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CRYPTOSPORIDIUM IN DRINKING WATER (DWQ 9006)

*Progress Report to the Department of the Environment
period April 1992 to September 1992*

DoE 3204
October 1992



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Report No: DoE 3204

October 1992

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SUMMARY

The report reviews progress on the DoE Contract funded as part of the national programme resulting from the deliberations of the Group of Experts chaired by Sir John Badenoch. The period covered is April to September 1992.

During this period a full progress report on the national programme and other work has been prepared for the Steering Committee and published by DWI.

Further progress has been made on the investigation of the feasibility of preparing a species-specific monoclonal antibody, the survey of occurrence on Loch Lomond and the application of enumeration techniques developed in the earlier stages of the programme.

Preparations have also been made for studies on the effectiveness of point-of-use devices, the susceptibility of environmentally stressed oocysts and new concentration techniques for oocysts, all being subject to revision of the contract.

The report on the determination of the infective dose in primates carried out in Kenya is still awaited.

Future research will be discussed at the Steering Committee meeting in November.

1. BACKGROUND

This report summarises progress on the contract funded by the Department of the Environment and managed by WRc. The period covered is April to September 1992. The work in progress forms part of the National Programme on Cryptosporidium which arose out of the deliberations of the Group of Experts chaired by Sir John Badenoch.

2. OBJECTIVES AND PROGRAMME OF WORK

Objectives

- (i) To administer and co-ordinate the national programme and ensure quality control.
- (ii) To examine new methods of separation and examination of oocysts, viability of environmentally exposed oocysts and determination of the minimum infective dose in primates and mice.
- (iii) To examine the removal of oocysts in classical water treatment processes at the laboratory scale.

To summarise existing available information on chemical by-products which may arise as a result of use of ozone as a disinfectant in water treatment.

To consider and identify the most effective point of application of ozone in the water treatment process for disinfection and minimising adverse reactions.

To identify by literature search and correspondence with manufacturers and suppliers, filters suitable for use in contact with potable water, capable of retaining micro-organisms down to 1 micron in size, and to have a critical expert appraisal of their likely potential applications for potable water filtration.

To examine by means of laboratory and pilot plant studies the effectiveness of disinfectants on cryptosporidium oocysts subject to exposure in the environment and passage through water treatment plants.

- (iv) To prepare reports at regular intervals including a final overview report of the national programme.
- (v) To oversee all catchment studies.

Programme of Work

1. Administration

- (i) To supervise the programme, provide general technical co-ordination and ensure quality control.

- (ii) To supervise and arrange the letting of contracts to nominated subcontractors and obtaining agreement to their programme of work from DoE. (Competitive tendering to be adopted where feasible.)
2. Identification and Viability
- (i) Development of methods of separation and identification which are more specific and sensitive than currently used techniques.
- 3A. Water Treatment Studies
- (i) Collate and summarise published information and data from DoE funded work at WRc on chemical by-products arising from action of ozone applied at levels used for disinfection of water on the organic contaminants of water, including aliphatic and aromatic substances, amino-acids, heterocyclic compounds, humic and fulvic acids and selected pesticides, and report.
 - (ii) Review literature to determine most effective points of application of ozone for disinfection while bringing about maintenance or improvement of water treatment.
 - (iii) Confirm (ii) by studies of recent practice and experience in Europe and United States by correspondence and attendance at the AWWA Conference.
 - (iv) Provide review report on the above.
- 3B. Review of Filters
- (i) Survey appropriate technical publications to identify sources of filters.
 - (ii) Obtain technical brochures and information from manufacturers and suppliers of filters intended for a range of technical applications.
 - (iii) Review information obtained, make initial selection of potentially useful filters.
 - (iv) Obtain further detailed information on composition, performance and durability in water, of those selected initially.
 - (v) Make expert critical assessment and appraisal of the selected filters, if necessary consulting certain authorities in the water industry.
 - (vi) Provide report (25 copies) after approval of draft, also camera ready copy if requested and separate summary for FWR.

3C Disinfection of environmentally stressed oocysts

- (i) The contractor will evaluate through laboratory tests the effect of oocyst age on their susceptibility to disinfectants (such as chlorine or ozone).
- (ii) Investigations will be carried out, using a pilot plant, on the effect of treatment on the susceptibility of oocysts to disinfectants (such as chlorine or ozone).

3. PROGRAMME OF WORK FOR THE PERIOD APRIL - SEPTEMBER 1992

The items of the programme reviewed during this period are:

- development of a species-specific enumeration method - SPDL/Strathclyde University;
- application of enumeration techniques - SPDL/Strathclyde;
- infective dose in primates - SPDL/Strathclyde/KETRI Kenya;
- Loch Lomond survey quality assurance - PHLS Rhyl;
- behaviour of environmentally stressed oocysts - WRc;
- point-of-use treatment - Warren Spring Laboratory;
- investigation of concentration techniques - Sunderland University;
- co-ordination and reporting.

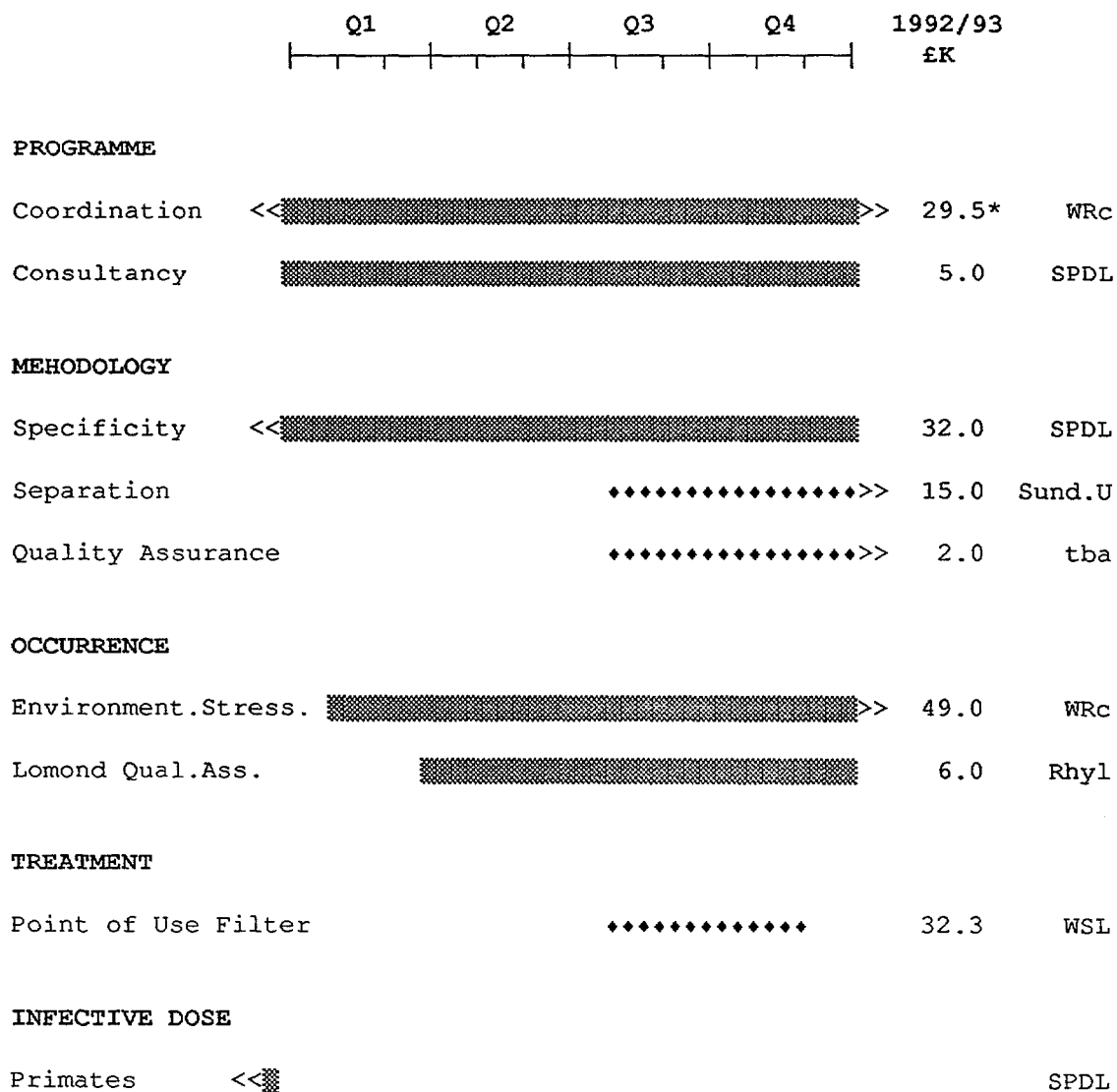
The timescale and cost in relation to the year 1992/93 of each item of the programme is shown in Figure 3.1.

4. RESEARCH PROGRESS

4.1 Development of a species-specific enumeration method

Suitable strains of mice have been immunised with extracts of the walls of *C. parvum* and *C. baileyi*. Antibodies from the mice have been fused with myeloma cells to create monoclonal antibodies. The results of the fusions will be evaluated between September and December.

The reactivity of three commercial antibodies to cell wall extracts has been compared using a number of regimes. It was shown that not all the antibodies attached to the same type of site, suggesting that the use of two antibodies was more effective than one. The affinity of the antibodies for the cell wall sites appears to be low and thus the cross reactions reported by some workers is not surprising.



[Shaded Bar] Studies in progress

[Dotted Bar] Proposed studies

* includes proposed £1.8K to cover coordination of proposed studies

SPDL - Strathclyde University/SPDL

Sund.U - Sunderland University

tba - to be arranged

Rhyl - PHLS Ryhl

WSL - Warren Spring Lab.

Figure 3.1 Programme of Research 1992/93

4.2 Application of enumeration techniques

Attention has been given to the application of two enumeration techniques developed in the programme carried out at Stobhill under the contract with SPDL/Strathclyde University.

In the search for a new rapid identification method for cryptosporidium oocysts it was concluded that the use of a close-coupled device (CCD) imaging camera could improve the final microscopy stage. Discussions have been held between the Department, WRc, SPDL and a camera company to assess the requirements for producing a prototype camera. Although software exists for automating camera movements and focusing, software would have to be written for identification of oocysts attached to monoclonal antibodies labelled with fluorescent dyes or oocysts stained with dyes used for detecting whether oocysts are alive or dead. In addition to the cost of software production it would be necessary to fund trials on two microscope/camera/computer systems, one at the camera company and one at SPDL. Funding would be necessary initially to draw up an accurate estimate of the final costs of the development programme. Due to funding problems this initial stage has not proceeded.

The test for viability using PI and DAPI dyes, developed at SPDL has been on trial at three water utility laboratories (Strathclyde R.C., Lothian R.C. and Severn Trent Water Ltd). Full evaluation has been hampered by a lack of oocysts in environmental samples. However, the method has been applied to the limited number of oocysts detected and found to be effective. The dye test can be incorporated readily into the SCA method. As with all enumeration methods it is still reliant on effective initial separation and concentration of oocysts and low numbers are difficult to detect, especially in the presence of suspended solids, although the dyes enhance detection to some degree.

The method is now being used on oocysts detected in the Loch Lomond study and by Severn Trent Water and Thames Water Utilities in other cryptosporidium investigations.

4.3 Infective dose in primates

Although SPDL believe that further tests on primates have been carried out, the final report on the study has not yet been received and is now some five months overdue. Strenuous efforts are being made by SPDL to obtain the report from KETRI in Kenya.

4.4 Quality assurance Loch Lomond

The survey of Loch Lomond has continued with continuous samples taken from the water abstraction point at Ross Priory since April 1992. Eight sewage works effluents have also been sampled at monthly intervals. Samples of faecal material and slurry have also been examined from areas around the feed rivers and the Loch itself.

On five occasions in the later part of May and once in August low levels of oocysts, equivalent of 1 in 83 to 1 in 220 litres, were observed at Ross Priory, about 50% appeared

to be viable. No oocysts were reported in finished waters. No outbreaks of Cryptosporidiosis have been reported in humans or animals.

Quality assurance has been in place since early June involving split filters on about 12% of samples from Ross Priory and sewage effluents. One such sample has been reported as positive by SPDL and negative by Rhyl. Full details are not yet available but the indications are that one oocyst was observed in a sample of about 80 litres.

4.5 Behaviour of environmentally stressed oocysts

The studies of Le Chevallier in the USA on full-scale water treatment plant indicated that oocysts which had been environmentally exposed and subject to treatment processes might be more amenable to conventional disinfection than laboratory experiments might indicate. This could be an important practical factor and the Steering Committee feels that it should be explored further. Of all the possible methods of experimentation it was concluded that seeded pilot plant trials would be the only practical approach.

Agreement has therefore been reached with the Foundation for Water Research (FWR) that the pilot plant under construction at Medmenham for the investigation of the effectiveness of treatment stages, should also be used to study the effects on oocysts of environmental exposure. As well as seeding the plant with oocysts exposed to the environment for given periods, some work will also be done on the effects of exposure itself on oocyst condition. Use will be made of the SPDL viability test in this work. The pilot plant is expected to be commissioned in October and work will commence as soon as the experiments can be fitted into the FWR programme.

Early results suggest that oocysts may disintegrate at a moderate rate during storage. The presence of autofluorescing algae in the test waters has produced complications in interpreting both the enumeration and the viability tests.

4.6 Point-of-use treatment

Following the review of available filters carried out by Warren Spring Laboratory, a programme was submitted by them for a follow-up study on removal efficiency of units representative of those selected. Agreement has been reached for this work to proceed as soon as a revision to the contract has been completed and an acceptable subcontract has been drawn up. The intention is to measure effectiveness of removal using surrogate particles initially. Consideration will also be given to maintenance and safe disposal of the filter units. It is hoped that the work will commence in early Autumn and will be completed in four months.

4.7 Investigation of concentration techniques

Cryptosporidia oocysts can readily be removed from sampled water by filtration but available techniques for recovery from the filter are not efficient. A programme of work has been submitted by Sunderland University to investigate the efficacy of a range of

eluants for the release of oocysts from representative filters. This work will proceed when a revision to the contract has been completed and an acceptable subcontract has been agreed.

4.8 Co-ordination and reporting

Substantial effort has been put into finalising the report from the Steering Committee published by the Drinking Water Inspectorate in July 1992. The report presented a complete review of the elements of the national programme together with summaries of results from other programmes.

SPDL sought and was given permission to submit for publication five papers resulting from the work on enumeration methods. These covered:

- the results on the viability tests;
- enhanced chemiluminescence methods;
- use of the CCD camera;
- survival of oocysts under environmental pressure; and
- an experimental container for environmental effect experiments.

A short article was also submitted to 'Water Bulletin' on the laboratory trials of the viability technique. A review paper was produced by Professor Smith on these aspects of the work. A poster presentation was given on this work by SPDL to a meeting of the IAWPRC in Washington in May 1992. A poster was also presented to the same meeting on the studies of infectivity in lambs, carried out by the Moredun Institute in collaboration with PHLS Rhyl.

The results of the U.K. national survey have been accepted for publication in the IWEM Journal and were presented to a meeting of IWSA in Amsterdam in September. At the same meeting a paper was presented on the work on the effects of different disinfectants on cryptosporidium carried out under the parallel DoE contract.

5. FUTURE WORK

During the next reporting period the work on specificity at SPDL will have been concluded together with the Warren Spring filter study and the Loch Lomond survey work.

Progress will be made with the pilot plant work on environmentally stressed oocysts and the studies on separation techniques at Sunderland will have commenced. It is also anticipated that a decision will be made on the potential for developing an automated method for enumeration of oocysts.

As currently written the cryptosporidium contract is due to finish in March 1993. However, funds have been sought by DWI for extension into 1993/94. A meeting of the Steering Committee is due to be held on 10 November and it is planned that the

programme for 1993/94 will be discussed at that meeting. Following these discussions an extension of the contract containing a full work programme will be drawn up.

6. FINANCIAL POSITION

A submission has been made for a revision of contract to accommodate the new work at Sunderland and Warren Spring. This would give a total budget for 1992/93 of £170,800. With commitment in 1993/94, initially, of £7,500.

At this stage it is expected that WRc costs will be as budget. Subcontract costs are always difficult to forecast but expenditure on the SPDL and Warren Spring contracts plus the quality assurance work for Loch Lomond are expected to be on budget. Expenditure on the Sunderland contract will depend upon the start date which in turn will depend upon the date when the revision is agreed by the Department and the time taken to recruit staff at Sunderland. Overall, at this stage, a significant underspend is not expected in this financial year.