Cryptosporidium in Water

Final report to the Department of the Environment
CRYPTOSPORIDIUM IN WATER

Final report to the Department of the Environment
January 1990 to March 1994

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Contract Duration: January 1990 to March 1994

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SUMMARY

After the outbreak of waterborne cryptosporidiosis in Swindon and Oxfordshire in 1989 a Group of Experts, under the Chairmanship of Sir John Badenoch, was established jointly by the Secretary of State for the Environment and the Secretary of State for Health. The Group’s report made a number of recommendations and identified areas where more research was required. As a result of the recommendations a national programme of research was established involving work funded by the industry and government.

This report presents the results of studies carried out between January 1990 and March 1994 on those parts of the National Programme funded by the Department of the Environment. The responsibilities under the contract also required WRc to provide the secretariat to the National Cryptosporidium Research Programme Steering Committee.

In the areas of isolation, identification and enumeration of oocysts the work has confirmed that the established methods are generally imprecise and unreliable, although cross-flow filtration with separation aided by magnetisable particles, shows promise. A literature review identified a number of recently developed technologies that have potential for improved recovery and identification techniques. One major achievement of the studies was the development of a test for viability of oocysts using a dye inclusion/exclusion technique.

The dose required to initiate infection in humans is still unclear with contradictory results arising from the two studies in this programme. The results do, however, support evidence of differences of strain or virulence within the species of Cryptosporidium parvum.

Studies of occurrence indicate that oocysts are frequently present in low numbers in surface waters and sometimes in borehole water. Although they survive long periods they are more susceptible to the extremes of environmental conditions, and viability decays more rapidly than oocyst numbers.

It was not possible to link an epidemiological study with the survey of occurrence, although a retrospective study of cases of cryptosporidiosis was made.

An initial evaluation of the removal of oocysts by water treatment using bench-scale studies indicated that a well operated plant ought to achieve an overall removal of up to five logs.

The relationship between the work under this contract and other studies is discussed.
1. BACKGROUND

After the outbreak of waterborne cryptosporidiosis in 1989 a Group of Experts, under the Chairmanship of Sir John Badenoch, was established jointly by the Secretary of State for the Environment and the Secretary of State for Health. The report of the Group made a number of recommendations and identified areas where more research was required. As a result of the recommendations a national programme of research was established involving work funded by the water industry and government.

This report presents the results of studies carried out between January 1990 and March 1994 on those parts of the National Programme which were funded by the Department of the Environment under their contract PECD 7/7/357, which was managed by WRc and supervised by the Drinking Water Inspectorate.
2. OBJECTIVES AND PROGRAMME OF WORK

2.1 Objectives

The contractor will administer and co-ordinate the national programme of work agreed by the Department's Cryptosporidium Research Steering Committee.

The contractor will carry out research or appoint and manage subcontractors to carry out research and ensure that reports are provided at regular intervals including a final overview report of the national programme.

2.2 Programme of work

The programme of work has evolved during the course of the studies as the steering committee has identified fresh needs arising from the results of studies within the programme and elsewhere. Much of the work has been carried out by specialist subcontractors and the resulting programme is described under headings representing areas of study and therefore is not necessarily in chronological order.

The main contractor is required to:

- adequately supervise and control the programmes of work, including the letting and supervision of subcontracts;
- provide technical and secretarial support to the Cryptosporidium Research Steering Committee; and
- provide interim and final reports on the programme elements as required by the Cryptosporidium Research Steering Committee.

The main contractor is also required to appoint a consultant to advise on aspects of identification, viability and infectivity of oocysts as well as the application of analytical methods developed within the programme.
3. **RESEARCH ITEMS**

Tabulated below are those research requirements identified by the Group of Experts or the National Programme Steering Committee which were funded by DWI as part of this contract.

<table>
<thead>
<tr>
<th>Research requirements</th>
<th>Research activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary isolation</strong></td>
<td></td>
</tr>
<tr>
<td>Concentration of oocysts by magnetic particles and cross-flow filtration.</td>
<td>The evaluation of antibody-coated magnetic particles and/or cross-flow filtration to concentrate oocysts from water samples.</td>
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<tr>
<td>Improved recovery of oocysts.</td>
<td>The evaluation of recovery of oocysts from filters and other concentration techniques and the use of elution techniques used in virology.</td>
</tr>
<tr>
<td><strong>Enumeration</strong></td>
<td></td>
</tr>
<tr>
<td>Development of rapid methods for oocyst separation and identification</td>
<td>The assessment of electronic imagery to enhance conventional monoclonal antibody enumeration techniques and the coupling of light emitting chemicals to monoclonal antibodies.</td>
</tr>
<tr>
<td>Test for oocyst viability.</td>
<td>The development of a relatively simple test for viability of oocysts that does not require large numbers of oocysts or experimental animals.</td>
</tr>
<tr>
<td>Development of a specific test for Cryptosporidium parvum.</td>
<td>The development of an enumeration method specific for Cryptosporidium parvum using a monoclonal antibody.</td>
</tr>
<tr>
<td>Review of molecular biology techniques for Cryptosporidium enumeration</td>
<td>A critical review of techniques currently used for oocyst enumeration and the potential for approaches based on molecular biology.</td>
</tr>
<tr>
<td>Research requirements</td>
<td>Research activity</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Infective dose</strong></td>
<td></td>
</tr>
<tr>
<td>Use of disease-free lambs for assessing</td>
<td>The assessment of the minimum infective dose of <em>Cryptosporidium parvum</em> using gnotobiotic lambs fed with milk seeded with oocysts.</td>
</tr>
<tr>
<td>infective dose.</td>
<td></td>
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<tr>
<td>Infective dose using primates.</td>
<td>The determination of infective dose of <em>Cryptosporidium parvum</em> oocysts by feeding trials using vervet monkeys of different age groups.</td>
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<td><strong>Water treatment</strong></td>
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<tr>
<td>Laboratory scale tests for removal of</td>
<td>The determination of the efficiency of removal of oocysts during water treatment using jar test equipment and laboratory filter columns.</td>
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<td>oocysts in water treatment.</td>
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<tr>
<td>Feasibility of the use of ozone.</td>
<td>A desk study to review available information on ozonation by-products and ozone applications.</td>
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<tr>
<td>Effect of environmental stress on oocyst</td>
<td>The investigation of the effect of individual and combined environmental stress factors on the survival of <em>Cryptosporidium parvum</em> oocysts and their behaviour, during water treatment.</td>
</tr>
<tr>
<td>viability and behaviour.</td>
<td></td>
</tr>
<tr>
<td>Effectiveness of point-of-use filters for</td>
<td>A desk study to identify suitable point-of-use filters followed by laboratory trials of representative filters using yeast cells as a surrogate for <em>Cryptosporidium</em> oocysts.</td>
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<tr>
<td>oocyst removal.</td>
<td></td>
</tr>
<tr>
<td><strong>Occurrence and epidemiology</strong></td>
<td></td>
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<tr>
<td>Survey of occurrence in lowland waters</td>
<td>The co-ordination of a survey over a period of 15 months of the occurrence of oocysts at ten lowland surface water and six borehole sites.</td>
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<tr>
<td>and boreholes.</td>
<td></td>
</tr>
<tr>
<td>Research requirements</td>
<td>Research activity</td>
</tr>
<tr>
<td>-----------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Epidemiology of cryptosporidiosis in areas covered by the lowland survey.</td>
<td>The determination of correlations between the occurrence of oocysts in distributed water and levels of cryptosporidiosis in the community using data from the survey of lowland waters and boreholes, 'spotter' General Practices and the PHLS reporting system.</td>
</tr>
<tr>
<td>Quality assurance on the survey of lowland waters and boreholes.</td>
<td>Duplicate analyses for quality assurance of representative samples from the survey of the occurrence of Cryptosporidium oocysts in lowland waters and boreholes.</td>
</tr>
<tr>
<td>Quality assurance on a survey of occurrence in an upland catchment.</td>
<td>Duplicate analyses for quality assurance of representative samples from a study to assess the occurrence and origins of Cryptosporidium oocysts in an upland source.</td>
</tr>
</tbody>
</table>
4. CONTRACT ARRANGEMENTS

4.1 Timescales and funds

The contract commenced during January 1990 and the active research finished during March 1994. The total cost of the contract was some £801K, the distribution between the financial years is shown in Table 4.1. The cost of each area of work is included in Table 4.2 of Section 4.3.

Table 4.1 Outturn of the contract

<table>
<thead>
<tr>
<th>Financial year</th>
<th>Cost (£)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989/90</td>
<td>39 985</td>
</tr>
<tr>
<td>1990/91</td>
<td>337 643</td>
</tr>
<tr>
<td>1991/92</td>
<td>114 917</td>
</tr>
<tr>
<td>1992/93</td>
<td>122 671</td>
</tr>
<tr>
<td>1993/94</td>
<td>186 011*</td>
</tr>
<tr>
<td>Total</td>
<td>801 227*</td>
</tr>
</tbody>
</table>

* Estimated outturn

4.2 Areas of work and subcontractors

The subcontractor, cost and timespan of the studies for each area of the programme of work are identified in Table 4.2.
<table>
<thead>
<tr>
<th>Table 4.2</th>
<th>Work areas; contractors, costs and duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contractor</td>
</tr>
<tr>
<td>PRIMARY ISOLATION</td>
<td></td>
</tr>
<tr>
<td>Magnetic Particles/</td>
<td>SPDL</td>
</tr>
<tr>
<td>Cross-flow Filtration</td>
<td></td>
</tr>
<tr>
<td>Improved Concentration</td>
<td>Sund U</td>
</tr>
<tr>
<td>ENUMERATION</td>
<td></td>
</tr>
<tr>
<td>Rapid Methods</td>
<td>SPDL</td>
</tr>
<tr>
<td>Viability</td>
<td>SPDL</td>
</tr>
<tr>
<td>Specificity</td>
<td>SPDL</td>
</tr>
<tr>
<td>Molecular Biology</td>
<td>LSHTM</td>
</tr>
<tr>
<td>INFECTIVE DOSE</td>
<td></td>
</tr>
<tr>
<td>Lambs</td>
<td>MRI-PHLS</td>
</tr>
<tr>
<td>Primates</td>
<td>SPDL-KETRI</td>
</tr>
<tr>
<td>WATER TREATMENT</td>
<td></td>
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<tr>
<td>Laboratory Scale</td>
<td>UCL</td>
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<tr>
<td>Use of Ozone</td>
<td>WRc</td>
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<tr>
<td>Environmental Stress</td>
<td>WRc</td>
</tr>
<tr>
<td>Point-of-use Filters</td>
<td></td>
</tr>
<tr>
<td>- Feasibility</td>
<td>WSL</td>
</tr>
<tr>
<td>- Evaluation</td>
<td>WSL</td>
</tr>
<tr>
<td>OCCURRENCE</td>
<td></td>
</tr>
<tr>
<td>Co-ordination of Lowland Survey</td>
<td>WRc</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>CDSC</td>
</tr>
<tr>
<td>QA Lowland Survey</td>
<td>SPDL or PHLS</td>
</tr>
<tr>
<td>QA Upland Catchment</td>
<td>PHLS</td>
</tr>
<tr>
<td>CO-ORDINATION OF PROGRAMME</td>
<td></td>
</tr>
<tr>
<td>Co-ordination of DoE Studies,</td>
<td>WRc</td>
</tr>
<tr>
<td>Contract Management,</td>
<td></td>
</tr>
<tr>
<td>Report preparation and</td>
<td></td>
</tr>
<tr>
<td>Steering Committee Secretariat</td>
<td>SPDL³</td>
</tr>
<tr>
<td>Specialist Advice</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
</tr>
</tbody>
</table>

1 Funded and directed jointly with the Foundation for Water Research (FWR).
2 With financial contributions from FWR, the National Rivers Authority and participating companies.
3 Professor H. V. Smith.

CDSC Communicable Disease Surveillance Centre, London.
KETRI Kenyan Trypanosoma Research Institute, Kenya.
LSHTM London School of Hygiene and Tropical Medicine.
MRI Moredun Research Institute, Edinburgh.
PHLS Public Health Laboratory Service, RvL.
SPDL Scottish Parasite Diagnostic Laboratory, Glasgow.
Sund U University of Sunderland.
UCL University College, London.
WSL Warren Spring Laboratory, (now part of AEA Technology, Harwell).
5. CONTRACT MANAGEMENT AND THE NATIONAL CRYPTOSPORIDIUM RESEARCH PROGRAMME

5.1 The National Cryptosporidium Research Programme

This contract formed the major part of the contribution of the Drinking Water Inspectorate (DWI) to the National Cryptosporidium Research Programme which was established following recommendations by the Group of Experts. The National Programme included the DWI funded studies in the field of Cryptosporidium in relation to water supplies as well as other studies which were independently managed. The latter were funded by bodies such as the Foundation for Water Research (FWR), the National Rivers Authority (NRA) and the Ministry of Agriculture Fisheries and Food (MAFF), together with other divisions within the Department. The Research Steering Committee comprised of nominees of the Water Services Association, the Water Companies Association and the Scottish Association of Directors of Water and Sewerage Services, representing the water industry together with nominees of MAFF, NRA, the Department of Health and DWI representing the organisations with responsibilities for water quality before or after treatment. The secretariat, technical support and co-ordination of the programme was provided by WRC. The committee met 11 times between April 1990 and June 1994.

The terms of reference were:

- To review progress and content of research programmes including the balance between projects and resources available.
- To advise researchers.
- To maintain an awareness of other related work.
- To report to the Group of Experts on the progress of the work.

During most of the period of the contract Professor H V Smith of the Scottish Parasite Diagnostic Laboratory (SPDL) and Strathclyde University has been retained as a consultant, on an ad hoc, basis to advise the Research Steering Committee and WRC on matters relating to the biology of oocysts.

A report of the first two years of the committee’s work which was drafted by WRC and published by DWI in July 1992. Two other reports have been produced to brief the Committee. The first summarised the additional evidence on Cryptosporidium, from inside and outside the National Programme since the publication of the report of the Group of Experts. The second presented a synopsis of the studies, funded by the Department and by others, within the National Programme.

The full four-years work will provide a major contribution to the second report of the Group of Experts which is being drafted by WRC as part of another contract. Publication of this report is anticipated in the Summer of 1995.
5.2 Contract management

The contract ran from January 1990 to March 1994 and the programme of work ranged from improved techniques for the recovery and identification of oocysts through removal during water treatment to epidemiological studies of the disease in the community.

The programme was divided into 20 subcontracts or subdivisions which involved 12 organisations, individually or in combination with others. One study, infectivity to primates, operated partially as a sub-subcontract from a sub-contractor. The scientific content of the subcontractors reports was generally high. However, most were delivered late and the subcontractors needed to be reminded frequently of the timescale of their commitments, particularly the provision of progress reports. Almost all the subcontractors assumed that the contract was in fact a grant, which could be invoiced on a pro-rata basis up to the maximum of the estimated cost, and most administration units needed to be reminded to submit invoices at the end of each quarter. However, there were no requests for revision of longer subcontracts to take inflation into account. With one subcontractor, the SPDL, changes in policy meant that at different times their funds were administered by different organisations.

The contractor or subcontractors carrying out investigations in specific areas of the programme of work have submitted full reports at the completion of each study. These have been listed chronologically in Section 8.1 as a contract report series (CR). The main achievements in each area are summarised briefly and the implications reviewed in Section 6.

Although the materials required for Cryptosporidium analysis are not expensive, the tests are tedious and time consuming, and hence costly. They are also imprecise. The need for the subcontractors to carry out large numbers of analyses in various forms has been a major contribution to the relatively high cost of the contracts.

During the four years the contract has run there has been a considerable increase in the knowledge about Cryptosporidium and water, arising from research funded through this contract and from other studies in the UK and elsewhere. This increase in knowledge has allowed the research programme to be reduced in future years to a size that can be managed from resources within the DWI and therefore this final report is produced at the end of WRc’s management role.

Contractor’s reports (see Section 8.1); CR 10, CR 18 and CR 19.
6. SUMMARY OF RESEARCH RESULTS AND DISCUSSION

6.1 Primary Isolation

Recovery of oocysts from environmental samples still remains a problem. The provisional method of the Standing Committee of Analysts (SCA) was based on methods used in the USA which have subsequently been shown to have a low and irregular recovery efficiency. Data from a study at Sunderland University comparing different filters for the recovery of oocysts from spiked tap waters show a range of recovery from 5.0% to 37.8% (Table 6.1). These results are in line with studies reported by other workers.

Table 6.1 Recovery of Cryptosporidium oocysts from spiked tap water by various filters (from CR 17).

<table>
<thead>
<tr>
<th>Filter type</th>
<th>Nominal pore size (μm)</th>
<th>Mean recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycarbonate membrane</td>
<td>2</td>
<td>6.0</td>
</tr>
<tr>
<td>Cellulose nitrate membrane</td>
<td>5</td>
<td>10.3</td>
</tr>
<tr>
<td>Cellulose nitrate membrane</td>
<td>3</td>
<td>24.1</td>
</tr>
<tr>
<td>Cellulose acetate membrane</td>
<td>1.2</td>
<td>37.8</td>
</tr>
<tr>
<td>Pleated polypropylene cartridge</td>
<td>15</td>
<td>5.0</td>
</tr>
<tr>
<td>Wound polypropylene cartridge</td>
<td>1</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Trials with tangential flow, or cross-flow, filters at SPDL have produced higher levels of recovery from potable quality water (Table 6.2). The effectiveness of the unit declines with time and the results are not so promising from turbid waters. These problems probably arise from the fouling of the filter elements which are currently only available with sub-micrometre pore diameters.

The concentration of oocysts from the material washed from the filters still presents problems. This relies on a number of centrifugations and if the material contains much other debris, a flotation stage. The outcome of these stages relies to some extent upon the skills of the technician. The use of antibody-labelled magnetisable particles appears to have potential to overcome some of these problems. SPDL have evaluated several types of magnetisable particle and several monoclonal antibodies in a range of water simulations. Table 6.3, as an illustration of this potential, summarises the results from one set of combinations.
Table 6.2  Recovery of oocysts from laboratory grade 1 water using a tangential flow filter (from CR 14)

<table>
<thead>
<tr>
<th>Trial No</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td>87</td>
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<tr>
<td>5</td>
<td>42</td>
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<td>7</td>
<td>34</td>
</tr>
<tr>
<td>9</td>
<td>31</td>
</tr>
</tbody>
</table>

Table 6.3  Comparison of oocyst recovery from 1 ml concentrates of seeded pond water using conventional techniques or antibody coated magnetisable beads (from CR 14)

<table>
<thead>
<tr>
<th>Oocyst concentration</th>
<th>Conventional technique</th>
<th>Magnetisable particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ml⁻¹</td>
<td>4.9%</td>
<td>24.3%</td>
</tr>
<tr>
<td>100 ml⁻¹</td>
<td>8.2%</td>
<td>58.3%</td>
</tr>
</tbody>
</table>

With current technology this technique is limited to the examination of the deposit as the concentrator has limited capacity. However, the manufacturer has indicated that it should be possible to design a concentrator with a capacity of one to four litres, which would allow the techniques to be applied prior to the current sucrose flotation stage. There are also a number of problems relating to the labelling of the beads to be overcome before the technique would be available for routine use.

The work at Sunderland University demonstrated that using flocculation with calcium carbonate high levels of oocysts could be recovered; an average of 73% from seeded tap water and 42% from seeded river water. This method is limited by the volume size of 10 litres. The originators, Thames Water, claim that although the sample size is limited, the test is more effective than the more usual method because of its higher recovery efficiency.

Subcontractors’ reports (see Section 8.1); CR 14 and CR 17.
6.2 Identification and enumeration

The techniques for oocyst identification and enumeration involve concentration of the oocysts and other deposits resulting from primary separation followed by tedious microscopic examination of the final deposit which at the same time requires skilled interpretation. A telephone survey by WRC indicated that the time involved for a single sample could range between 0.5 and 4.5 hours with the median value near to the higher end of the range.

SPDL investigated the attachment of a photoluminescent chemical to the monoclonal antibody and exposing the final slides to a suitable detector, such as X-ray film. Although the attachment of the chemiluminescent agent was reasonably successful and as low as 5 oocysts per slide could be detected, the number of false positives meant considerable microscopic examination was still required and it would be necessary to develop high affinity antibodies before the technique can be adopted for routine use. The use of a charge-coupled device (CCD) to digitise the microscope image and compare it with a database initially appeared attractive, particularly because it would utilise existing techniques based monoclonal antibodies and the viability stain. Discussions at the time with a company developing CCD imaging suggested that a considerable amount of effort would be required to develop the software to control the microscope and interpret the database. Subsequent discussions with other developers suggest that such developments would only require small extensions to existing software, but it involves ‘near-market’ development which might be difficult with public funding.

During the last four years companies within the water industry have supported developments in enumeration and identification outside the National Programme.

The most notable is the application of flow-cytometry, which, together with flow activated cell sorting, is in routine use by at least two companies and is being installed in a number of others. Another system which shows promise and is being actively developed for the detection of Cryptosporidium oocysts in water is the electro-rotation assay. This assay should be able to distinguish between oocysts of different species and to determine their viability.

Prior to the establishment of the National Programme the means of determining viability of oocysts was by animal infection or the somewhat tedious microscopic technique of excystation. During the course of the National Programme a relatively simple dye inclusion-exclusion test using the vital dyes 4'6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) was developed at SPDL. The test has proved to be a very useful research tool, both within the National Programme and in other studies, although a minor disadvantage has been identified in that the test is a measure of the cell wall integrity and this may not degenerate until some time after the cessation of nucleic acid activity. It has not been possible to correlate the results of the test with infectivity or with virulence but it has been shown to have a very good correlation with excystation and recent work in Canada has demonstrated that the latter overestimates infectivity compared with animal tests.

Because the infective dose to man is low and a small proportion of oocysts can survive for long periods in the environment, the Industry, from an operational point of view, is unsure how to utilise the results of the test if it is applied to finished waters.
Only one *Cryptosporidium* species, *C. parvum*, infects man. Other species, *C. muris* and *C. baylei*, infect small mammals and birds respectively, and the oocysts of these species are also likely to be found in source waters. The Group of Experts thought it desirable to have a test to identify *C. parvum* oocysts to species level and consequently a number of organisations were invited to tender for a research study. From the responses it was apparent that a number of studies were underway, in the UK and the USA, to develop a gene probe. A subcontract was placed with SPDL to develop a species-specific monoclonal antibody. SPDL were not able to identify a suitable epitope for the development of a monoclonal antibody but did identify lectins that were worthy of further investigation. The lack of information in the scientific press suggests that no gene probe has yet been developed.

In recent years there have been many developments in molecular biology for the identification of micro-organisms. Following a call for bids, a contract was placed with the London School of Hygiene and Tropical Medicine to review these fields in relation to the identification and enumeration of *Cryptosporidium* oocysts. They conclude that whilst a number of systems have the potential to enumerate or identify oocysts there is still a need for efficient concentration techniques and most systems need further refinement to reduce capital or labour costs and to improve sensitivity. Polymerase chain reaction (PCR) was seen as a potential means of distinguishing between species but at present requires elaborate laboratory facilities. Their conclusions are summarised in Table 6.4.

**Subcontractors’ reports** (see Section 8.1); CR 7, CR 8, CR 11 and CR 16.

### 6.3 Infective dose

At the time of the Swindon/Oxfordshire outbreak there had been only one small study in primates, which had indicated that the infective dose was very low. Two studies were established as part of the National Programme. The first, using day-old gnotobiotic lambs, simulated the most vulnerable part of the population, the very young was carried out at the Moredun Research Institute, Edinburgh in association with the Public Health Laboratory at Rhyl. The second used monkeys over a range of ages which it is believed had not been exposed to *Cryptosporidium* oocysts in a simulation of the general, but susceptible, population. This was carried out at the Kenyan Trypanosome Research Institute in association with SPDL. The study with lambs confirmed earlier reports that the infective dose is very low possibly as low as 1-3 per lamb. This was not supported by the results from the monkey study where illness was not detected after the monkeys had received doses of $10^4$ or $10^5$ oocysts. There were, however, several differences between the studies. The lambs were new-born, gnotobiotic and kept in isolators in controlled conditions. The monkeys were mainly over 12-months old and most had spent part of their life in the wild, although they had been kept in captivity prior to the study. The oocysts used in the lamb study had been previously passaged through ovines whereas those used in the monkey study were derived from human sources, and there is now emerging some evidence that the species of the host may influence the infectivity of oocysts. Examination of the sera of the monkeys suggested that they developed a mild symptomless infection. The results are compared in Tables 6.5 and 6.6.
<table>
<thead>
<tr>
<th><strong>Technique</strong></th>
<th><strong>Advantages</strong></th>
<th><strong>Disadvantages</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoenzyme analysis</td>
<td>Potential for species differentiation</td>
<td>Requires large number of oocysts</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Time and labour saving</td>
<td>Expensive, Requires microscopy</td>
</tr>
<tr>
<td>Magnetisable particles</td>
<td>Time and labour saving</td>
<td>Small scale only, Requires microscopy</td>
</tr>
<tr>
<td>Dielectrophoresis</td>
<td>Potentially labour saving</td>
<td>Expensive, Needs considerable development</td>
</tr>
<tr>
<td>Electro-rotation assay</td>
<td>Time and labour saving, Highly sensitive, Potential for viability test</td>
<td>Needs considerable development</td>
</tr>
<tr>
<td>DNA analysis</td>
<td>Potential for species-specificity test</td>
<td>Requires large number of oocysts</td>
</tr>
<tr>
<td>Polymerase chain reaction</td>
<td>Highly sensitive, Potential for viability test, Potential for species-specificity test</td>
<td>Complex facilities required</td>
</tr>
<tr>
<td>Cooled-charge device</td>
<td>Spatial visualisation of oocyst, High potential for automation</td>
<td>Expensive, Software not available</td>
</tr>
<tr>
<td>Enhanced chemiluminescence</td>
<td>Time and labour saving</td>
<td>Low resolution, False positives detected</td>
</tr>
</tbody>
</table>
Table 6.5  Acquisition of infection by lambs (from CR 2)

<table>
<thead>
<tr>
<th>Days of exposure</th>
<th>Cumulative number infected(^1) at 50 oocysts (^{-1})</th>
<th>Cumulative number infected(^1) at 5 oocysts (^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

\(^1\) After first 24 hours the lambs were fed *ad libitum* a diet of two parts water spiked at 5 or 50 oocysts per litre mixed with one part evaporated milk.

\(^2\) Each group comprised 10 lambs and infection was defined as the shedding of oocysts.

Table 6.6  Response of vervet monkeys (from CR 12)

<table>
<thead>
<tr>
<th>Animals in group</th>
<th>Age</th>
<th>Oocyst dose(^1)</th>
<th>Faeces examination(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;5 yr</td>
<td>nil</td>
<td>42 day</td>
</tr>
<tr>
<td>3</td>
<td>&gt;5 yr</td>
<td>(10^4)</td>
<td>42 day</td>
</tr>
<tr>
<td>3</td>
<td>&gt;5 yr</td>
<td>(10^5)</td>
<td>42 day</td>
</tr>
<tr>
<td>2</td>
<td>subadult</td>
<td>(10^{4.2})</td>
<td>28 day</td>
</tr>
<tr>
<td>2</td>
<td>subadult</td>
<td>(10^5)</td>
<td>28 day</td>
</tr>
<tr>
<td>5</td>
<td>3-8 month</td>
<td>(10^5)</td>
<td>30 day</td>
</tr>
</tbody>
</table>

\(^1\) The oocysts were administered as a single dose *per os*.

\(^2\) Faeces from each animal were examined daily for oocysts.

The results of these studies will be superseded by a study in the USA funded by their Environmental Protection Agency (US EPA) involving healthy, but *Cryptosporidium*-antibody free, adult volunteers. One of the 5 volunteers who received 30 oocysts, the lowest dose, became infected. The researchers determining the LD\(_{50}\) to be 214 oocysts. Extrapolation of the data suggests that a small portion of the susceptible population, about 1/250, may be infected by one oocyst. The next stage of the study will include volunteers who have *Cryptosporidium* antibodies. A further stage will study re-infection in the initial volunteers. The data from the infectivity studies are a necessary part of the equation in modelling risks to water consumers.

**Subcontractors’ reports** (see Section 8.1); *CR 2, CR 12 and CR 15*. 

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6.4 Water treatment

An initial evaluation of water treatment systems for the removal of oocysts from source water was carried out at bench-scale by University College, London. Extrapolation of the data predicts that rapid sand filtration alone should achieve a 2.6 log removal. This should increase to better than 5 log removal when the water has been subjected to coagulation prior to filtration. Slow sand filtration was predicted to be more efficient at oocyst removal. The researchers, however, point out that, because of the similarity of the oocyst size to that of particles in low turbidity waters, it cannot be assumed that no oocysts are present in treated water. Typical results using alum and sand filters are shown in Tables 6.7 and 6.8 respectively.

Table 6.7 Removal of oocysts by coagulation with alum in jar tests (from CR 3)

<table>
<thead>
<tr>
<th>Alum (M)</th>
<th>Polymer (µg l⁻¹)</th>
<th>pH</th>
<th>Oocysts (l⁻¹)</th>
<th>Removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x10⁻⁵</td>
<td>-</td>
<td>7.98</td>
<td>5160</td>
<td>87</td>
</tr>
<tr>
<td>2x10⁻⁵</td>
<td>50</td>
<td>7.95</td>
<td>1010</td>
<td>97.5</td>
</tr>
<tr>
<td>2x10⁻⁵</td>
<td>100</td>
<td>8.00</td>
<td>8980</td>
<td>78</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>-</td>
<td>7.44</td>
<td>80</td>
<td>99.8</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>50</td>
<td>7.42</td>
<td>120</td>
<td>99.7</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>100</td>
<td>7.39</td>
<td>330</td>
<td>99.2</td>
</tr>
</tbody>
</table>

Table 6.8 Removal of oocysts by bench-scale slow sand filtration (from CR 3)

<table>
<thead>
<tr>
<th>Sand (mm)</th>
<th>Flow rate (m h⁻¹)</th>
<th>Schmutzdecke</th>
<th>Oocyst removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.27</td>
<td>0.3</td>
<td>No</td>
<td>98.4</td>
</tr>
<tr>
<td>0.27</td>
<td>0.15</td>
<td>Yes</td>
<td>97.6</td>
</tr>
<tr>
<td>0.14</td>
<td>0.3</td>
<td>No</td>
<td>99.7</td>
</tr>
<tr>
<td>0.14</td>
<td>0.15</td>
<td>Yes</td>
<td>99.6</td>
</tr>
</tbody>
</table>

Studies on oocyst removal using a large pilot-scale plant have been funded by FWR (FWR report FR 0457). This report estimates that a well operated water treatment process should achieve an oocyst removal in excess of 90%, and over most of the operating period the pilot plant achieved removals of in excess of 99.8% using a two-stage coagulation system. Turbidity measurements and particle size analysis could not detect the presence of oocysts but identified changes in plant conditions leading to particle penetration which should act as
a warning to the operators. Calculations indicate that well-settled backwash waters, returned over a long period, are unlikely to increase significantly the oocyst load to the plant.

Work prior to the establishment of the National Programme by the (then) Water Research Centre, the Department and elsewhere demonstrated that chlorine had little effect upon the viability of Cryptosporidium oocysts and that ozone was the most promising disinfectant for their control. As a result of these observations the National Programme included a desk study to review ozonation by-products and ozone application (CR 1). The authors conclude that the risk to health from ozonation by-products was not sufficient to preclude the use of ozone to control Cryptosporidium oocysts. However, the positioning of ozone in the treatment chain, particularly in existing plants, to produce a satisfactory compromise between design criteria and plant and process constraints may be difficult. Studies with ozone have continued within the orbit of the National Programme at the pilot-scale level with funding from FWR (see Hall, Pressdee and Carrington, (1994) FWR report FR 0457), and more recently UK Water Industry Research Ltd (UK WIR). A comprehensive study of the disinfection of Cryptosporidium oocysts is underway in Canada under the direction of the Research Foundation of the American Water Works Association.

The report of the Group of Experts identified the need to protect the most vulnerable populations within of the community and recommended that point-of-use devices should be evaluated for this purpose. A desk study conducted by (the then) Warren Spring Laboratory identified a number of ceramic, membrane or cartridge style depth filters with potential (CR 5). Representative filters were further evaluated using yeast cells as a surrogate for Cryptosporidium oocysts. These studies show that the filters would probably retain oocysts, but the plumbing needed to be designed to avoid hydraulic shock and care was needed during maintenance to protect the operator and the system from contamination (CR 9). The efficacy of such devices has yet to be confirmed using oocysts.

Subcontractors’ reports (see Section 8.1); CR 3, CR 1, CR 5 and CR 9.

6.5 Environmental stress

Following reports from the USA that oocysts exposed to environmental stress were more readily inactivated during water treatment DoE and FWR jointly funded studies carried out by WRC to determine the factors that influence the survival of oocysts and the effects of exposure to the environment upon their removal or inactivation during water treatment (CR 13). To identify the influence of individual factors, suspensions of oocysts from two batches were stored under conditions such that only one factor differed from the standard of 10 °C, pH 7 and darkness. The numbers and viability of the oocysts were examined at intervals over a period of 16 weeks for batch C1/92 and 38 weeks for batch C1/93/3. Table 6.9 summarises the slope of the least squares regression lines of these investigations. Oocysts survived better at the more moderate temperatures and the pH of the suspending medium had little effect. Continuous low level light did not enhance or retard decay. The rate of change in viability was greater than the rate of change in density.
There were differences in the rates of change between the two batches of oocysts used. It is likely that there were commonalities in the origin of the two batches of oocysts. Attempts to obtain oocysts from another source failed when an alternative supplier did not confirm that they were unable to deliver until late in the study.

### Table 6.9  Slope of the least squares regression lines of the density or viability of oocysts stored under various conditions *(1)*
(from *CR 13*).

<table>
<thead>
<tr>
<th>Storage condition <em>(1)</em></th>
<th>Batch C1/92 Density</th>
<th>Batch C1/92 Viability</th>
<th>Batch C1/93/3 Density</th>
<th>Batch C1/93/3 Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At pH 7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-25 °C</td>
<td>-0.20</td>
<td>-9.87</td>
<td>-0.07</td>
<td>-4.84</td>
</tr>
<tr>
<td>4 °C</td>
<td>-0.04</td>
<td>-0.60</td>
<td>-0.03</td>
<td>-0.35</td>
</tr>
<tr>
<td>10 °C</td>
<td>-0.03</td>
<td>-1.93</td>
<td>-0.03</td>
<td>-0.52</td>
</tr>
<tr>
<td>20 °C</td>
<td>-0.13</td>
<td>-6.45</td>
<td>-0.03</td>
<td>-0.71</td>
</tr>
<tr>
<td>30 °C</td>
<td>-0.15</td>
<td>-7.76</td>
<td>-0.03</td>
<td>-0.92</td>
</tr>
<tr>
<td><strong>At 10 °C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4</td>
<td>-0.50</td>
<td>-1.33</td>
<td>-0.02</td>
<td>-1.27</td>
</tr>
<tr>
<td>pH 7</td>
<td>-0.03</td>
<td>-1.13</td>
<td>-0.01</td>
<td>-0.79</td>
</tr>
<tr>
<td>pH 10</td>
<td>0.00</td>
<td>-1.60</td>
<td>-0.02</td>
<td>-1.22</td>
</tr>
<tr>
<td>River Water</td>
<td></td>
<td></td>
<td>-0.01</td>
<td>-1.13</td>
</tr>
</tbody>
</table>

*(1)* See text for explanation of storage conditions.

As part of this study oocysts were also exposed to the total environment by containing oocyst suspensions in bottles, which allowed the passage of light and water, immersed in lagoons of river water. Meteorological and similar measurements were made at frequent intervals. The survival of oocysts was inversely related to air temperature and/or light intensity. These results are summarised in Table 6.10.
Table 6.10  Survival of oocysts under a range of environmental conditions
(from CR 13)

<table>
<thead>
<tr>
<th></th>
<th>Exposure period</th>
<th>Exposure (weeks)</th>
<th>Temperature (max-min)</th>
<th>Decline in numbers (log)</th>
<th>Change in viability during exposure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb0</td>
<td>Nov-Apr</td>
<td>22</td>
<td>27.8-1.7</td>
<td>1.4</td>
<td>67-62</td>
</tr>
<tr>
<td>Pb1a</td>
<td>Apr-Jul</td>
<td>14</td>
<td>27.9-6.2</td>
<td>5.0</td>
<td>94.1-39.7</td>
</tr>
<tr>
<td>Pb1b</td>
<td>Apr-Oct</td>
<td>31</td>
<td>27.9-3.1</td>
<td>4.5</td>
<td>94.1-47.2</td>
</tr>
<tr>
<td>Pb2a</td>
<td>Jun-Sep</td>
<td>14</td>
<td>27.8-12.9</td>
<td>2.1</td>
<td>89.2-42.5</td>
</tr>
<tr>
<td>Pb2b</td>
<td>Jun-Dec</td>
<td>24</td>
<td>27.8-7.1</td>
<td>2.6</td>
<td>89.2-58.3</td>
</tr>
<tr>
<td>Pb3</td>
<td>Aug-Nov</td>
<td>9</td>
<td>20.3-3.9</td>
<td>1.6</td>
<td>96.0-66.9</td>
</tr>
<tr>
<td>Pb4</td>
<td>Oct-Jan</td>
<td>11</td>
<td>13.0-3.1</td>
<td>2.0</td>
<td>87.7-52.0</td>
</tr>
</tbody>
</table>

These findings are in line with those of other studies. A small amount of work at SPDL (CR 7) has reported similar findings after freezing oocysts and they have reported differing survival times by oocysts in stools from different sources. They also demonstrated a decline during storage in semi-permeable containers in river water but they did not record the meteorological conditions at the time.

Oocysts that had been subjected to environmental stress were also passed through the pilot-scale treatment plant, exposed to ozone or exposed to chlorine. There was no observable difference compared to the control oocysts with regard to removal during water treatment or inactivation by ozone. However, the exposed oocysts were more susceptible to chlorine, for example oocysts exposed to the aquatic environment for nine weeks during the late summer of 1993 and then exposed to chlorine at a residual concentration of 0.5 mg l⁻¹ for 30 minutes showed a 31.8 per cent reduction in viability compared to similarly exposed oocysts that were not chlorinated.

Subcontractors' reports (see Section 8.1); CR 13 and CR 7.

6.6  Occurrence of oocysts

Frequency of occurrence and levels of oocysts.

At the time of the establishment of the National Programme there was little information relating to the occurrence of oocysts in source waters. The discharge of oocysts into a watercourse, particularly from animal derived sources, is likely to be intermittent. This
indicates that a large number of samples are required and the relatively high cost of analysis means that a reliable survey is expensive. With funding from DWI, FWR and the NRA, as well as contributions in kind from the participating water companies, a total of ten sites on three lowland rivers were sampled three times weekly for 15 months between January 1990 and April 1991. The rivers and sites were selected as being typical of the range of lowland waters used as sources for potable water in the UK. The analyses were carried out by the local water company and the study was co-ordinated and collated by WRc. The sites are described in Table 6.11 and the results are summarised in Table 6.12.

<table>
<thead>
<tr>
<th>Table 6.11</th>
<th>River stretches included in the survey (from CR 6)</th>
</tr>
</thead>
</table>

**River A**

<table>
<thead>
<tr>
<th>Site</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Reservoir intake. Upstream mainly rural, some small communities, large town some 60 km away.</td>
</tr>
<tr>
<td>A2</td>
<td>Major tributary upstream of A1. Upstream mainly rural.</td>
</tr>
</tbody>
</table>

**River B**

<table>
<thead>
<tr>
<th>Site</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>B3</td>
<td>Waterworks intake. Upstream mainly rural.</td>
</tr>
<tr>
<td>B4</td>
<td>Downstream of site B3. Upstream tributaries draining rural or urban areas, and including a major sewage treatment works.</td>
</tr>
<tr>
<td>B5</td>
<td>Waterworks intake downstream of B4. Upstream area urbanised.</td>
</tr>
<tr>
<td>B6</td>
<td>Waterworks intake about 15 km downstream of B3 and a sewage treatment works. Upstream area urbanised.</td>
</tr>
</tbody>
</table>

**River C**

<table>
<thead>
<tr>
<th>Site</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C7</td>
<td>Canal immediately downstream of a town.</td>
</tr>
<tr>
<td>C8</td>
<td>Canal immediately downstream of sewage effluent discharge.</td>
</tr>
<tr>
<td>C9</td>
<td>River below confluence with canal. Waterworks intake.</td>
</tr>
<tr>
<td>C10</td>
<td>River above confluence with canal. Upstream mainly rural.</td>
</tr>
<tr>
<td>Site</td>
<td>Number of samples</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------</td>
</tr>
<tr>
<td>River A</td>
<td></td>
</tr>
<tr>
<td>Site A1</td>
<td>183</td>
</tr>
<tr>
<td>Site A2</td>
<td>192</td>
</tr>
<tr>
<td>River B</td>
<td></td>
</tr>
<tr>
<td>Site B3</td>
<td>171</td>
</tr>
<tr>
<td>Site B4</td>
<td>160</td>
</tr>
<tr>
<td>Site B5</td>
<td>180</td>
</tr>
<tr>
<td>Site B6</td>
<td>180</td>
</tr>
<tr>
<td>River C</td>
<td></td>
</tr>
<tr>
<td>Site C7</td>
<td>108</td>
</tr>
<tr>
<td>Site C8</td>
<td>106</td>
</tr>
<tr>
<td>Site C9</td>
<td>110</td>
</tr>
<tr>
<td>Site C10</td>
<td>106</td>
</tr>
</tbody>
</table>

In River A and River C the incidence of oocysts was low and for long periods they were absent. In River B about 50% of the samples were positive. The levels of oocysts in the positive samples was also low, generally well below one per litre. Only in River B were there enough positive samples to carry out statistical analysis of the results. There was a significantly increased occurrence of oocysts in the period February-June 1990 when compared with the period July 1990 - March 1991. However, this result may have been influenced by changes in the sampling protocol. There was a weak correlation between rainfall and the number of oocysts observed, but there was no correlation between rainfall and the number of positive samples, and comparisons of river flow with the levels of oocysts or the number of positive samples gave similar results.

This study is probably the only comprehensive study of the occurrence of oocysts that has been carried out on lowland waters. However, similar patterns of results have been reported from ad hoc or less comprehensive studies from Australia, Canada, Germany and The Netherlands, as well as some studies in the USA. A geographically wide ranging investigation in the US which included “industrialised rivers” recorded levels as high as 480 per litre. A more recent study on one of the rivers used in the UK survey and some results made available by water supply companies indicate that on rare occasions the levels in UK surface waters can be as high as 70-80 per litre.
An upland water was also monitored as part of the National Programme, but not with DWI funding. A raw water intake on Loch Lomond was monitored continuously, by changing the filter every 48-72 hours, over a two-year period. Of the 290 samples, 32 were positive. The concentration of oocysts ranged between 0.0019 l⁻¹ and 0.12 l⁻¹, with a mean value of 0.018 l⁻¹.

At the same time as the major survey of UK lowland waters, a survey of occurrence in boreholes was carried out. Six boreholes representing a range of rock types and bore integrity were sampled once-weekly over the latter 12 months of the same period. The sites are described in Table 6.13 and the results are summarised in Table 6.14.

**Table 6.13**  
**Ground water sources survey (from CR 8)**

<table>
<thead>
<tr>
<th>Site</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D11</td>
<td>Unconfined chalk, fast recharge, suspected surface water connection.</td>
</tr>
<tr>
<td>D12</td>
<td>Chalk borehole, confined London Clay.</td>
</tr>
<tr>
<td>D13</td>
<td>Partially confined Northern Chalk, very fast recharge, possible contamination of source, sheep graze nearby.</td>
</tr>
<tr>
<td>E14</td>
<td>Chalk well and adit, urban location with some rural catchment, prone to coliform contamination.</td>
</tr>
<tr>
<td>E15</td>
<td>Chalk borehole, rural catchment with sheep and cattle grazing, prone to coliform contamination.</td>
</tr>
<tr>
<td>E16</td>
<td>Chalk borehole of excellent quality, rural catchment with livestock grazing.</td>
</tr>
</tbody>
</table>

**Table 6.14**  
**Occurrence of oocysts in six boreholes, April 1990 - April 1991 (from CR 8)**

<table>
<thead>
<tr>
<th>Site</th>
<th>Number of samples</th>
<th>Number positive</th>
<th>Range of positive values (oocyst l⁻¹)</th>
<th>Arithmetic mean of positive results (oocyst l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D11</td>
<td>44</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D12</td>
<td>42</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D13</td>
<td>34</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E14</td>
<td>46</td>
<td>3</td>
<td>0.004-0.026</td>
<td>0.01</td>
</tr>
<tr>
<td>E15</td>
<td>48</td>
<td>2</td>
<td>0.007-0.922</td>
<td>0.47</td>
</tr>
<tr>
<td>E16</td>
<td>44</td>
<td>3</td>
<td>0.009-0.39</td>
<td>0.20</td>
</tr>
</tbody>
</table>
The boreholes returned negative results except for eight samples from area E over a short time period in May-June 1990. These results were surprising, particularly as one of the boreholes was in deep chalk yielding water of excellent quality.

Investigation by the water company and the examining laboratory, which in this case was a different organisation, failed to identify any likely cause of contamination.

**Quality assurance**

Quality assurance was carried out on the comprehensive lowland water survey, the upland water study and the survey of boreholes. One sixth of the total number of filters were quartered, and two quarters were examined in the sampling bodies laboratory and the other quarters were examined in a reference laboratory, either SPDL or PHLS Rhyl. The number of positive samples from Rivers A and C, the upland water and the boreholes were too small to be able to make meaningful comparisons. Of the 109 comparisons from River B, 49 gave differences in positive and negative between the company and the reference laboratory. Statistical analysis showed that the company laboratory was more likely to record a positive result and where both laboratories recorded a positive, the company laboratory result was likely to be higher.

Associated with the upland water study a number of sewage effluents which ultimately entered the loch were monitored, 18 samples were examined in duplicate. Although both laboratories were following the techniques in the provisional method of the SCA, examination of their protocols indicated that there were differences in the techniques. This resulted in significant differences in the portion of the original sample that was examined. Consequently there was a significant difference between the results from the two laboratories.

The results from the Quality Assurance exercises reinforce the need for a simple, reliable and reproducible method of oocyst recovery and identification which can be applied in an unambiguous way by routine laboratories, particularly where comparisons need to be made between similar or related samples. However, a standard methodology should not be so rigid as to exclude the application of new developments or technologies.

**Epidemiology**

When the study of lowland waters and boreholes was initiated it was intended that, if the level of oocysts exceeded 10 l⁻¹ at a site, intensive monitoring would take place to try to identify the source. Although the target level was subsequently reduced to 5 l⁻¹ these investigations were never triggered. It was also arranged that if significant numbers of oocysts were found in a source the Communicable Disease Control Centre (CDSC) would conduct epidemiological studies in that area. To provide baseline data the CDSC carried out a descriptive epidemiology study on cases of the disease within five representative water supply zones receiving water from the monitored sources. The data were collected on a specially designed surveillance form during an interview with the patient. The responses of the 191 patients interviewed between April 1990 and June 1991 are summarised in Table 6.15.
Table 6.15  The interview response of 191 cryptosporidiosis patients. (derived from CR 4)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Percent of respondents</th>
<th>Criteria</th>
<th>Percent of respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case status</strong></td>
<td></td>
<td><strong>Tapwater consumption</strong></td>
<td></td>
</tr>
<tr>
<td>Primary case</td>
<td>71</td>
<td>None</td>
<td>14</td>
</tr>
<tr>
<td>Secondary case</td>
<td>27</td>
<td>1-2 glasses d⁻¹</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-4 glasses d⁻¹</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-6 glasses d⁻¹</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;6 glasses d⁻¹</td>
<td>3</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td><strong>Foods consumed</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>54</td>
<td>Unpasteurised milk</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>Raw meat</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sausages</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cold meat</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hamburgers</td>
<td>40</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td><strong>Animal contact</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>9</td>
<td>Pets, sick or young</td>
<td>17</td>
</tr>
<tr>
<td>1-4 years</td>
<td>55</td>
<td>Farm</td>
<td>20</td>
</tr>
<tr>
<td>5-14 years</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-44 years</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-65 years</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;65 years</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td><strong>Travel</strong></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>98</td>
<td>Nights away</td>
<td>35</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>58</td>
<td>Local</td>
<td>12</td>
</tr>
<tr>
<td>Adults (80%)</td>
<td></td>
<td>UK</td>
<td>16</td>
</tr>
<tr>
<td>Children (50%)</td>
<td></td>
<td>Abroad</td>
<td>6</td>
</tr>
<tr>
<td>Vomiting</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults (30%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children (59%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The study confirmed other reports that young children are more susceptible than other age groupings. The CDSC suggest that not only do they represent the primary cases but also pass the infection to their susceptible peers at school or playgroup. A higher proportion of cases lived in households where there was a child under five years (36%) than that shown in the 1981 census for all households in England and Wales (12.8% of households contained a child under five). The CDSC do point out, however, that parental concern ensures that children with diarrhoea are more likely to see a doctor than adults. It is unfortunate that it was not possible to include any control (uninfected) persons in the study.
As part of the survey of the upland water, where the quality assurance was funded as part of this contract, some epidemiological studies were carried out by the Scottish Centre for Infection and Environmental Health. During November and December 1992 there was an increase in the levels of cryptosporidiosis in the communities receiving the water. Although oocysts were detected in the water in supply the uneven distribution of cases between the supply zones mitigated against water being a common source of infection.

The epidemiological studies have confirmed that there are potentially many sources of oocysts available to the community other than water.

Subcontractors’ reports (see Section 8.1); CR 6 and CR 4.
7. FURTHER STUDIES

Since the report of the Group of Experts on Cryptosporidium in Water in 1990 a considerable amount of research has been carried out within the UK National Programme and elsewhere in the UK and in other countries. The role of WRC within the contract in providing the Secretariat and co-ordination of the National Programme has required them to be aware of ongoing work and consequently to be able to identify remaining gaps in knowledge. This has also been a requirement of another contract awarded by DWI (43/2/35), to draft a report updating the Group of Experts 1990 report.

Some of the areas where more information is required are set out below.

Primary isolation

- There is still a need for a rapid, reliable and reproducible isolation method. The application of cross-flow filtration with magnetisable particles shows promise, as does the electro-rotation assay.

- Quality assurance exercises have demonstrated the need for a consistently reproducible technique, particularly where comparisons between laboratories have to be made. It is understood that the relevant panel of the Standing Committee of Analysts has been reconvened.

- The absence of infection or low attack rate in some circumstances could be explained by differences in virulence or strain of the oocyst. There is some evidence that this may occur.

Infecive dose

- There is some conflicting evidence as to the dose of oocysts required to initiate infection. Current trials in the USA may provide an answer.

Water treatment

- Work is in hand in the FWR and UKWIR programmes to identify optimum regimes for plant operation. However, there is a need for dialogue between plant operators, engineers and scientists to identify areas of concern and possible solutions. A "workshop" is being held on this topic in February 1995.

- Point-of-use devices have value in the protection of the vulnerable population. There is a need to complete the studies carried out so far, using Cryptosporidium oocysts.
Disinfection

- A considerable amount of research on disinfection in relation to water supply has been reported, particularly on the use of ozone. However, the use of different definitions of viability, e.g. excystation or animal infection, has led to different research teams reporting divergent results. There is a need for the various reports to be reviewed in the light of the techniques used.

Occurrence

- Catchment control is a major factor for restricting access of oocysts to waters. There is a need to evaluate the effectiveness of current practices and regulations.

- The National Survey monitored the occurrence of oocysts over an intensive period. These data could usefully be extended by including the results of long term monitoring.

- Oocysts are likely to occur infrequently in potable waters in small numbers. In such circumstances a small number of infections may occur in the community, but at a level that would not be detected by schemes detecting outbreaks of disease. A case can be made for long term monitoring of water quality and infection in the consumers of a few water treatment plants.

- Sewage effluent is potentially a major source of oocysts in water. No large surveys have been carried out.
8. REPORTS PREPARED

8.1 Contractors and subcontractors reports

In addition to the regular six-monthly progress reports submitted by WRc to the Department the following reports have been produced by the contractor or subcontractors carrying out investigations in specific areas of the research programme.


CR 7 Scottish Parasite Diagnostic Laboratory (1991) Final report on DoE sponsored research on Cryptosporidium spp. oocysts undertaken at the Scottish Parasite Diagnostic Laboratory.


CR 9 Warren Spring Laboratory (undated) Assessment of cartridge filters for the removal of a Cryptosporidium surrogate.


CR 11 Campbell A.T. and Smith H.V. (undated) Production of high affinity, species specific monoclonal antibodies against oocysts of Cryptosporidium parvum.

CR 13 Carrington, E.G. and Ransome, M.E. Factors influencing the survival of Cryptosporidium oocysts in the environment. [Published by the Foundation for Water Research, Marlow, Report number FR 0456.]


CR 17 University of Sunderland (1994) Evaluation of different filtration techniques for the concentration of Cryptosporidium oocysts from water.


8.2 Report of the National Research Programme Steering Committee


8.3 Papers in peer-reviewed journals

The following papers describing studies wholly or mainly funded by the contract have appeared in peer-reviewed journals.


National Cryptosporidium Survey Group (1992) A survey of Cryptosporidium oocysts in surface and ground waters in the UK. JIWEM 6, 697


