)	agricultural run off associated with snow melt	3 - 11	Anon, 1996c <i>Cryptosporidium</i> capsule 1 (11) 1-3

¹The number of confirmed cases. The number in brackets is the estimated total number of cases.

²The number of oocysts detected in the drinking water during or shortly after the outbreak.

³Cases of acute diarrhoea not confirmed as cryptosporidiosis.

⁴Oocysts were detected in ice that had been frozen during the outbreak.

⁵Epidemiologically-linked suspected cases

ND - not determined, NA - not available.

Table 2: Recreational-waterborne outbreaks of Cryptosporidiosis

Date	Location	Number of infections ¹	Attack Rate	Oocysts per 100 litres ²	Reference
1988	Los Angeles, Swimming Pool	44	73%		Sorvillo <i>et al.</i> , 1992
1988	Doncaster, UK Swimming Pool	67			Joce <i>et al.</i> , 1991
1990	British Columbia. Childrens pool		8% - 78%		Bell <i>et al.</i> , 1993
1992	Cotswolds, UK. Swimming Pool	8			Hunt <i>et al.</i> , 1994
1992	Wave Pool, USA, Exact location not provided	17			MacAnulty <i>et al.</i> , 1994.
1993	Hotel Swimming pool USA. Location not provided	51	43%	ND	MacKenzie <i>et al.</i> , 1995
1993	Dane County, Wisconsin USA Swimming Pool				Bongard, <i>et al.</i> , 1994
1994	Lake Water in State Park New Jersey.	66	33%		Kramer, <i>et al.</i> , 1995
1995	Sutherland, Sydney, NSW.	74			Anon, 1995
1995	Kansas. Swimming Pool				Wilberschied 1995
1995	USA, Cobb County Water Park	58 (>3,400)	12% - 26%	2 Crypto 7.6 Giardia	Beach <i>et al.</i> , 1996.

¹The number of confirmed cases. The number in brackets is the estimated total number of cases.

Table 3: Summary of results of surveys of levels of *Cryptosporidium* found in raw and drinking water, 1986 to 1993.

Location	Year	Samples	Oocysts	Comments	Reference
US	1986	surface waters	2.1-5000 oocysts/L		Rose <i>et al.</i> , 1986
US	1987	4 rivers	2-112 oocysts/L	Samples from four rivers in Washington and California.	Ongerth and Stibbs, 1987
US/ Canada	1991	66 treatment plants 14 US states 1 Canadian province	87% raw	Combined immunofluorescence. Higher in source waters, getting industrial & sewage effluents. Significant correlations between Giardia & Crypto densities & raw water quality parameters.	LeChevallier <i>et al.</i> , 1991a
US/ Canada	1991	66 treatment plants (as above)	21% drinking	Compliance with filtration criteria outlined by US EPA Surface Water Treatment Rule doesn't ensure freedom from oocysts. 78% would have met turbidity regulations.	LeChevallier <i>et al.</i> , 1991b
US	1991	35 samples from 4 locations on 2 rivers	34-100% range 0.2-65/L	Highest in early sampling period (spring) with post rainfall runoff. Lowest in controlled public water supply watershed. 10 times higher in adjacent non-controlled. Downstream influenced by dairy farming nearly 10 times higher than upstream. Continuous not intermittent oocysts. Watershed management affected oocysts significantly.	Hansen and Ongerth, 1991
US	1991	257 samples from 17 states	55% surface 43/100L 17% drinking 0.5-100/1.1L	More frequently in waters receiving sewage & agricultural discharges as opposed to pristine. No correlation between crypto and water quality indicators.	Rose <i>et al.</i> , 1991
US	1991			Cryptosporidium or Giardia in 86% of locations surveyed over 16 months in Kansas	Todd <i>et al.</i> , 1991
UK	1991	Raw and treated waters	40.1-40.5%	A 12 month survey. 40.1% of treated waters positive for <i>Cryptosporidium</i> .	Smith <i>et al.</i> , 1991
UK	1991	Sewage effluents raw waters raw potable water treated waters	100% 55% 46% 22%	Range of 0.14-359 oocysts/L	Gilmour <i>et al.</i> , 1991

UK	1991	raw water	7.8%	Range up to 88 oocysts/L	Poulton <i>et al.</i> , 1991
Germany	1991	Surface waters	78%		Gornik and Exner, 1991
North Greece	1992	26 samples 3 rivers (15 samples) 2 lakes (11 samples)	5 lakes +ve 0 rivers	Monoclonal antibody based immunofluorescence assay was used	Arvanitidou <i>et al.</i> , 1992
Brazil	1993	18 freshwater	4 (22%)	Acid-fast and immunofluorescence.	Newman <i>et al.</i> , 1993
Kenya		11 waste stabl. ponds 66 wastewater samples 11 raw waste water 55 pond effluent	6	Ranges Raw waste water 12.5-72.97/L pond effluents 2.25-50/L Giardia: Raw waste water 212.5 - 6212.5 cysts/L Pond effluent 3.15 - 230.7 cysts/L	Grimason <i>et al.</i> , 1993
Spain	1993	8 rivers and lakes 9 drinking (tap)	4 (50%) Range 0.3-3.1/L 3 (44%) Range 0.04-0.2/L	No crypto in final effluent from pond system. Minimum retention period for removal of Crypto sp. was 37.3 days. Highest oocyst conc. found in rivers Giardia also in both sample types, up to 2.1 cysts/L in river, 0.3/L in drinking.	De Leon <i>et al.</i> , 1993
Canada, Yukon	1993	27 Pristine 42 drinking raw & treated sewage	0 2 (5%) Range 0-74/L	Daily monitoring of raw sewage. Occasional oocysts after springtime contaminate drinking water Giardia: 26 - 3,022 cysts/L in raw sewage.	Roach <i>et al.</i> , 1993
US	1993	15 surface water sites sampled 10 times over 1 year (n=147)	30 (20%) Range 0.2-9.8/L	No correlation found between Crypto and Giardia occurrence. Only one site free of both parasites for whole survey period. Both 2-3 times higher in river sources. Giardia found in 23% of samples, range 0.4-6.3/L	Atherholt <i>et al.</i> , 1995.

Table 4: Occurrence of *Cryptosporidium* oocysts (all forms) and *Giardia* cysts at individual sites around Australia.

Site	Use	No.	Crypto oocysts		Giardia cysts	
			No. +ve	Max	No. +ve	Max
Katherine Donkey Camp	(F,R)	12	0	-	0	-
Darwin Darwin R Dam	(P)	14	1	4	0	-
Manton Dam	(R)	13	0	-	1	4
Cairns Barron River	(R)	10	0	-	1	1
Lake Morris	(P)	10	0	-	0	-
Brisbane Mt Crosby Weir	(F)	13	6	10	0	-
North Pine	(F,R)	13	3	4	1	2
Melbourne Tarago Reservoir	(R,F)	4	0	-	0	-
Yarra Valley	(P)	4	2	2	0	-
Ballarat Giles Creek	(F)	7	2	2	0	-
Devil's Creek	(F)	9	4	2	1	1
West Moorabool River	(P)	1	0	-	1	5
Blue Eye Creek	(P)	2	0	-	0	-
Adelaide First Creek	(F,R)	16	5	10	1	1
Houlgrave Res. Onkaparinga R.	(F)	16	2	1	1	1
Perth Lower Helena Reservoir	(R)	6	1	1	1	1
Bickley Dam	(R)	5	0	-	0	-
Canning Dam	(P)	10	2	1	0	-
Harris Dam	(P)	1	0	-	0	-
Karatha Harding Dam	(P)	6	1	1	0	-
Hobart National Park	(P)	2	1	10	0	-
Bryn Estyn	(P)	2	0	-	0	-
North West Forth River	(R,F)	2	2	10	2	4
Forth River (Treated)	-	3	0	-	0	-
Launceston Rocky Creek	(F,R)	5	2	30	1	10
Distillery Creek	(F,R)	5	2	30	3	20

Table 5 : Sample analysis statistics for each of the sites sampled for protozoan parasites.

SITE	No. Samples	Average Pellet Volume (ml)	Pellet Volume Range (ml)	Average Percent Analysed	Percent Analysed Range
Donkey Camp	12	0.74	0.1 - 2.5	71.7%	20 - 100
Darwin R. Dam	14	0.69	0.2 - 1.5	41.4%	20 - 100
Manton Dam	13	0.75	0.2 - 2.5	42.3%	20 - 100
Barron R.	10	0.29	0.1 - 1.0	62.5%	50 - 100
Lake Morris	10	0.29	0.1 - 0.25	81.3%	50 - 100
Mt Crosby Weir	13	0.57	0.1 - 1.0	44.6%	20 - 100
North Pine	13	0.49	0.25 - 0.5	51.2%	20 - 100
Tarago Res.	4	0.79	0.1 - 1.0	37.5%	20 - 100
Yarra Valley	4	0.60	0.2 - 1.0	60.0%	20 - 100
Giles Ck	7	-	-	67.1%	20 - 100
Devil's Ck	9	-	-	74.4%	20 - 100
W Moorabool R.	1	-	-	100%	-
Blue Eye Ck.	2	-	-	30%	10 - 50
First Ck.	16	0.68	0.1 - 2.0	66.3%	10 - 100
Onkaparinga R.	16	2.30	0.4 - 12.0	28.0%	5 - 100
L. Helena Res.	6	0.95	0.1 - 2.0	58.3%	10 - 100
Bickley D.	5	1.0	0.4 - 2.0	47.1%	10 - 100
Canning D.	10	0.56	0.2 - 1.0	77.0%	20 - 100
Harris D.	1	1.0	-	50%	-
Harding D.	6	0.72	0.3 - 1.0	83.3%	50 - 100
Hobart N.P.	2	0.3	0.2 - 0.5	83.3%	50 - 100
Bryn E.	2	0.3	0.1 - 0.5	75%	50 - 100
Forth	2	0.1	0.1	100%	-
Forth (Treated)	3	<0.1	-	100%	-
Rocky Ck.	5	0.15	0.1 - 0.25	100%	-
Distillery Ck.	5	0.4	0.1 - 1.0	75%	25 - 100

Table 6: Numbers of samples from catchments classified as Protected (P), and influenced mainly by Farming (F) or Recreational (R) uses, or both (R,F), found to be +ve or -ve for *Cryptosporidium* or *Giardia*.

Type of Site	Sites	Samples	Cr.+ve	Cr.-ve	Ga. +ve	Ga. -ve
P	10	52	7	45	1	51
R only	4	34	1	33	3	31
F only	4	45	13	32	2	43
Total R	11	91	16	75	9	82
Total F	11	102	28	74	8	94
R/F (incl)	15	136	29	107	11	125
Total	25	188	36	152	12	176

Table 7: Numbers of *Cryptosporidium* oocysts and *Giardia* cysts per positive sample for P, R and F sites, and for both disturbed types combined (R/F).

Type of Site	Crypto +ve	Total Oocysts	No./+vS ample	Giardia +ve	Total Cysts	No./+ve Sample
P	7	19	2.7	1	5	5
Total R	16	133	8.3	9	45	5
Total F	28	170	6.1	8	41	5.1
R/F (incl)	29	171	5.9	11	44	4.2
Total	36	190	5.2	12	52	4.3

Table 8: Sampling areas ranked by latitude, and proportion of samples found positive for *Cryptosporidium* oocysts (Cr +ve) and *Giardia* cysts (Ga +ve).

SITE	Latitude	Cr +ve	Ga +ve
Darwin	12° 24'	0.04	0.04
Katherine	14° 27'	0	0
Cairns	16° 55'	0	0.05
Karatha	20° 44'	0.17	0
Brisbane	27° 28'	0.35	0.05
Perth	31° 56'	0.14	0.05
Adelaide	34° 55'	0.22	0.06
Ballarat	37° 35'	0.32	0.11
Melbourne	37° 55'	0.25	0
Forth	41° 12'	1.0	1.0
Launceston	41° 27'	0.4	0.4
Hobart	42° 53'	0.17	0

Table 9: Recovery of *Cryptosporidium* oocysts and *Giardia* cysts (as %) from 10L water samples spiked with 500 cysts or oocysts after collection, and concentrated by the designated laboratory. (Each value is the mean of 3 identical experiments. An unspiked sample was also concentrated and analysed, and the results are corrected for background levels).

Laboratory	% Recovery <i>Cryptosporidium</i>		<i>Giardia</i>	
	Mean	(S.D.)	Mean	(S.D.)
Adelaide	54	(27)	41	(41)
Perth	20	(12)	33	(22)
Darwin	46	(4.4)	66	(7.6)
Brisbane	64	(5.2)	57	(5.1)
Hobart	62	(4.8)	50	(8.8)
TOTAL	49.2	(17.8)	49.4	(13.0)

Table 10: Results of analysis of 10 litre samples from 4 Sydney sites. (Data from AWT Report No. ES 96/21, used with kind permission of Australian Water Technologies,).

Site	Use	No.	<i>Crypto</i> oocysts		<i>Giardia</i> cysts	
			No. +ve	Range	No. +ve	Range
Coxs River	(R,F)	25	2	<1-10	0	<1
Kedumba River	(R,F)	24	0	<1	0	<1
Werribi Creek	(R,F)	29	1	<1-5	1	<1-1
Bend Creek	(P)	23	1	<1-1	0	<1
Total		101	4	<1-10	1	0-1

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UWRAA RESEARCH REPORTS

Report Number	Title	Author	Report Number	Title	Author
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UWRAA RESEARCH REPORTS

Report Number	Title	Author	Report Number	Title	Author
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UWRAA RESEARCH REPORTS

Report Number	Title	Author	Report Number	Title	Author
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UWRAA RESEARCH REPORTS

Report Number	Title	Author	Report Number	Title	Author
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