Cryptosporidium in Water - Synopsis of Studies within the National Research Programme. January 1990 to March 1994
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INTRODUCTION

The National Cryptosporidium Research Programme was initiated in January 1990. The funding has been provided by the Department of the Environment, through the Drinking Water Inspectorate, the Foundation for Water Research, the National Rivers Authority, the Ministry of Agriculture, Fisheries and Food and the Scottish Office Agriculture and Fisheries Department, a number of individual water companies and UK Water Industry Research Ltd. The programme has been linked together by the National Programme Research Steering Committee and the studies have been carried out in a number of institutions, selected for their specialised knowledge, expertise or facilities.

The Steering Committee has asked WRc to produce a summary of the work to assist it in reviewing future needs and advising the Group of Experts.

In the following pages the 25 major studies have been drawn together under 6 general headings. For each study a synopsis of the objectives and outcome is provided together with details of the funding and the contractor.
PRIMARY ISOLATION

1. Improved initial concentration

Funder: FWR £80K  
Contractor: WRc  
Duration: April 1991 - March 1992  
Nature of study: To investigate techniques that had the potentially for a more efficient initial recovery of oocysts.  
Outcome: Of the techniques examined, which included pleated polypropylene filters, glassfibre filters, membrane filters, sand columns, vortex-flow filters, cross-flow filters and continuous centrifugation, only cross-flow filtration showed a marked improvement over the widely-recommended wound polypropylene filters.

2. Separation of oocysts by magnetic particles and cross-flow filtration

Funder: DoE £30K  
Contractor: Scottish Parasite Diagnostic Laboratory  
Duration: May 1993 - March 1994  
Nature of study: Use of antibody-coated magnetic particles and/or cross-flow filters to concentrate oocysts from water samples.  
Outcome: Use of magnetic particles significantly increases recovery from treated water samples compared with conventional methods. Cross-flow filtration has potential for the concentration of oocysts from potable water, but currently available filter elements are unsuitable for raw waters.

3. Improved recovery of oocysts

Funder: DoE £27K  
Contractor: University of Sunderland  
Duration: May 1993 - May 1994  
Nature of study: To evaluate the use of elution techniques used in virology for the recovery of oocysts from filters and other concentration methods.  
Outcome: Initial results indicate improved recovery compared to conventional techniques. The final report is awaited.
4. Development of rapid method for oocyst separation and identification

Funder: DoE £75K
Contractor: Scottish Parasite Diagnostic Laboratory
Duration: April 1990 - March 1991

Nature of study: Use of electronic imagery to enhance conventional monoclonal antibody enumeration techniques and coupling of light emission chemicals to monoclonal antibodies.

Outcome: The light emission approach was limited by the strength of the available antibodies. Use of a CCD camera showed promise in giving enhanced images of oocysts and sporozoites but further development of software is required to produce a commercial prototype. An estimate of the work required was drawn up but no further action taken.

5. Test for oocyst viability

Funder: DoE £30K
Contractor: Scottish Parasite Diagnostic Laboratory
Duration: April 1990 - March 1991

Nature of study: The development of a relatively simple test for viability of oocysts that did not require large numbers of oocysts or experimental animals.

Outcome: A staining technique that has been widely acclaimed and correlates well with excystment has been developed.

6. Development of a specific test for Cryptosporidium parvum

Funder: DoE £64K
Contractor: Scottish Parasite Diagnostic Laboratory
Duration: April 1991 - March 1993

Nature of study: To develop an enumeration method specific for Cryptosporidium parvum using a monoclonal antibody.

Outcome: It was not found possible to develop a suitable antibody which did not link with other species of Cryptosporidia. The study did identify a sugar which appears to be specific to Cryptosporidium parvum
which could provide a measurement technique based on binding to lectins but this has not been pursued further. A number of other organisations carrying out studies to develop a gene probe were identified.

7. **Review of molecular biology techniques for Cryptosporidium enumeration**

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<th>Funder:</th>
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<td>Contractor:</td>
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**Nature of study:** Critical review of techniques currently used for oocyst enumeration and potential for approaches based on molecular biology.

**Outcome:** Current methods were compared showing advantages and disadvantages. The need for further work was demonstrated to develop a method suitable for environmental samples with low oocyst concentrations. Molecular techniques were shown to have considerable potential particularly PCR, although the risk contamination indicate the need for care. Further work on electrorotation assay and dielectrophoresis was also recommended.
INFECTIVE DOSE

8. Use of disease-free lambs for assessing infective dose

Funder: DoE £15K
Contractors: Moredun Research Institute and Public Health Laboratory Service (Rhyl)
Duration: June 1990 - May 1991
Nature of study: Assessment of minimum infective dose of Cryptosporidium parvum using gnotobiotic lambs fed with milk seeded with oocysts. Infection was defined by the presence of oocysts in faeces.
Outcome: Tests using a range of oocyst numbers indicated that infection could be caused by as little as one oocyst. Disease-free lambs were seen to be a useful test animal for other studies such as treatment and disinfection but this has not been pursued in the national programme.

9. Infective dose using primates

Funder: DoE £20K
Contractors: Scottish Parasite Diagnostic Laboratory and Kenya Trypanosomiasis Research Institute
Duration: June 1990 - March 1993
Nature of study: Establishment of infective doses for Cryptosporidium parvum oocysts by feeding trials using isolated vervet monkeys of different age groups.
Outcome: Infection could not be established in any age group of primates using doses up to $10^5$ oocysts. The results of blood tests for immunity are awaited.
WATER TREATMENT

10. Laboratory scale tests or removal of oocysts in water treatment

Funder: DoE £48K  
Contractor: University College London  
Duration: February 1990 - March 1991

Nature of study: The efficiency of removal of oocysts was examined using jar test equipment and laboratory filter columns.

Outcome: Oocysts were shown to be removed by coagulation and settling as effectively as other similar particles. Direct filtration through rapid gravity sand and granular activated carbon filters both with and without coagulant showed effective removal although efficiency was considerably increased by the presence of a coagulant. Results were extrapolated for full depth filters and indicated removals between 2.3 and 5.7 log, rather higher than subsequent work indicates.

11. Laboratory scale tests of treatment processes

Funder: Foundation for Water Research £68K  
Contractor: WRc  
Duration: April 1991 - March 1992

Nature of study: Measurement of oocyst removal using both coagulation and flotation jar tests. Inactivation studies in jar test ozonation experiments.

Outcome: Removal by coagulation and sedimentation of oocysts seeded in upland and lowland waters ranged mostly between 1.55 log and 3 log with the bulk of the results in the upper end of the range. Flotation of upland water also produced removals between 1.3 and 3 log. Tests using ozone in batch reactors and dynamic contactors indicated rather poor inactivation of seeded oocysts as measured by excystation considering the ozone doses and contact times used.

12. Alternative disinfectants to chlorine

Funder: DoE £190K  
Contractor: WRc  
Duration: April 1989 - March 1991

Nature of study: A bench-scale study to determine the efficiency of a wide range of disinfectants against the oocysts of cryptosporidia.
Outcome: Ozone was the only disinfectant to show potential for further consideration. Some of the others examined such as chlorine dioxide, hydrogen peroxide, ultraviolet irradiation and iodine had effects upon oocyst viability but, in general terms, the levels required were so high that the waters would be rendered unpalatable or unsuitable for some purposes, or they may exceed other guidelines or legislation.

13. Feasibility of the use of ozone

Funder: DoE £11K
Contractor: WRc
Duration: February 1990 - May 1990

Nature of study: This desk-study reviewed available information on ozonation by-products and ozone application.

Outcome: The report concluded that ozone reacts with many organic compounds to form by-products, some of which may have health effects, but the risk did not preclude the use of ozone for the control of cryptosporidia.

14. Cryptosporidium removal in full scale treatment plant

Funder: Foundation for Water Research £66K
Contractor: Scottish Parasite Diagnostic Laboratory and WRc
Duration: February 1989 - March 1991

Nature of study: Opportunistic sampling was carried out on full scale works in Scotland. The types of work included microstraining, coagulation sedimentation and filtration and slow sand filtration. Measurement of oocyst concentrations were made in raw and treated water.

Outcome: Comprehensive studies of removal in treatment were not possible because of the intermittent presence of oocysts in the raw waters studied and the complexities of the analytical procedure. However, the work showed the presence of oocysts in raw and treated waters on all types of plant indicating some penetration. A slow sand filter plant was much closely studied and the results suggested removals of around 92%.
15.  Pilot scale studies of removal/inactivation during treatment

Funder:  Foundation for Water Research £600K
Contractor:  WRc
Duration:  April 1991 - March 1994

Nature of study:  Measurement of the removal and inactivation of live oocysts fed to a pilot plant which included slow sand filtration, coagulation, floc blanket clarification, flotation, sand and activated carbon filtration, ozonation and membrane separation.

Outcome:  The plant was operated for 15 months from November 1992. Supportive work was also carried out on laboratory jar test, filtration and ozonation equipment. The results showed that all chemical treatment schemes achieved better than 2 log removal. With 300 to 800 oocysts per litre in the feed water oocysts were non-detectable in the treated water for 50% of the time and less than 0.1 per litre for 70% of the time. The slow sand filter stream gave similar removal to the chemical treatment stream. Ozonation was shown to be less effective based on the viability dye test than a number of other studies with high CT values for achieving greater than 1 log inactivation.

16.  Pilot scale studies on oocyst removal

Funder:  UK Water Industry Research Ltd £173K
Contractor:  WRc
Duration:  April 1993 - March 1994

Nature of study:  Continuation of the FWR funded studies with special emphasis on effect of flow rates on processes and influence of recycling of settled sludge and backwash supernatent waters.

Outcome:  Currently in progress.

17.  Effect of environmental stress on oocyst viability and behaviour

Funder:  DoE and FWR £103K
Contractor:  WRc
Duration:  September 1993 - March 1994

Nature of study:  Investigation of effect of individual and combined environmental stress factors on survival of seeded Cryptosporidium parvum oocysts and their behaviour during treatment.
Outcome: In the natural environment oocyst numbers and viability decline and the effect is greater in warmer and/or brighter conditions. In the tests on individual factors extremes of temperature affected viability but pH and low level light did not. Stressed oocysts are not removed to a different extent in treatment compared with unstressed oocysts but are slightly more susceptible to chlorine.

18. Effectiveness of point-of-use filters for oocyst removal

Funder: DoE £42K
Contractor: Warren Spring Laboratory
Duration: September 1991 - March 1994

Nature of study: Following a desk study to identify suitable point-of-use filter tests were carried out on three filters using yeast cells as a surrogate for Cryptosporidium oocysts

Outcome: Small pore size or ceramic filters could effect up to 3.5 log removal of yeast cells, but hydraulic shock could release particles. For optimum filter life a prefilter would be required.
19. Survey of lowland rivers and boreholes

Funders: FWR, NRA, 5 Water Companies £320K
Contractors: Water Companies, SPDL, PHLS Rhyl
Duration: January 1990 - March 1991

Nature of study: Three lowland river stretches and six boreholes were sampled three times a week for rivers and weekly for boreholes over a 15 month period and oocysts determined. Quality assurance was provided on 1 in 6 samples by independent laboratories.

Outcome: All rivers were shown to contain oocysts at times but with a maximum concentration of 4 litre. Boreholes were negative apart from a few samples over a short period in three of the boreholes. Quality assurance was effective, although this involved 70% negative results. Of the 30% positive results 80% showed agreement between the water company and the reference laboratories.

20. Epidemiology in areas covered by the occurrence survey

Funder: DoE £77K
Contractor: PHLS Communicable Disease Surveillance Centre
Duration: April 1990 - December 1991

Nature of study: Surveillance was undertaken using the PHLS reporting system and spotter-practices in areas served with water obtained from sources in the lowland rivers and boreholes survey. The intention was to correlate occurrence of oocysts and increased levels of cryptosporidiosis in the communities.

Outcome: Cases of cryptosporidiosis were correlated with a number of factors but no evidence was produced that water was a more likely source of infection than other risk exposures. Absence of high number of oocysts in the raw water limited the follow up work that could be carried out.

21. Occurrence in an upland catchment

Funders: FWR and Central Scotland Water Development Board £95K
Contractors: WRc, SPDL
Duration: April 1991 - March 1993

Nature of study: Survey of Loch Lomond to assess occurrence and origin of Cryptosporidium oocysts and their viability with parallel work on disease in animals and the community.
Outcome: The number of oocysts found were low. Although oocysts were detected in sewage effluent and bovine faecal specimens and some oocysts passed into supply, these could not be correlated with any identifiable outbreak of disease in the community. The majority of oocysts detected at the water abstraction point were non-viable. Quality assurance work showed that results could depend on the detailed method of analysis used.

22. Impact of farming events upon oocyst numbers in surface waters

Funders: DoE and NRA £75K
Contractor: WRc
Duration: February 1993 to July 1994

Nature of study: A river in an area with a high density of cattle has been monitored at seasons when the levels of oocysts were likely to be low and when they were likely to be high. The results will be compared with meteorological conditions, farming events and water quality parameters.

Outcome: The study is still in progress. There is probably a correlation between the presence of oocysts and other indicators of pollution such as BOD, but there is no clear relationship between the presence of oocysts and the particle size distribution within the waters.
SLURRIES AND WASTE WATERS

23. Occurrence and fate of Cryptosporidium oocysts in farm wastes

Funder: MAFF and SOAFD £320K
Contractors: Scottish Agricultural Colleges, SPDL, Moredun Research Institute, Institute of Grassland and Environmental Research
Duration: April 1992 - March 1995
Nature of study: The occurrence and survival of oocysts in slurries and subsequent release to the environment will be studied and effects of treatment Guidelines for safe disposal will be produced.
Outcome: Project is still in progress. Oocysts have been detected in large numbers in young cattle and sheep and a seasonal pattern in cases in animals has been confirmed.

24. Levels of oocysts in market wastes

Funder: FWR £50K
Contractor: WRc
Duration: April 1991 - March 1993
Nature of study: The waste treatment facilities at a large animal market were monitored regularly over 15 months to relate levels of oocyst in the waste with market activities.
Outcome: The study confirmed the potential of the waste from markets and similar situations to contain high levels of oocysts. However, the market managers ceased to cooperate late in the study and it was not possible to correlate oocyst numbers with animal throughput, amount of bedding material, water use or season.

25. Treatment and utilisation of sewage sludge

Funder: DoE £86K
Contractor: WRc and Scottish Parasite Diagnostic Laboratory
Duration: October 1991 - March 1992
Nature of study: A laboratory study to determine the fate of oocysts during primary sewage treatment, to assess their viability following sludge treatment and their survival and viability in sludge treated soils.
Outcome: Primary sedimentation was ineffective at removing oocysts from the liquid phase. Thermophilic aerobic digestion and pasteurisation carried out according to the DoE Code of Practice for Agricultural Use of Sewage Sludge inactivated oocysts. It is possible, that a small proportion of oocysts may survive mesophilic anaerobic digestion and subsequent storage, and remain viable in soils.