Public Health Laboratory Service

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Advice on proceedings of the WSAA strategic workshop on viability tests and genetic typing of Cryptosporidium.

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Aims

The aim of this document is to provide interpretation of the implications of the workshop for *Cryptosporidium* research in the UK.

Scope

A written report of the workshop proceedings is available (Anon, 2000). This document interpretation of the workshop and does not replicate that report.

Executive summary

The Water Services Association of Australia (WSAA) has outlined a strategy for the the development of guidelines setting out conditions required to inactivate *Cryptosporidium* oocysts and for improved hazard analysis, health surveillance and incident management through the application of predictive molecular epidemiology. To facilitate achievement of these aims, a workshop was held in Sydney in March 2000 to explore issues of viability measurement (specifically, capability of infecting a human) and genetic typing as a tool for strain identification and molecular epidemiology of cryptosporidial infections.

Collaborative approaches to viability studies and genotyping of *Cryptosporidium* were proposed within Australia and internationally. The UK should be involved, since advances in surveillance and epidemiology of cryptosporidiosis, and the creation of a national reference collection of oocysts, provide an excellent base for expanded studies. Validated viability, typing and infectivity determinants are required in the UK and elsewhere to provide sensitive and specific methods for environmental samples and application to risk assessment. Cell culture methods should be investigated since they appear to offer a good model for human infectivity with genotype 2, and the opportunity for comparison of cell culture with human infectivity trials involving genotype 1 should be created. UK representation at the forthcoming ASM Cryptosporidium Conference in October 2001 is essential for transfer of technology both ways.

The work described in this report was funded by the Department of the Environment, Transport and the Regions. The Drinking Water Inspectorate was responsible for contract management.

Background and introduction

In order to further define their research strategies to address issues of *Cryptosporidium* in water supplies (Gill and Langford, 1999), the Water Services Association of Australia (WSAA) sponsored a workshop in Sydney in March 2000. This was attended by key personnel from the Australian water industry, researchers in Cryptosporidium and epidemiology, and by international experts. Ultimate aims of the WSAA strategy are:

1. the development of guidelines setting out conditions required to inactivate oocysts

2. improved hazard analysis, health surveillance and incident management through the application of predictive molecular epidemiology.

The workshop focused on two broad areas of research to facilitate achievement of these aims:

1. viability measurement (specifically, capability of infecting a human)

2. genetic typing as a tool for strain identification and molecular epidemiology of cryptosporidial infections.

The workshop followed that held at Tadley Court, UK in August 1999 "Towards a standardized experimental design for viability and inactivation studies on *Cryptosporidium* in water" (Anon, 1999a) which provided a good model and much background for the Sydney workshop. One of the conclusions was that human cell culture provided a reliable measurement of oocyst viability, but while methods for genotype 2 are fairly well established, those for genotype 1 need further development.

The Sydney workshop was run under five objectives for discussion:

1. to define the research and technology transfer necessary to refine and establish *in vitro* procedures for the maintenance of *C. parvum* isolates of both genotypes 1 and 2.

2. Develop strategies for the evaluation of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and Fluorescence *In Situ* Hybridisation (FISH) by comparison with *in vivo* and *in vitro* techniques and by multi-laboratory trials

3. Develop strategies for multi-centre trials to compare the specificity and sensitivity of current PCR detection techniques

4. Develop strategies for multi-centre trials to compare the specificity and sensitivity of current PCR genotyping techniques

5. Explore strategies for determining the prevalence and distribution of human infectious and non-infectious genotypes in different water sources, and populations of human and animal hosts.

By the end of the workshop, consensus outcomes for each objective were agreed and the requirements to achieve each outcome defined. These are summarized below with interpretation and implications for *Cryptosporidium* research in the UK.

Objective 1. To define the research and technology transfer necessary to refine and establish *in vitro* procedures for the maintenance of *C. parvum* isolates of both genotypes 1 and 2.

To evaluate oocyst inactivation under a range of environmental and storage conditions and disinfectant treatments, reliable measurement of oocyst viability is required. Many methods of viability measurement have been used in the past but inherent problems in their application have been identified (Anon, 1999a). The ultimate measure is human or animal infectivity. Human infectivity is clearly unfeasible for routine assessment and cell culture with human enterocytes has shown promise as a laboratory model for human infection of genotype 2, with better correlation with volunteer studies than a mouse model. Cell culture for genotype 1 isolates is being developed by Dr George Di Giovanni and co-workers of the American Water Services Company (AWSC). PCR has also been applied to cell culture (CC-PCR) to permit the detection of low numbers of viable organisms in the original seed and hence a potential viability test for environmental samples.

The outcome of objective 1 would be a routine and reproducible cell culture procedure for detection and viability assessment of *Cryptosporidium* genotypes of public health significance.

Requirements to achieve the outcome

The importance of oocyst isolate/strain, origin and history, preparation method and age, and purity for use in viability and disinfection studies has been highlighted (Anon, 1999a). The same principles will apply to the development of cell culture methods. The importance of consistency in the seed used for method development has also been stressed. To establish a cell culture method in Australia it will be necessary to

- Identify the capabilities required to undertake the work and their presence in Australian laboratories and obtain a commitment to technology transfer from AWSC and thence between laboratories
- Establish collaboration with overseas laboratories to investigate the technique in different environments
- Establish a standard strain (eg Iowa) for development work and inter-laboratory comparability trials
- Establish suitable cell lines
- Follow isolation and cell culture protocols
- Hold a collaborative workshop on cell culture applications for *Cryptosporidium.*

Implications for the UK

The cell culture model is being used more widely in the field of *Cryptosporidium* viability and infectivity studies. In addition to standard strains such as Iowa, other strains should also be investigated.

Work is in progress in the US on human infectivity with genotype 1 isolates (being supported in part by the PHLS Cryptosporidium Reference Unit by the supply of suitable isolates), and data from those studies should be used to provide a measure of correlation between viability/infectivity models for this genotype.

Although cell culture does not result in bulking up of oocysts numbers, it is timely that UK research involving cell culture is expanded to investigate the provision of a model for both animal and human infectivity, and potentially a gold standard. Other potential applications are far reaching and include not only a measure of infectivity, but also provide a tool for disinfection studies and drug therapies, and investigation of the effect of strain variation. Phenotypic differences between isolates can be studied in parallel with molecular studies.

Laboratories in the UK with a special interest in *Cryptosporidium* should be identified and included in collaborative studies.

Objective 2. Develop strategies for the evaluation of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and Fluorescence *In Situ* Hybridisation (FISH) by comparison with *in vivo* and *in vitro* techniques and multi-laboratory trials.

While cell culture methods developed under Objective 1 will be essential for provision of a gold standard, RT-PCR and FISH may be more applicable to simultaneous detection and viability assay in environmental samples. In order to further investigate these methods and undertake such a comparison, *in vivo* and *in vitro* techniques must be established, with standard protocols for the source and preparation of oocysts in infectivity and viability studies. Variables such as oocyst strain, storage conditions, laboratory conditions need to be investigated to establish the robustness (sensitivity and specificity, reproducibility and reliability) of the tests.

The outcome of this objective would be an evidence-based decision on adoption of routine procedures for identification of *C. parvum* and confirmation of viability.

Requirements to achieve the outcome

- Success of objective 1 to provide a gold standard
- Compare the application of *in vivo* and *in vitro* methods within and between laboratories using experiments designed by specialists including a Biometrician/statistician
- Use standard protocols

Implications for the UK

The UK water industry is concentrating on maintaining effective barriers to prevent the presence of *Cryptosporidium* oocysts in potable water, and a treatment standard has been set to regulate this (Anon, 1999b). This refers simply to the presence of the parasite in treated water, reflecting the physical barrier nature of the treatment, since there is no specific treatment at water treatment plants to reduce oocyst viability. However, questions about the public health significance of oocysts detected are being asked increasingly, and the significance to human health of their presence in water and other environmental samples needs to be evaluated. Information about oocyst viability, species or strains would be needed to contribute to an assessment of the risk to human health, but first more information is required about the viability and strains of oocysts in the environment. In addition, some immunocompromised people may be susceptible to strains or species previously not identified as infective for humans, and this needs to be taken in to account.

Techniques such as RT-PCR and FISH allow the simultaneous detection and a conservative (over) estimation of oocyst viability. Further development and application of RT-PCR or FISH in parallel with other detection techniques should be investigated in the UK for the potential to provide useful information about oocysts in the environment, and ultimately for health risk assessment. In particular the specificity of the method needs to be clearly identified (to target particular species or strains), and tailored depending on the application of the technique.

Objective 3. Develop strategies for multi-centre trials to compare the specificity and sensitivity of current PCR detection techniques.

PCR detection techniques are currently applied to clinical and environmental samples but their specificity and sensitivity need to be evaluated on application to new sources since even minor differences in water (for example) can affect performance.

The outcome of this objective would be evaluated PCR methods for environmental samples.

Since this objective is closely linked to Objective 4, further consideration follows in that section.

Objective 4. Develop strategies for multi-centre trials to compare the specificity and sensitivity of current PCR genotyping techniques

Researchers worldwide have developed PCR genotyping techniques using different genetic loci. Choices vary locally but a multi-center study will better identify the most useful loci, particularly for characterizing environmental isolates.

The outcome of this objective would be identification of the most sensitive loci for use, and evaluated PCR detection and genotyping techniques for environmental isolates.

Requirements to achieve the outcome

- Information from different countries comparative usefulness of various loci
- Use of cloned DNA and reference genotyping methods as a standard for comparison
- Use of standard methods, protocols and primer sets
- Use of differential display proteomics for identification of new loci

Implications for the UK

In Australia and US, HSP 70 and variable regions of 18s RNA have been widely used in genotyping. In the UK other loci such as TRAP C-2 and COWP genes are preferred options for routine genotyping of clinical isolates, and when combined with exposure data have produced valuable epidemiological information.

For environmental isolates particular sensitivity and specificity are required since cross reactive organisms (sharing DNA sequences) not found in clinical isolates may be present, and techniques need to be developed specifically for such work. In addition, the question of the detection of particular subtypes because of dominance of strain or method sensitivity needs to be addressed. Genotyping work is currently being taken forward in the UK by a collaborative group (PHLS Cryptosporidium Reference Unit, Scottish Parasite Diagnostic Laboratory, Scottish Centre for Infection and Environmental Health and Glasgow University) funded by the DWI to investigate application of micro-satellite DNA markers. This work should be taken in to account in determining international strategies, and the UK would have a valuable role to play.

Objective 5. Explore strategies for determining the prevalence and distribution of human infectious and non-infectious genotypes in different water sources, and populations of human and animal hosts.

Knowledge of genotype distribution is essential for catchment management, which involves identifying sources of oocysts, tracking their distribution and transmission. The significance of the prevalence of genotypes in animal and environmental samples for human health needs to be investigated in this context, and the isolates characterized in terms of infectivity and virulence. Creation of a reference collection of oocysts from human, animal and environmental sources would provide a valuable resource to support this. Early detection of outbreaks and their management requires predictive epidemiology that would be supported in part by such studies.

The outcome of this objective would be identification of genetic markers for infectivity and virulence.

Requirements to achieve the outcome

- Established methods for measuring infectivity
- Genotyping of human clinical isolates, environmental isolates and the establishment of an oocyst collection with isolates from human, animal and environmental sources
- Investigation of genetic markers for infectivity and virulence

Implications for the UK

To provide comprehensive assessment of oocyst sources and fate in the environment, studies need to be undertaken specific to geographical areas and catchments. UK surveillance for human case of cryptosporidiosis is more comprehensive than in many other countries, and epidemiology of infection with the application of typing techniques is more advanced. Oocysts from animal and human sources are currently being collected in the UK by a collaborative group (PHLS Cryptosporidium Reference Unit, Scottish Parasite Diagnostic Laboratory, Scottish Centre for Infection and Environmental Health and Glasgow University) as part of a project funded in part by the DWI, with a minimum dataset to support the development of typing methods applicable to epidemiological studies. This work is creating a national reference collection in the UK as a resource for further genetic studies.

The prevalence of *Cryptosporidium* in different hosts and environmental sources in the UK has been explored previously but could usefully be expanded by surveillance and coordinated with human epidemiological studies and surveillance data, with the application of typing to better understand the natural history of *Cryptosporidium* and cryptosporidiosis and disease transmission. Although COWP and TRAPC-2 gene loci are providing useful data about clinical samples, the investigation of other genetic loci and sequence data in the UK may also be useful particularly for environmental studies.

With detailed background knowledge about sources of *Cryptosporidium,* genetic markers of infectivity and virulence could be investigated from clinical cases and other sources and contribute to the provision of better baseline data for risk assessments.

References

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