



CRYPTOSPORIDIUM

INTRODUCTION

Cryptosporidium is recognized as a significant and widespread cause of diarrheal illnesses in several animal species, including humans. The organism is a protozoan of the subclass *coccidia* and is found in the environment as a dormant oocyst (pronounced "oh-oh-cyst").

Transmission of the oocyst from host to host occurs by the fecal-oral route. The active phase of Cryptosporidium's life cycle begins when an oocyst is ingested. Reproduction of oocysts inside the intestine results in the disease called Cryptosporidiosis. Cryptosporidiosis may cause a short-term diarrheal illness in otherwise healthy persons. However, in persons with weak immune systems, such as infants, the elderly and AIDS patients, Cryptosporidiosis may cause a life-threatening, cholera-like illness.

The species *Cryptosporidium parvum* is responsible for all well-documented cases of Cryptosporidiosis in mammals. Cross-transmission studies have shown that *C. parvum* is able to infect humans and animals alike, from person-to-person contact and animal-to-person contact. In addition, infected animals can be the source for Cryptosporidium contamination of water supplies.

Cryptosporidium was first described and named by the American parasitologist E.E. Tyzzer over 80 years ago. For many years, it was considered nothing more than a curiosity, and was thought to rarely infect animals or humans. In 1971, interest in Cryptosporidium took hold after R.J. Panciera and his colleagues reported its association with diarrhea in cattle. In 1976, the first cases of human Cryptosporidiosis were reported.

Since that time, major outbreaks of Cryptosporidiosis have been identified in the United States and Europe. An outbreak in Milwaukee affected over an estimated 400,000 people and caused 4,400 hospitalizations. The total estimated cost (Milwaukee Journal estimate) was over \$54 million.

In direct response to waterborne outbreaks, the Environmental Protection Agency (EPA) added Cryptosporidium to their list of Priority Substances Which May Require Regulation Under the Safe Drinking Water Act (Federal Register, Vol. 56, No. 9, Jan. 14, 1991). Regulation of Cryptosporidium is expected to be included in the Enhanced Surface Water Treatment Rule ("ESWTR"). The Surface Water Treatment Rule regulates *Giardia lamblia*, a pathogenic protozoan sharing a similar epidemiology and ecology with Cryptosporidium. Currently, water systems using a surface water source must be able to remove or inactivate 99.9% of *Giardia lamblia* from their water supply.

THE CRYPTOSPORIDIUM OOCYST

In the life cycle of Cryptosporidium, the oocyst is responsible for the spread of the organism to new "hosts". The oocysts of this intracellular parasite are circular shaped and smaller than a red blood cell (approximately 3 to 5 microns). The Cryptosporidium oocyst contains four sporozoites that are released through the oocyst membrane after ingestion. These sporozoites infect and multiply, asexually, in intestine of the host. When mature, the organism either forms a thin-walled oocyst or a thick-walled oocyst.

The thin-walled oocyst remains within the host and repeats the infection cycle. The thick-walled, environmentally-resistant oocyst is passed in the feces. These oocysts can survive in the environment for months if kept cool and moist. They are highly resistant to routine chlorination of drinking water and are small enough to pose a problem for filtration systems. Oocysts can be transported in a fine mist and on rare occasions can cause respiratory infections.



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General

The disease caused by Cryptosporidium is called Cryptosporidiosis. It can be caused by the ingestion of as few as one to ten oocysts from feces of an infected animal or human.

Human Cryptosporidiosis

The most prevalent symptom of cryptosporidiosis in humans is diarrhea. Characteristically, the diarrhea is profuse and watery; may contain mucus but rarely blood; and is often associated with weight loss. Other less common features include abdominal pain, nausea and vomiting, and low-grade fever. Occasionally, nonspecific flu-like symptoms occur.

The duration of symptoms and outcome typically vary according to the immune response of the host. AIDS patients can experience a prolonged, life-threatening illness, whereas most immunocompetent persons experience a short-term illness with complete spontaneous recovery. Infection usually lasts from 3 to 12 days; however, infections lasting over a month are known to occur in otherwise healthy individuals.

Respiratory tract infections with this parasite have been associated with chronic coughing and bronchitis. In children, symptomatic intestinal and respiratory infections can occur during the acute phase of measles, a cause of transient immunosuppression. Clinically, the infection most closely resembles giardiasis (the disease caused by *Giardia lamblia*). Although self-limited, its debilitating effects can be severe enough, especially in children, to justify therapeutic intervention.

Epidemiology

Cryptosporidium is found throughout the world but the prevalence of human cryptosporidiosis is not yet known. Most epidemiological surveys have been based on examination of stool specimens either from persons with diarrhea or simply from those submitted to parasitology laboratories. These studies indicate that Cryptosporidium is a common cause of diarrhea worldwide, infecting as many as 7% of children in the developing world. Investigations of several day care centers in the United States have documented prevalence from 6% to 54%.

Animal-to-human as well as human-to-animal transmission of Cryptosporidium has been documented. The occurrence of infection -- in household contacts of infected persons, children attending day care centers, and hospital acquired sickness -- indicates that Cryptosporidium is highly infectious and transmissible from person to person. Indirect exposure to contaminated surfaces and food is a less frequent yet possible means of transmission. Asymptomatic (not showing outward signs of disease) Cryptosporidiosis

has also been documented. Cryptosporidiosis has been described in travelers, and infections are significantly more prevalent in children, and during warm, wetter months.

Diagnosis

Clinical diagnosis of Cryptosporidiosis requires identification of oocysts in feces. Various staining techniques can assist the microbiologist in identification. One widely used method is the Fluorescence Assay. This method employs an antibody, tagged with a fluorescent dye, which in turn seeks out Cryptosporidium oocysts in a fecal sample. Once the antibody has located the oocysts, a microscopist can examine the sample using fluorescence microscopy. The oocysts are easily identified because they will glow when exposed to the fluorescent light of the microscope.

Treatment

No therapy specific to cryptosporidiosis is available.

Environmental Detection

Sampling

Isolation of Cryptosporidium oocysts from water sources requires passage of a large volume (from 1 to over 1000 liters) of water through a concentrating filter. Source water conditions and contamination expectations help to determine the volume to be taken and the expected recovery efficiency. The yarn-wound filter has been the most common type of filter but is rapidly being replaced by the new EPA method 1623 which makes use of a capsule filter, vortex flow filtration or membrane disk filtration more popular. The new method 1623 is a raw drinking water method which calls for a 10 liter grab sample. Since the method is intended for raw water there is no designated sample volume for finished waters. Concentration of the sample usually takes place in the laboratory.

Isolation

After concentration the filter element is washed and the sediment is concentrated by centrifugation. Oocysts are isolated from the concentrate pellet by density gradient centrifugation or, in the case of Method 1623, by Immunomagnetic bead separation.

Examination

From the isolation step the final pellet has commonly applied to a filter membrane for application of a fluorescent-based anti-cryptosporidium antibody and examined by fluorescence microscopy. Other stains and optics may be used in conjunction with the fluorescent-based reagent in order to identify internal structure and/or the potential for viability (discussed below). The EPA 1623 method does not use filter membranes but does use well slides to which the sample is applied directly, stained with the antibody and a viability stain DAPI (described below) and examined by fluorescence microscopy. Alternatively, some testing laboratories, predominantly outside of the United States (U.K. and Australia), prepare the sample using a fluorescent-based anti-cryptosporidium antibody but examine the sample using a mechanical process called flow cytometry.

Spiking Studies

Since chlorine disinfection has little affect on Cryptosporidium under normal treatment, there is great interest in determining the effectiveness of other types of treatment systems in either removing or inactivating the oocyst in water. By challenging various types of treatment systems with the high concentrations of oocysts, log removal (usually at least $3 \log_{10}$) data can be generated from which regulatory decisions, or system purchase, or modification decisions can be made.

Physical Removal

Physical removal data is generally produced at the bench or pilot scale. Large scale challenge studies are difficult because of the lack of availability of oocysts and/or the expense in producing numbers greater than 10^7 or 10^8 total oocysts. The condition of the oocysts can range from formalin fixed (ie. dead) to what BioVir calls "natural". Natural oocysts are oocysts isolated from fecal material and stored in a buffered solution. These oocysts have been carefully handled in order to maximize the number of "live" organisms in the seed stock.

Formalin fixed oocysts allow for greater study flexibility, when compared to natural oocysts, because of safety issues associated with working with potentially infective material. They do not pose a health threat and consequently can be shipped anywhere and handled by anyone with a minimum of training. The use of "fixed"

oocysts during a filtration study does compel scientific argument however, as to their loss of elasticity and resultant filterability. The argument being that formalin fixed oocysts are more rigid than natural oocysts and are therefore more easily removed during the filtration process. Whether this is true or not, one should weigh filterability against safety in making a study design decision.

Disinfection

Similar to physical removal studies, disinfection data is generally produced at bench or pilot scale. Again, large scale challenge studies are difficult because of the lack of availability of oocysts and/or the expense in producing numbers greater than 10^7 or 10^8 total oocysts.

The current methods for determining Cryptosporidium oocyst viability and / or infectivity (see following discussion) each have their advantages and disadvantages from a scientific perspective. Issues with respect to viability versus infectivity for disinfection studies are currently be discussed withing the scientific community. The method or methods which are ultimately employed in a disinfection study should be based on regulatory requirements, or lack thereof, budgetary constraints, and time constraints.

If a study is required to address regulatory issues, BioVir always recommends that the applicable regulatory body be involved in the planning stages in order to avoid completion of a project that may not satisfy the regulator.

Issues of Viability

Animal Infectivity

Infectivity studies are normally conducted in neonatal mice. This is considered the most conclusive method regarding *infectivity* of the organism because the gut of the mice are examined for evidence of infection. It is an expensive, labor intensive endeavor however requiring many mice and few environmental laboratories have the technical expertise to offer this service. BioVir began offering this service in 1999 in its NIH approved facility.

Excystation

During laboratory induced excystation, the oocyst is exposed to an environment similar to that of a host gas-

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trointestinal system. Therefore, excystation is a measure of the ability of an oocyst to react to chemical changes in its immediate environment. Since the sporozoites within the oocyst are the only living things (not the oocyst itself) excystation really measures the intact physical and biochemical nature of the oocyst and not the viability of the sporozoites.

Excystation as a check for viability is considered by many to be inconclusive in the presence of negative results. Positive results evoke discussions regarding *viability* versus *infectivity*. However, if a very conservative approach is taken, one could argue that if excystation occurs infectivity can be assumed. Costs associated with this testing are quite reasonable when compared to the Animal Infectivity method indicated above.

“Viability” Stain

Vital stains are chemicals which interact, or not, with cells, such as oocysts, in such a manner as to indicate viability of the organism in question. In the case of cryptosporidium oocysts, a combination of 4'6-diamidino-2-phenylindole (DAPI) and Propidium Iodide (PI) is commonly used. PI is excluded by live organisms and DAPI is absorbed by intact DNA. Similar to Excystation this is not a 100% test but use of this method is growing. This method is the least costly to employ of the methods described here.

Vital stains used in conjunction with excystation and DIC/Hoffman microscopy will reveal if the sporozoites are present, contain DNA and that, if placed into the right environment, will excyst and release the sporozoites.

Cell Culture

Propagation of cryptosporidium in vitro is currently being studied by many research laboratories. The technique shows promise and is much less expensive than a live animal study. However, detection of the active forms of cryptosporidium's life cycle require specialized antibody sets which are not widely available. Other post cell culture detection techniques are proving to be inconsistent.

ADDITIONAL INFORMATION

For more information concerning Cryptosporidium spiking studies, sampling, detection, immunofluorescent assay and current regulation, please call BioVir Laboratories at 1-800-GIARDIA (442-7342).

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