A REVIEW OF LABORATORY DIAGNOSIS OF FUNGAL DIARRHOEA

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ABSTRACT
Cryptosporidium spp is widely accepted as a cause of fungal diarrhoea. Many techniques have been applied to detect oocysts in faeces, but the difficulties of discriminating between non-cryptosporidial bodies, acid fast bodies like cryptosporidia, and cryptosporidia remain. A simple examination in two stages, suitable for routine use is described, using auramine phenol and carbol fuchsin for screening and a kinyoun staining method for confirmation. A further method, using Giemsa stain, is of value for confirmation of identity, especially where fluorescence microscopy is unavailable. A modification of the formol-ether method of concentration is also described. Immunofluorescence and thin section electron microscopy provide definitive identification.

Key Words: Cryptosporidium, Diarrhoea and Laboratory Diagnosis

INTRODUCTION
Food poisoning syndrome results from ingestion of water and wide variety of food contaminated with pathogenic microorganisms (bacteria, viruses, protozoa and fungi), their toxins and chemicals. Food poisoning must be suspected when an acute illness with gastrointestinal or neurological manifestation affect two or more persons, who have shared a meal during the previous 72 hours. The term as generally used encompasses both food-related infection and food-related intoxication. Some microbiologists consider microbial food poisoning to be different from food-borne infections. In microbial food poisoning, the microbes multiply readily in the food prior to consumption, whereas in food-borne infection, food is merely the vector for microbes that do not grow on their transient substrate. Others consider food poisoning as intoxication of food by chemicals or toxins from bacteria or fungi. Consumption of poisonous mushroom leads to mycetism, while consumption of food contaminated with toxin producing fungi leads to mycotoxicosis. Some microorganisms can use our food as a source of nutrients for their own growth. By growing in the food, metabolizing them and producing by-products, they not only render the food inedible but also pose health problems upon consumption. Many of our foods will support the growth of pathogenic microorganisms or at least serve as a vector for their transmission. Food can get contaminated from plant surfaces, animals, water, sewage, air, soil, or from food handlers during handling and processing.
Cryptosporidiosis, also known as crypto, (Cryptosporidiosis, 2009) is a parasitic disease caused by Cryptosporidium, a protozoan parasite in the phylum Apicomplexa. It affects the intestines and is typically an acute short-term infection. It is spread through the fecal-oral route, often through contaminated water; (Cryptosporidiosis, 2009) the main symptom is self-limiting diarrhoea in people with intact immune systems. In immunocompromised individuals, such as AIDS patients, the symptoms are particularly severe and often fatal. Cryptosporidium is the organism most commonly isolated in HIV positive patients presenting with diarrhoea. Treatment is symptomatic, with fluid rehydration, electrolyte correction and management of any pain. Despite not being identified until 1976, it is one of the most common waterborne diseases and is found worldwide. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exist in the small intestine and result in an infection of intestinal epithelial tissue.

LABORATORY DIAGNOSIS
In diarrheal diseases, the preferred specimen is the feces. It should be properly collected. A properly collected specimen should be collected in the right container, which should be clean, of sufficient size for collecting and with a tight-fitting lid. A rectal catheter can be used. The stool should be freshly passed and sent to the laboratory at once. Although the stool is preferable to the rectal swab specimen, in practice there are situations in which a rectal swab must be used as: (1) when it is desirable to collect the feces immediately in the absence of a bowel movement; (2) when transport of the stool to the laboratory would pose problems; (3) when there may be delay in transporting the stool to the laboratory; and (4) for practical purpose, when too many stool samples are to be collected at one time. Carefully collected rectal swab specimens may sometimes be preferred for bacteria, which invade the mucosa of the lower intestine because the swab sample is collected with a scrubbing motion of the intestinal mucosa. To collect rectal swab specimens, use a cotton-tipped swab moistened with the transport medium contained in the test tube carrier. Insert the swab through the rectal sphincter, rotate and withdraw. A good collecting device for rectal swab

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specimens is a plastic tube with a scoop attached to the tube cap; this is made in Japan. Another advantage of the rectal swab is that there is no problem if it cannot be sent to the laboratory at once since the collecting tube contains a transport medium, which can act as a preservative.

There are many diagnostic tests for Cryptosporidium. They include microscopy, staining, and detection of antibodies (Cryptosporidiosis, 2009). Microscopy can help identify oocysts in fecal matter (Brooks et al., 2004). To increase the chance of finding the oocysts, the diagnostician should inspect at least 3 stool samples (Murray et al., 2005). There are several techniques to concentrate either the stool sample or the oocysts. The modified formalin-ethyl acetate (FEA) concentration method concentrates the stool (Winn et al., 2006). Both the modified zinc sulfate centrifugal flotation technique and the Sheather’s sugar flotation procedure can concentrate the oocysts by causing them to float (Murray et al., 2005). Another form of microscopy is fluorescent microscopy done by staining with auramine (Brooks et al., 2004).

Other staining techniques include acid-fast staining, (Chen et al., 2003) which will stain the oocysts red (Winn et al., 2006). One type of acid-fast stain is the Kinyoun technique (Gideon, 2009). Giemsa staining can also be performed (Ryan & Ray, 2004). Part of the small intestine can be stained with hematoxylin and eosin (H & E), which will show oocysts attached to the epithelial cells (Winn et al., 2006).

Detecting antigens is yet another way to diagnose the disease. This can be done with direct fluorescent antibody (DFA) techniques (Cryptosporidiosis, 2009). It can also be achieved through indirect immunofluorescence assay (Murray et al., 2005). Enzyme-Linked Immuno Sorbent Assay (ELISA) also detects antigens (Brooks et al., 2004). Polymerase chain reaction (PCR) is another way to diagnose cryptosporidiosis. It can even identify the specific species of Cryptosporidium (Cryptosporidiosis, 2009). If the patient is thought to have biliary cryptosporidiosis, then an appropriate diagnostic technique is ultrasonography. If that returns normal results, the next step would be to perform endoscopic retrograde cholangiopancreatography (Chen et al., 2003).

CONCLUSION
The laboratory is very important in the diagnosis of diarrheal diseases, because without it the many etiologic agents causing diarrhoea cannot be identified and the medical people might have a hard time controlling the spread of diarrheal diseases. The laboratory is likewise needed to help in the treatment of specific diarrheas.

REFERENCES
Cryptosporidiosis Gideon (2009). Trial subscription required to access.