

SUMMARY OF RESEARCH DOCUMENTS ON *CRYPTOSPORIDIUM* AND *GIARDIA* ABSTRACTED BETWEEN 1995 AND 1997

The following is a summary of research papers on *Cryptosporidium* and *Giardia* which have been published between the beginning of 1995 and the present date. The papers have been summarised in broad subject areas and the full references are given after the text. Part of the summary is taken from abstracts prepared for a conference in California and in some cases, data was not presented in the abstract.

A portion of the work is centered around water treatment, in particular the optimisation of existing coagulation and flocculation practices. Novel treatments such as ultra-filtration and nano-filtration also appear. Disinfection studies have looked at combinations of disinfectants to obtain synergistic effects and the application of new technologies such as mixed oxidant species and ultra-violet light (UV). A variety of viability assays are available from mouse infectivity, tissue culture, excystation and dye exclusion. Of all of these perhaps tissue culture may be a valuable technique for the future. A number of outbreaks have been documented and a proportion of these involve swimming pools. Distributions of cysts and oocysts in surface waters, groundwaters, sewage effluents and slurries have been studied and in particular efforts have been made to collect data other than through routine sampling programmes. It is not always possible to know when oocysts are present in high numbers but efforts should be made to collect data when environmental conditions suggest raw water quality deterioration. In addition a clear understanding of the likely input of parasites to a raw water catchment is helpful.

Methods for detection continue to improve. Many authors highlight the need to understand recovery efficiencies and the factors that can lead to poor recoveries. Perhaps collation of this data might be useful as a 'How to improve your analysis' guide. Emphasis for the most part is on simplicity with reproducibility and the need for quality control. There are many variables in any detection method but it is more important that a laboratory knows what its variables are rather than the method it chooses. Better concentration techniques, the use of immunomagnetic separation (IMS) and flow cytometry have all helped improve detection and reduce analytical time. Determination of viability by tissue culture is an interesting development. The viability of a single oocyst can be determined from replication of sporozoites. Detection may be by means of enzyme-linked immunosorbent assay (ELISA) or using molecular techniques with viability assay times ranging from 24-48 hours. Molecular techniques are based on either DNA or RNA amplification or fluorescent *in-situ* hybridisation (FISH). These techniques also demonstrate potential viability and infectivity. The Standing Committee of Analysts and the United States Environment Protection Agency both have draft revised methods for the analysis of water samples. These are being completed at this time and should be available in the near future. The rate of progress with development will probably mean that they will be out of date before too long.

Studies of survival in the environment continue to demonstrate that whilst *Giardia* is a poor survivor, *Cryptosporidium* can survive well although aged oocysts are more susceptible to environmental factors and the effects of disinfectants.

1. SAMPLING

Walker, *et al.*, (1997) have described a sampling device, operating under a gravity head for sampling streams during periods of moderated sediment-generated turbidity.

2. DETECTION

Shepherd and Wyn-Jones, (1996) compared membrane filtration (142mm) with flocculation and cartridge filtration for recovering *Giardia* and *Cryptosporidium* from tap and river water. Flocculation gave the best recoveries of both parasites from both water types. A 1.2µm cellulose acetate membrane was found to be best for *Cryptosporidium* and a 3.0µm cellulose nitrate membrane best for *Giardia*. Ongerth, (1997) evaluated membrane filtration, cartridge filtration and chemical flocculation for the recovery of *Giardia* and *Cryptosporidium* from seeded river, reservoir and tap water samples. Chemical flocculation and membrane filtration gave comparable results whilst cartridge filtration gave poor recoveries. Investigation of losses during sample processing are discussed by Grimason, *et al.*, (1997). Highest losses occur during filtration with wound yarn cartridges (up to 36% pass through) and floatation (up to 41%). Filter elution and centrifugation also create losses. A coefficient of variance of up to 40% can be observed. Schaub, (1997) has compared two types of membrane filter, a stainless steel cartridge filter, a polysulphone cartridge filter and vortex flow filtration for recovering oocysts from 10 litre volumes of seeded tap water. Jonas *et al.*, (1997) describe the use of vortex flow filtration which can achieve >90% recovery together with IMS which can give similar recoveries as a rapid and reliable detection system for *Giardia* and *Cryptosporidium* in water. Vortex filtration, nucleopore filters (ongoing) and Envirocheck filters (Gelman) were examined by Matheson, *et al.*, (1997) as alternatives to flocculation and flat bed membrane filtration. Recoveries for each of the membranes was >50% for both parasites and gave significantly less variation than wound fibre cartridges. Clancey, *et al.*, (1997) describe trials in the USA and the UK using filtration with either vortex flow, Gelman Envirocheck, capsule filters or Costar 5 inch filters. The filters gave >70-96% recovery. Samples were further concentrated by IMS and could be stained with monoclonal antibody and DAPI/PI. Parton, *et al.*, (1997) describe an open cell reticulated foam filter which can concentrate oocysts from water with an efficiency of >95%. Samples are recovered in 5-10 minutes in volumes of 250ml and the process requires minimum equipment and operator skill. Johnson, *et al.*, (1995) compared two methods for concentrating parasites from 300 litre seeded samples of marine waters. The filterite negatively-charged filter was found to be simpler and faster with reduced interference from algae than the wound polypropylene cartridge.

New approaches to detection are described by Whitmore (1997), including hydrocyclones, magnetically stabilised fluidised beds, dynamic membranes and flow cytometry using two colour sorting. Graczyk, *et al.*, (1997a) outline the importance of using an eluting fluid for sample containers for residual oocysts.

Trials showed a mean of 34.7% retention in sample containers and recoveries increased to nearly 78% from 44.1% without elution. They also demonstrated (Graczyk, *et al.*, 1997b) that dissolution of cellulose acetate membranes with acetone and parasite concentration using ethanol does not affect stain intensity with monoclonal antibodies or infectivity in BALB/c mice.

Campbell and Smith, (1997) describe an inter-laboratory trial using IMS for the concentration of *Cryptosporidium* from seeded water samples. With low turbidity waters, IMS was found to be better than microscopy and flow cytometry. With increased turbidity, the efficiency of IMS diminished. Campbell, (1997) describes an improved immunomagnetic procedure for concentrating *Cryptosporidium* from high turbidity samples. Selective concentration of oocysts was achieved from waters with turbidities ranging from 60-6,000 NTU and for a wide range of different water types.

In a recent study comparing counting methods, Klonicki, *et al.* (1997) found that there were significant variations between haemocytometer, cellulose acetate and well slide counting. Recovery after cleaning with percoll/sucrose also varied. Hoffmann, *et al.*, (1997) discusses the use of flow cytometry compared with direct microscopy for the detection of *Giardia* and *Cryptosporidium* in water samples. Flow cytometry was found to take less time, cost less, and could analyse a greater volume of sample. An increase in sensitivity of almost three times was observed for both parasites. A review of flow cytometry has been published by Deere, *et al.*, (1996). Medema, (1997a) describes the detection of *Cryptosporidium* and *Giardia* in river and reservoir water using flow cytometry. Viability was demonstrated by DAPI/PI staining before sorting and could easily be assessed. A number of oocysts were observed to be DAPI and PI negative but internal contents could not be resolved. Deere *et al.*, (1997) discuss the use of two antibodies specific for *Cryptosporidium* labelled with different coloured fluorochromes to minimise non-specific sorting in flow cytometry together with a specific DNA probe to identify at genus or sub-species level and demonstrate viability. Vesey, *et al.*, (1997) describe the development of an IgG₁ monoclonal antibody which is less sticky and reduces the amount of non-specific binding. This helps microscopy and sorting with a flow cytometer. The authors also provide some guidance on how to evaluate antibodies and improve existing reagents.

Rodgers, *et al.*, (1995) tested 54 algal species and found that 24 showed some fluorescence. Two species showed bright green fluorescence. Blocking with goat serum was found to be successful. Dowd and Pillai, (1997) describe the use of propidium iodide with immunofluorescence to detect oocysts and determine viability. Three different inactivation methods were used in the study.

Clancy, (1997a) reports that in studies, laboratories produce highly variable data and in some trials cannot detect seeds as high as 4,000 oocysts and may therefore not detect oocysts in an outbreak. Health officials may misuse data particularly when advising AIDS patients about risk. Smith and Fricker, (1997) discuss the variabilities that current analytical techniques have. They provide

data on a range of analytical techniques from filtration to drying on a slide which can enable analysts to optimise some of their methodology and improve recoveries. Fricker, *et al.*, (1997) discuss some factors which can produce variations in assessing the recovery efficiency of various analytical methods. Spikes should be counted in replicates of 10 and the whole recovered pellet examined. The source, age and condition of oocysts will have an effect as will the time that the oocysts have been in water. Where laboratories are comparing methods they should use the same type of water. Veal *et al.*, (1997) describe rigorous quality control procedures for *Cryptosporidium* and *Giardia* analysis where one sample in ten is seeded for recovery. The information allows optimisation of different stages of analysis, comparison of recoveries from different water types and modification of results to present values corrected for recovery.

Sartory, (1997) was unable to find any relationship between sulphite reducing clostridia, enterococci and the presence of *Cryptosporidium* in surface and groundwater in samples taken for routine analysis over a two year period.

3. MOLECULAR TECHNIQUES

Mayer and Palmer, (1996) used immunofluorescence, PCR and nested PCR to investigate the removal of *Giardia* and *Cryptosporidium* from wastewater. Immunofluorescence demonstrated a 3-log reduction for *Giardia* and a 2- log reduction for *Cryptosporidium*. PCR gave 100% correlation for *Giardia* but slightly less for *Cryptosporidium*. Stinear *et al.*, (1996) describe an RT-PCR technique capable of detecting a single oocyst in reticulated, reservoir, borehole and river water. No product was obtained in oocysts fixed with formalin. *Cryptosporidium parvum* was grown in Caco-2 cells by Rochelle, *et al.*, (1997). PCR was used to detect the heat shock protein 70. A single infectious oocyst could be detected using this procedure. Additional data is given on *in-vitro* infectivity with *in-situ* PCR and probe hybridisation for the specific quantification of infectious *Cryptosporidium parvum* in water (Rochelle, 1997).

Battigelli (1997) describes an integrated ELISA/RT-PCR tissue culture procedure using human adenocarcinoma (HCT-8) cells. ELISA could assess viability down to 20 oocysts and RT-PCR could reduce this to one oocyst. A simple membrane filter procedure is described by Wiedenmann *et al.*, (1997) whereby filtered oocysts are stained and counted, the membrane dissolved in acetone and excystation of sporozoites is followed by PCR. The PCR can currently detect 10-20 viable oocysts but sensitivity is expected to be lower.

Chappell, *et al.*, (1997a) describe a study to assess whether DNA sequence variations were related to infectivity *in-vivo*. The Harley Moon strain was compared with the TAMU isolate and current data suggests that genetic variability revealed by random amplified polymorphic DNA does not correlate with differences in oocyst infectivity in mice and humans.

Vesey, *et al.*, (1997) used fluorescence *in situ* hybridisation (FISH) with a Cyl probe targeted to 18S ribosomal RNA to produce sporozoite fluorescence.

The technique was shown to correlate with excystation, be *parvum* specific and could be combined with immunofluorescence for detection. A rapid method for the extraction, amplification and detection of *Cryptosporidium parvum* DNA is described by Wick, (1997). Strand displacement amplification uses biotin labeled primers and the amplicon is captured on microtitre plates using complimentary oligonucleotides. Detection is with horseradish-peroxidase streptavidin. Calomiris, (1997) describes the use of compound D7 to enhance the recovery of oocysts from seeded turbid waters. As well as improving percoll/sucrose floatation recovery by 2-6 fold, compound D7 improves the sensitivity of DNA based detection by preventing inhibition. Chung, *et al.*, (1997) describe an improved method using 293mm, 8.0µm pore size cellulose acetate membrane filtration followed by solution of the membrane in acetone, DNA extraction and amplification. The overall sensitivity of the method was 100 oocysts per 100 litres. Smith *et al.*, (1997) used the combination of IMS to remove oocysts from inhibitory water concentrates with PCR to detect low levels (0.003-0.015/l) in raw waters.

Deere, *et al.*, (1997) describe a fluorescent *in-situ* hybridisation (FISH) technique, requiring only 1 hour for labelling. The technique is species specific and can demonstrate viability by targeting 18S ribosomal RNA.

4. **VIABILITY**

Fricker, *et al.*, (1997) describe an AWWARF and UK DWI joint funded project to assess methods for the determination of viability using CD-1 mice, excystation and vital dyes during disinfection studies. The project is being undertaken in the USA and UK during summer and autumn 1997. Smith, *et al.*, (1997) investigated the criteria for using neonatal CD-1 mice for infectivity studies. Variability of response to infection was demonstrated and parameters such as variation in infective dose, choice of the method for mouse analysis and stress could all influence the results obtained. Slifko, *et al.*, (1997) describe a foci detection method (FDM), using slide cultures of human ileocaecal adenocarcinoma (HCT-8) cells. Infection was determined by immunofluorescence. As few as 10 oocysts have been observed to set up infections. Jenkins *et al.*, (1997) in comparing dye permeability using DAPI/PI with excystation and mouse infectivity, demonstrated that dye permeability assay as an indicator of potential viability and infectivity was a useful tool. Belsoevic, *et al.*, (1997) describe a procedure for determining viability using SYTO-9, hexidium and MPR7 1059. Viability related to animal infectivity but not excystation. Staining was not affected by disinfectants. Black *et al.*, (1996) used dye exclusion with DAPI and PI, excystation and mouse infectivity to assess viability after disinfection. Dye exclusion and excystation were found to give comparable results but overestimated viability. Mouse infectivity was considered the most reliable measure. Campbell, (1997) found that following exposure of *Cryptosporidium* oocysts to low levels of ozone, the vital dye assay significantly over-estimated the viability of oocysts. With excystation the released sporozoites were non-motile and misshapen suggesting non-viability. Sporozoite to oocyst ratios were also found to be variable.

5. METHODOLOGY REVIEW

Watanabe (1996) discussed the validity of current test procedures for *Cryptosporidium* and *Giardia* and the newer test methods of flow cytometry, electroration assay, IMS and PCR. Jakubowski *et al.*, (1996) also review methods in a report by the Working Group on Waterborne Cryptosporidiosis. Viability assessment, surrogate indicators, sampling and processing techniques are reviewed. The report also presents relevant information on available antibodies. Smith and Hayes, (1997) review the limit of current isolation techniques, suggest modifications and additions and examine the development of new methods and the assessment of oocyst viability.

6. RAW AND TREATED WATER CONTAMINATION

Ong *et al.*, (1996) studied two adjacent catchments for *Giardia* and *Cryptosporidium*. Headwaters were not contaminated but creeks and water intakes contained *Giardia*, consistent with multiple source contamination. Both *Giardia* and *Cryptosporidium* were found in one catchment coinciding with calving activity. Wallis *et al.*, (1996) found *Giardia* in 73% of raw sewage samples, 21% of raw water samples and 18.2% of treated water samples. The corresponding values for *Cryptosporidium* were 6.1, 4.5 and 3.5%. Karanis and Seitz (1996) analysed raw, treated and backwash water from six treatment plants in Germany. Twenty one percent of drinking water samples were positive for *Giardia* and 36.4% were positive for *Cryptosporidium*. Eighty four percent of backwash waters were positive for *Giardia* and 82% were positive for *Cryptosporidium*. Chauret, *et al.*, (1995) analysed raw waters for the parasites together with a range of indicators. There was some correlation between *Cryptosporidium* and enterococci and *Giardia* and somatic coliphages and algae. These were not general but were catchment specific. There was no correlation between *Cryptosporidium* and *Clostridium perfringens*. LeChevallier and Norton, (1995) provide results of the American Water System's monitoring for surface and potable waters. A prevalence rate of 53.9% for *Giardia* and 60.2% for *Cryptosporidium* in surface waters is reported. The validity of the test procedures are examined and the importance of the Disinfectants/Disinfection By-products Rule not jeopardising microbiological quality is stressed. A South African study of sewage, raw and drinking water by Kfir, *et al.*, (1995) revealed that the average values for *Giardia* in 10 litres of sewage, effluent, surface water and treated water were 130, 120, 30, and 2 respectively. Values for *Cryptosporidium* were approximately one quarter of these.

Hancock, (1997) notes that in the 12 most recent outbreaks of *Cryptosporidium*, 33% were traced to contaminated wells. In addition, in 1993-94, 40% of outbreaks with both parasites were traced to groundwater. Her most recent survey showed that 7 of 74 wells contained *Giardia* (18 cysts/100 litres, average) and 17 contained *Cryptosporidium* (41 oocysts/100 litres, average).

Norton, (1997) increased the frequency of monitoring of a raw water from monthly to weekly/fortnightly to assess whether low frequency monitoring underestimated oocysts levels. This was found to be the case in that more than 50% of the short-term evaluation samples were greater than the monthly samples (up to 14 times).

In a study of waters and effluents in Israel, Zuckerman, *et al.*, (1997) found 12 of 15 stream samples were positive for *Cryptosporidium* (0.04-1.9 oocysts/l) and 8 for *Giardia* (0.05-0.78 cysts/l). Four out of 6 samples of a drinking water reservoir were also positive for *Cryptosporidium* (0.3-1.09 oocysts/l) and 5 were positive for *Giardia* (0.135-16.2 cysts/l). Sewage samples were positive and one sample of cowshed effluent contained 3,630 oocysts /l.

Crockett and Haas, (1997) describe a systematic approach to sources of protozoa in catchments and those conditions which could lead to increases in the concentrations of protozoa in surface waters and challenges to water treatment. In addition, they discuss (Crockett and Haas, 1995) the variations in sample collection and analysis in relation to the collection of data for the Enhanced Surface Water Treatment Rule. The authors note that the consequences of poor monitoring could result in expensive and unnecessary water treatment. States, *et al.*, (1997) detail the monitoring of two rivers in the US for *Giardia* and *Cryptosporidium*. The parasites were detected in more than 50% of river samples and although *Giardia* was not detected in the treated water, small numbers of *Cryptosporidium* were occasionally found and higher numbers were found in the backwash water. LeChevallier, *et al.*, (1997) examined the inlet and outlet of 6 open finished water reservoirs. Results for parasites and indicator bacteria increased through the reservoir. Nearly all the cysts and oocysts detected were either empty or the internal structures were poorly defined. They concluded that the health risks were low.

Bailey, (1997) tested raw and treated waters, effluents and sludge for *Cryptosporidium* in KwaZulu-Natal. Flocculation and immunofluorescence were used for detection. Faecal samples from 2 hospitals were also examined. Raw water data ranged from 0-80 oocysts/l with the highest concentrations in the summer months (rainfall). A wastewater effluent contained up to 150 oocysts/l, a pre-thickened sludge 7.0×10^5 and a post-thickened sludge 0.25×10^4 oocysts /l. Ten percent of patients stools were positive and 35% of children with the highest incidence during summer rainfall.

7. WATER TREATMENT REMOVAL AND MONITORING

Daniel, *et al.*, (1996) describe a risk assessment made in the U.S.A., studies of *Cryptosporidium* and *Giardia* removal at activated sludge and drinking water plants in France, and Japanese research examining the risk of *Echinococcus multilocaris* infection in water. The French study concluded that membrane filtration was needed for completely reliable removal of parasites and the Japanese study concluded that risk of infection was low. Hancock, *et al.*,

(1996) suggest using microscopic particulate analysis (MPA) to assess water treatment plant performance as an alternative to parasite detection. This can be done by centrifugate pellet and particulate count reduction between raw and treated water samples. Powell, (1996) describes a membrane-based filtration for water treatment using 0.2µm membranes. The technique is suitable for surface waters, groundwaters and backwash water, removing colour and suspended solids as well as bacteria and parasites. Bernhardt and Clasen, (1996) discuss the optimisation of water treatment regimes to prevent breakthrough of *Giardia* and *Cryptosporidium* into treated water supplies.

Ongerth and Pecoraro (1995) used laboratory based experiments to look at parasite removal from alum coagulant dosed waters using optimal and sub-optimal doses. Dosed waters were fed to a triple-media filter of anthracite coal, silica sand and garnet sand. Removal of *Giardia* ranged from 2.7 to 3.1 logs and *Cryptosporidium* from 3.05 to 3.6 logs. Halving the coagulant reduced the removal to 1.3 and 1.5 logs respectively. Jacangelo, *et al.*, (1995) investigated microfiltration and ultrafiltration for the removal of *Giardia*, *Cryptosporidium* and MS2 coliphage. Removal was increased by coating membranes with kaolinite. Dissolved air floatation with iron dosing was shown to remove 3.7 logs of *Cryptosporidium* under optimum conditions by Plummer *et al.*, (1995). Turbidity, UV absorption or dissolved organic carbon were suggested as surrogate parameters for analysis. Yates, (1997) used bench-scale and pilot-scale studies to optimise coagulant and polymer doses for particle and turbidity removal. Further pilot-scale work will include dual or tri-filtration media. Aerobic spores will be used as a surrogate for determining optimisation. *Cryptosporidium* seeding and removal will be used once conditions are optimised. Additional testing will include evaluation of pre-oxidant dosing (chlorine or ozone).

Surrogates of particle counting, turbidity and bacillus spores for *Cryptosporidium* were compared by Fox, (1997b). Jar tests and a pilot-scale plant were used to evaluate flocculation and filtration. Spiking with *Bacillus subtilis* spores and *Cryptosporidium* oocysts were also done. Scott, *et al.*, (1997) is studying a full-scale treatment plant to determine the removal of *Cryptosporidium*, aerobic spore formers and particles to optimise treatment processes and provide methods for treatment plant evaluation. Hijnen, *et al.*, (1997) used spores of sulphite reducing clostridia in raw water as a surrogate for parasite removal in a water treatment plant. Floatation, filtration, ozone and GAC gave an approximate 3 log reduction.

Frederiksen, (1997) augmented conventional water treatment with wound fibre nanofiltration with monitoring over a 15 month period. Particle counts, turbidity, spore and plate counts were monitored. Membranes remained intact despite over 50 acid washings. Further studies will include parasite monitoring. Drodz and Schwartzbrod, (1997) used a pilot tangential microfiltration system (0.2µm) for the removal of oocysts added to large volumes of river water. Removal of >4.3 logs was observed in 9 trials. The viability of the oocysts in the filter concentrate was unaltered but washing the membrane with sodium hydroxide and nitric acid significantly reduced viability.

Drury, (1997) discusses the difficulties of enumerating oocysts, the use of laboratory or pilot-plant studies for 'real-life' situations and the failure of surrogates to mimic *Cryptosporidium*. Good water treatment regimes and good liaison between water utilities, the Environment Agency and Environmental Health officials are stressed. Oxenford, *et al.*, (1997) have produced a report based on findings of an AWWARF sponsored research project and other international research to provide water treatment managers with 'bottom line' information on *Cryptosporidium*.

8. DISINFECTION

Fayer *et al.*, (1996) used saturated gaseous atmospheres of ammonia, carbon monoxide, ethylene oxide, formaldehyde and methyl bromide to challenge purified oocysts of *Cryptosporidium parvum* at 21 - 23°C for 24 hours. Oocysts exposed to ammonia, ethylene oxide or methyl bromide were non-infective for BALB/c mice whilst formaldehyde and carbon monoxide exposed oocysts were infective. Quinn, *et al.*, (1996) used dielectrophoresis at two frequencies to demonstrate ozone inactivation of *Cryptosporidium* oocysts. Aqueous chlorine, chlorine dioxide, sodium thiosulphate, chlorite and chlorate were assessed by Liyanage *et al.*, (1997) as disinfectants against *Cryptosporidium parvum* at pH 8.0 and 22°C. Infectivity was assessed using CD-1 mice. Only chlorine dioxide was shown to be effective. Gyurek, *et al.*, (1997) examined chlorine and monochloramine inactivation of *Cryptosporidium parvum* oocysts at pH 6.0 and 8.0 and 22°C. CD-1 mice were used to assess infectivity. Design graphs were produced to aid engineers to establish disinfection requirements for controlling *Cryptosporidium* in drinking water. Venczel, *et al.*, (1997) compare an electrochemically produced oxidant solution (MIOX; LATA Inc.) and free chlorine as disinfectants against *Cryptosporidium* and *Clostridium perfringens* spores at pH 7.0 and 25°C. Doses of 5mg/l were used with contact times of up to 24 hours. The mixed oxidant gave a 3 log inactivation of oocysts and spores in four hours whilst free chlorine had no effect on oocysts and a 1.4 log reduction of *Clostridium perfringens*.

Studies of the relationship between the inactivation of *Cryptosporidium* in natural waters and different combination of different disinfectants are being done by Oppenheimer, (1997). Ozone, chloramine and chlorine are being tested over a wide range of temperatures. Mouse infectivity will be used to assess viability. In bench-scale experiments, Finch, *et al.*, (1997a) found that pre-treatment of oocysts with ozone enhanced the disinfectant activity of free chlorine. A similar effect was found for pre-treatment with free chlorine followed by monochloramine. Viability was assessed by mouse infectivity. Additional data is described in Finch, (1997b). LeChevallier, (1997) proposes to look at *Cryptosporidium* inactivation using using a new system for generating pure chlorine dioxide. Two pH levels (6 and 8), two disinfectant concentrations (0.5 and 1.5mg/l) and two temperatures (10 and 20°C) will be assessed. Viability will be assessed by DAPI/PI staining, excystation and tissue culture. Pilot studies will also examine the levels of disinfection by-products. Miltner, (1997) working with a pilot-scale ozone plant found that *Bacillus*

subtilis spores were the most difficult microorganisms to inactivate followed by indigenous spores >*Cryptosporidium*>*Giardia*>poliovirus. Inactivation of spores was found to be temperature dependent.

Campbell *et al.*, (1995) studied ultraviolet radiation in a novel apparatus. Oocysts were removed by filtration, exposed to ultraviolet, backflushed from the filter and the process repeated. A reduction in viability by a factor of 100 was noted. Clancy, (1997b) examined pulse and medium intensity UV, plasma sparker technology (sonoluminescence), electron beam and pulsed electric field systems as alternative disinfection technologies. Viability was determined by mouse infectivity. Only UV methods were found to inactivate oocysts. In a further study (Clancy, 1997c) using a full scale plant treating 400 gpm, UV gave a 4.1 log reduction in oocysts viability using mouse infectivity. Assays by DAPI/PI and excystation gave only a 2 log reduction.

9. **PREVENTION OF INFECTION**

A Working Group on Waterborne Cryptosporidiosis (WGWC) have produced guidelines on issuing and rescinding boil-water orders (Pontius, 1996). The paper recommends forming a local task force to evaluate factors such as source water quality, treatment effectiveness, distribution system integrity, finished water quality and epidemiological evidence before issuing or removing boil water advice.

10. **RISK ASSESSMENT**

Gale, (1996) discusses the wide range of pathogens which may be in water and which should be modelled. Complications arise around pathogen densities in water and whether organisms are randomly distributed or clumped. The author suggests that emphasis should be shifted from dose-response curves to defining exposures to pathogen doses when making risk assessment models. The major contributing factors for human infection from drinking water are discussed by Teunis, *et al.*, (1997). They conclude that the uncertainty in the estimated removal efficiency of treatment processes is more important than other factors. Le Blanq, (1997) considers that risk assessment for infection from drinking water can be based on seroprevalence or epidemiological data. Her risk assessment results suggest that tap water has a minor role to play for *Giardia* but a potentially significant role for *Cryptosporidium*. Medema, (1997b) assesses all the relevant data necessary to build a risk model. Factors include concentration of the parasite in the raw water, recovery efficiency of the detection method, treatment removal and daily consumption of unboiled water. He concludes that in general risk is low but there are a few instances when risk may be high and here, reliability of water treatment needs to be controlled. Miller, (1997) collected data on risk factors for *Cryptosporidium* infection in New York. Of 475 cases in 1995, >80% had a compromised immune function and 698% were listed in the AIDS registry. The data is not case controlled.

11. OUTBREAKS

Fox and Lytle, (1996) report on the U. S. EPA investigations into the Milwaukee outbreak. Factors contributing to the outbreak are discussed and recommendation for improving the operation of the treatment works are summarised. Roefer *et al.*, (1996) report the investigations into an outbreak of cryptosporidiosis in the HIV-infected population in Las Vegas in 1994. There were no apparent treatment deficiencies or breakdowns. AIDS patients were considered to be at greater risk of infection through drinking tap water as opposed to bottled or filtered water. Solo-Gabriele and Neumeister (1996) review cryptosporidiosis outbreaks in the USA. Most people affected received surface water supplies and all treatment facilities were complying with federal and local regulations. Interestingly, wastewater was implicated as the source of contamination of raw or treated waters for about half the outbreaks. A case-control study of adults with HIV infection revealed that those who drank tap water were four times more likely to have cryptosporidiosis than those who drank bottled water (Goldstein, *et al.*, 1996). Weidenmann *et al.*, (1996) note that so far there have been no recorded outbreaks of *Cryptosporidium* in Germany. The investigation suggested that consumption of raw milk and contact with animals were major sources of infection rather than consumption of contaminated drinking water. Fewtrell and Delahunty (1995) report that between 1987 and 1992, there were 497 laboratory confirmed cases of *Cryptosporidium* in Blackpool, Wyre and Fylde. There was no correlation with water supply but water sport participation and animal contact were risk factors.

Bridgman, *et al.*, (1995) describe an outbreak in north-west England in 1993 giving 47 cases of *Cryptosporidium*. One groundwater source drained water from a field contaminated with animal faeces where there were fissures from mining subsidence. Water analysis was negative but a case-control study showed significant association with drinking unboiled tap water. Maguire, *et al.*, (1995) describe the investigation of an outbreak of 44 cases of *Cryptosporidium* in South London in 1991. Fifteen primary cases were supplied by one water company. There was no association with the amount of tapwater drunk and no water quality problems had been identified by the company. Steiner, *et al.*, (1997) review *Cryptosporidium parvum*, *Giardia*, *Entamoeba histolytica* and *Cyclospora cayetanensis* as causes of waterborne diarrhoeal disease. Fox, (1997a) has researched waterborne outbreaks in the USA and the treatment lapses which allowed oocysts into the drinking water. The data can be used to assist utilities to manage water treatment systems to minimise the risk of outbreaks.

Frost, (1997) plans to study seroprevalence of antibodies against two specific *Cryptosporidium* antigens. Of the two populations selected, one receives water from a heavily contaminated surface source and the other from a deep well source. Five hundred sera will be collected and tested over a five month period. An additional study is reported using surplus sera from NHANES III involving seven cities, Frost, (1997). Statistically significant differences were observed possibly due to geographical variation in endemic levels of infection and possibly a significant contribution from waterborne transmission.

12. INFECTION

Chappel, *et al.*, (1997b) infected volunteers and used the immunological response to understand the host-parasite interaction. A challenge of 29 serologically negative adults with 30-1,000,000 oocysts (Iowa strain) gave 18 with oocysts in faeces and of these 7 had diarrhoeal symptoms. Faecal IgA was positively associated with faecal shedding. Serum response did not correlate with shedding or illness. Over 73% were positive for IgM, 45% for IgA and 21% for IgG. One year on, 19 were re-challenged with 500 oocysts. Only 3 had evidence of oocyst shedding and 7 had diarrhoea. Swabby-Cahill and Cahill, (1997) used C57B1/6 mice for routine passage and stock production of *Cryptosporidium* for investigating minimum infective dose. A full review of the epidemiologic aspects of human cryptosporidiosis has been published by Meinhardt, *et al.*, (1996).

13. SURVIVAL

Heisz, (1997) suspended oocysts in river water in the dark at different temperatures. Oocysts were counted using a counting chamber and viability assessed by excystation. At higher temperatures there was a 2.5 log reduction in viability at 30 days and a 1.2 log reduction at lower temperatures in the same period. Abbeaszadegan, (1997) studied the survival of *Cryptosporidium parvum* and *Giardia muris* in natural waters, sludges and sediments. *Giardia* rapidly becomes undetectable in river water (3 weeks) whereas *Cryptosporidium* numbers were only reduced by 0.6 logs. Aged oocysts and cysts were more susceptible to chlorine than fresh ones. *Giardia* is very susceptible to freezing. Viability was assessed by excystation. Medema, *et al.*, (1997) found that the time required for a 1 log reduction of *Cryptosporidium* in river water was 40 - 160 days at 15°C and 100 days at 5°C. Die-off of *Escherichia coli* and enterococci was faster than *Cryptosporidium* but *Clostridium perfringens* die-off was slower. Johnson, *et al.*, (1997) found that *Giardia* cysts were inactivated by salinity, where the contents hyperplasmolyse, and by light (cysts survive for 72 hours in the dark and 3 hours in the light). Two month old oocysts required 13 - 16 days for 90% reduction whereas 4 month old oocysts required 3-4 days. The order of survival was *Cryptosporidium*>poliovirus>*Giardia*>*Salmonella*.

14. PRIVATE WATER SUPPLIES

Clapham, (1997) examined 15 private water supplies. *Cryptosporidium* was found in 20 (14%) of samples taken and 9 of the 15 supplies (60%) contained oocysts during the survey. *Giardia* was present in 8 of the supplies. Enterococci and sulphite reducing clostridia were significantly correlated to *Cryptosporidium*.

15. SWIMMING POOLS

The recent occurrence of cryptosporidiosis at five public pools led Kebabjian (1995) to make a number of suggestions on the management of faecal

contamination in pools. Closure for up to one day is suggested to permit proper filtration and disinfection of pool water. An increase of *Cryptosporidium* incidence from 0.5-1% to 15-17% was investigated by Medema, (1997c). Drinking water, distribution systems and water treatment operations were satisfactory but a case control study revealed that swimming in pool was the only risk factor. MacKenzie, *et al.*, (1995) detail an outbreak of cryptosporidiosis involving fifty one people at a hotel. Use of the swimming pool was the only significant risk factor. Unrecognised faecal accidents were suggested as the cause.

16. WASTEWATER

Rider *et al.*, (1996) describe a wastewater treatment system which removes 99.9% of *Giardia* cysts and *Cryptosporidium* oocysts. The filter system comprised a dual-stage, deep bed sand filter. Stadterman *et al.*, (1995) found that a laboratory activated sludge plant removed 98.6% of seeded *Cryptosporidium parvum* oocysts. In a comparison of different treatment regimes, activated sludge and anaerobic digestion were found to be the most effective means of removing oocysts, the latter destroying 99.9% in 24 hours. Hirata (1997) looked at raw sewage, primary, secondary and final effluents for *Giardia* and *Cryptosporidium*. *Cryptosporidium* was only found in one sample (28 oocysts/l) whereas *Giardia* was found in all samples (125-4,500 cysts/l). Conventional activated sludge reduced cysts by 2 logs. *Clostridium perfringens* was suggested as a good surrogate. Bukhari, *et al.*, (1997) produced data to show small numbers of oocysts in both the influent and effluent samples from sewage works whereas *Giardia* cysts were detected more frequently and at higher concentrations. Oocysts were only detected at one site in sewage sludge whereas cysts were found at all the sites examined.

Madireddi *et al.*, (1997) constructed a pilot plant to treat a municipal secondary effluent for augmentation of a lake used as a drinking water source. Extensive treatment including ultrafiltration and nanofiltration gave 21-22 log removal of bacteriophages and 8-10 log removal of *Giardia* and *Cryptosporidium*. During a one year study, at a water reclamation facility employing biological treatment, sand filtration and chlorination, Rose, *et al.*, (1996) found that total and faecal coliforms were reduced by >7 logs, coliphages and enteroviruses by >5 logs and *Giardia* and *Cryptosporidium* by >3 logs. The risk of infection by exposure to 100ml of water was calculated as between 16^{-6} and 10^{-8} .

17. FAECAL MATERIAL

Bukhari and Smith, (1997) discuss ways in which agricultural wastes could pollute water. Additional information on survival of oocysts in naturally contaminated materials will be presented. The two will be linked to transmission to hosts by the waterborne route. Bodley-Tickell, *et al.*, (1997) found *Cryptosporidium* in almost 70% of rural surface waters tested. Numbers were found to be higher in Autumn coinciding with calving, slurry spreading and rainfall. Levels range from 0-16.7 oocysts/l with a mean value of

approximately 1.0. The results indicate that wildlife may have a substantial input to small rural waters.

18. **SOIL**

Mawdsley, *et al.*, (1996) dosed soil cores in the laboratory with high numbers (10^8) of *Cryptosporidium* oocysts. Small numbers of oocysts were detected in the leachate from clay loam and silty loam but not from a loamy sand soil. Variation in leaching were observed with replicate cores. The majority of oocysts were found in the top 2cm of soils.

19. **FOOD**

Harp, (1996) demonstrated that oocysts were killed by heating in water and milk to 71.7°C for 5, 10 and 15 seconds. Viability was assessed by infectivity in mice. Water as a vehicle for various foodborne agents is discussed by Palumbo, *et al.*, (1997). An overview of wastewater treatment processes is presented and approaches to reconditioning plant processing water for reuse in food processing is included.

20. **STATISTICAL ANALYSIS**

Nahrstedt and Gimbel, (1996, 1997) and Gimbel and Nahrstedt (1996) describe a statistical method for determining the reliability of analytical results and a strategy for the improvement of analytical methods. Medema, (1997d) used a chemical pollutants model to calculate the concentration of oocysts and cysts in surface waters receiving domestic sewage effluent. Calculated figures were found to be in good agreement with actual measurements although the model underestimates the concentrations in agricultural discharges where concentrations may be significantly higher. Sakaji and Chun (1997) outline an integrated action plan for when oocyst counts in waters are higher than historical data. Particular reference is paid to good communication systems. Stuart, (1997) modelled the effect of body-contact recreation on the concentration of pathogens, including *Cryptosporidium*, at the outlet to a reservoir under construction. The effects of boating (limited activity) and full recreational activities were modelled.

21. **TYPING CRYPTOSPORIDIUM**

McLauchlin, *et al.*, (1997) describe the use of SDS-PAGE Western-blotting analysis to sub-type *Cryptosporidium parvum*. This and a similar system can be used to recognise multiple types of the parasite and antibodies in sera. The technique has been used to investigate waterborne outbreaks. Bonnin, *et al.*, (1996) used PCR with restriction-fragment length polymorphism (RFLP) to type 23 isolates of *Cryptosporidium parvum*. Ten calf isolates were shown to have the same profile but 13 human isolates had two patterns, one identical to the calf isolates but the second different. Spano, *et al.*, (1997) also used PCR with RFLP to distinguish *Cryptosporidium wrayii* from *Cryptosporidium parvum* and were able to distinguish two isolates of *parvum*, one associated

with animal and one with human infections. Carraway, *et al.*, (1996) and Morgan, *et al.*, (1995) were also able to differentiate human and animal groups, the latter using RADP. In addition, Carraway, *et al.*, (1997) were able to show that there was a change in the genetic profile of *Cryptosporidium parvum* following transmission from cattle to humans.

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