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Review article

A review of the biology and detection methods of *Cryptosporidium* and *Giardia* spp in water sources and situation in Tanzania.

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Abstract

Cryptosporidium and *Giardia* species are the two most common enteric protozoan pathogens which affect humans worldwide. In both developed and developing countries. In immunocompetent individuals they cause self limiting diarrhea, which may disappear after 2-3 days, but it is potentially life threatening in immunocompromised persons and children under the age of five years. Contaminated water plays a critical role in the transmission of these pathogens in the form of oocysts and cysts respectively. In the past 20 years, a number of detection methods have been developed to detect both *Cryptosporidium* spp and *Giardia spp* in the water samples. These methods have been used for monitoring the occurrences of *Cryptosporidium spp* and *Giardia spp* in the raw water intake and understand removal efficiencies at different level of the treatment train. This article aims at reviewing the biology and current detection methods of *Cryptosporidium* and *Giardia spp* in the water and current situation in Tanzania.
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Keywords: *Cryptosporidium*, *Giardia* , PCR , Tanzania

Introduction

Every year, unsafe water, together with lack of proper sanitation, kills at least 1.6 million children under the age of five years. More also, 2.6 billion people, which is approximately 40% of the world population, do not use a toilet, but defecate in the open spaces or unsanitary places (1) Fecal pollution of surface water causes serious public health problem in developing countries like Tanzania, where large number of households lack access to better water supply and depend on untreated surface water or shallow unprotected ground water for domestic uses, this situation make them susceptible to water pathogens like protozoan, bacteria and virus(1,2)

Both Cryptosporidium spp and Giardia spp are common gastrointestinal protozoan pathogens affecting both human and animals. They are important causes of diarrhea in developed and developing countries. These protozoa mainly affect the small intestine, causing persistent diarrhea and enteritis and these two parasitic protozoa can easily transmit through untreated water(3) . Oocysts of Cryptosporidium and cysts of Giardia are ubiquitous found in water and their concentration is related to the level of fecal pollution or human activities which is closer to the water sources. The human activities which are potential sources of Cryptosporidium spp and Giardia spp include agricultural activities/animal operations, combined sewer overflows or wastewater treatment plant discharges, other potential sources are wild animals, and storm water runoff (2) *Cryptosporidium* are now reconsider as worldwide water pathogen(4) In children, the infection is fulminates and may be life-threatening(5)

Cryptosporidium

Members of genus *Cryptosporidium* are small coccidian protozoan parasites that infect a microvillous region of epithelial cells in the digestive and respiratory track of vertebrate. In this genus there more than 30 species, many with several subtypes, which infect human and a wide range of animals. Although *Cryptosporidium parvum* and *Cryptosporidium hominis* (previously known as *C. parvum* genotype 1) are the most prevalent species causing disease in humans, infections by *C. felis*, *C. meleagridis*, *C. canis*, and *C. muris* has also been reported(6–8).

Morphology and life cycle

Oocysts of Cryptosporidium spp are small spherical to ovoid in shape measuring 4-6µm in diameter(9). The life cycle of Cryptosporidium consists of sexual and asexual stages (figure 1) and is completed in the single host. The Life cycle comprises of six major development stages which are excystation, merogony, gametogony, fertilization and zygote development, formation of environmentally resistant oocyst wall, and sporogony(6,10–14)

Pathogenesis

Different infection studies with healthy volunteers have tried to show different degrees of pathogenecity among Cryptosporidium species and they demonstrated a clear relationship between the probability of infection and ingested oocyst dose of a bovine C. parvum strain. Dupont et al., demonstrated that after a dose of 30 oocysts, one of

five subjects which was 20 percent, became infected, whereas at a dose of 1000 or more oocysts, seven of seven became infected which was 100%. The study also revealed that there was not any statistical significance between the size of inoculum and the time of the onset of infection and the duration of oocysts excretion. Teunis et al model showed even a single oocyst can result into the probability of infection. Although there was a clear dose–response relation for infection, occurrence of symptoms of intestinal illness in the volunteers was not dose-related(15–17).

Cryptosporidiosis

The incubation period of the disease varies widely, but it is average of seven days. The disease is characterised by watery diarrhea that may sometimes be profuse and prolonged. Diarrhea and abdominal ache are normally the symptoms which cause patients to look for medical attention, leading to a laboratory diagnosis of cryptosporidiosis. Other clinical features include nausea, vomiting, and low-grade fever. The severity, persistence, and ultimate outcome of the infection is typically dependent on a variety of parasite characteristics and host factors. Host factors include both the immune status and frequency of exposure of the infected individual (15,18,19).

In immunocompetent individuals, will experience a transient self limiting illness of 2 to 3 weeks and eventually the immune system will clear the parasites. Infection in immunocompromised patients with defective in cellular immunity due to (congenital or AIDS or chemotherapy) or defective in humoral immunity, cryptosporidosis can be potentially life threatening disease which associated with persistent and heavy diarrhea (17,20).

More than 90% of all human cryptosporidiosis caused by *C. hominis* and *C. parvum*, although other species of *Cryptosporidium* have been identified in human and may cause illness both to immunocompromised individuals and immunocompetent individuals. Other species which have been revealed to associate with human illness including *C. meleagridis, C. felis, C. canis, C. suis, C. muris, C. andersoni* (21,22)

Routes of transmission of *Cryptosporidium*

There are multiple routes of *Cryptosporidium* transmission, hence create a complex interplay between the various routes of transmission. These transmission routes can be subdivided into direct route and indirect route. Direct route occurs by fecal - oral contact from an infected host to a susceptible host, including animal to animal or animal to human (zoonotic), human to human or human to animal (anthroponotic) transmission(7,21).

Indirect transmission involves coming in contact with fecal contaminated materials with viable *Cryptosporidium* oocysts, including water and food. Contaminated environment plays an important role in the indirect transmission route; this is caused by released feces, sewage, waste water slurry, agriculture activities, and wildlife sources. The oocysts can be overflow to the water sources following to heavy rainfall events(2,7,23,24).

Water is still the key driver for the *Cryptosporidium* transmission and almost all *Cryptosporidium* outbreaks associated with contaminated water(25–29). Here, the fundamental message is that, contaminated water play a big role in the transmission of *Cryptosporidium*. Improving water supply system, therefore, is expected to bring down the majority of cryptosporidiosis cases.

Giardia

Members of the genus Giardia are microscopic unicellular flagellated protozoan parasite in the phylum Retortamonada, they colonized and reproduce in the small intestine of more than 40 animal species and cause giardiasis throughout the world. Giardia is also known as *Giardia intestinalis*, *Giardia lambia*, or *Giardia duodenali* (30,31)

Giardia life cycle consists of two stages, the cyst (resting form) and trophozoite (feeding or proliferative stage) (6,12,32,33).

Giardiasis

The infective dose of Giardia is very low, 10-25 cysts may be enough to initiate the infection. Not everyone infected with Giardia develops the infection, generally the incubation period range from 3 days to 25 days or longer. The median is 7-10 days. In healthy persons, the symptoms can last between 4-6 weeks and occasionally longer. Typical symptoms are diarrhea (Fatty, yellowish), which may become chronic, nausea, abdominal cramps and bloating. Frequent passing of loose, pale greasy stools, fatigue and weight loss can also occur. Complications such as damage to cells that line the intestine can arise from prolonged infection and can lead to malabsorption of food nutrients(17,34–37). Studies showed that, excretion of cysts ranges between 106 to 108 per gram of positive stool sample(38).

Route of transmission of *Giardia*

Giardia is transmitted by a cyst form that is 10-12 µm long, from one host to another and the major route of transmission is person to person fecal oral transmission due to the poor hygiene system or person to person in the community. This is very frequent in the area with poor sanitation infrastructure. Transmission can also occur from person to person through certain type of sexual contact (eg oral- anal contact). The waterborne outbreak has been reported for many years and the outbreak has resulted from consumption of fecally contaminated water, such as stream/lake water and swimming pools that are open to contamination by human and feces(39,40).

Detection of *Cryptosporidium spp* and *Giardia spp*

Sensitive and specific method for detection of oocysts and cysts in the contaminated water is very important to prevent and control contamination. The overall detection procedure consists of a number of stages, namely; water sample collection and concentration, separation of oocysts and cysts from contaminants debris, detection of oocysts and cysts(6,41)

US-Environmental Protection Agency (US-EPA) recommended a monitoring procedure for *Cryptosporidium spp* and *Giardia spp* in water which consist of stages including filtration, concentration, immunomagnetic separation (IMS) and detection with DAPI and fluorescent antibodies. This method is now employed widely due to its high

efficiency, rapid process, with requirement of less expertise compares to other methods, the drawback of this method; it is costly and not affordable by many laboratories especially in the developing countries(42,43).

Antibody coated magnetic beads are used in order to separate oocysts and cysts from other organisms and debris, and then identified with immunofluorescense assay technique (FA) under epi-fluorescence microscope. Epi fluorescence microscope must have UV filter block option to confirm oocysts and cysts using 2,4-diamidino-2-phenylindole(DAPI), a vital dye staining(44). DAPI staining is useful to confirm *Giardia*-positive cyst or *Cryptosporidium* –positive oocysts samples because one can sometimes visualize the internal structures(42,45,46). But DAPI staining is not consistently positive in *Giardia* in environmental samples (unpublished data). Microscope counts must be done with a well trained microscopist, who ensures that particles being counted are oocysts or cysts, and algae, yeast and other debris are excluded from the counts that are made(6).

The microscope technology does not offer any insights about the genotype characterization of a given oocyst or cyst and it has a limit of oocysts and cysts detection, molecular based techniques offer alternative, sensitive and accurate way of detecting and characterize the oocysts and cysts from both water samples. These molecular techniques can help to identify the source of contamination in the water by the help of genotype and host-adapted Cryptosporidium spp (45,47) These molecular techniques including Nested PCR-sequencing, Nested PCR- RFLP and TaqMan real time PCR (43,48–51)

The Nested PCR is widely used to detect Cryptosporidium oocysts and Giardia cysts in water concentrate. Initially high quality DNA is extracted followed by two steps PCR, which amplify a portion of small-subunit (SSU) ribosomal RNA gene of Cryptosporidium spp and for molecular typing of Giardia spp, semi- nested PCR is performed to amplify a portion of ß-gene. Sequencing for both strands is performed for all successful PCR products for the genotype analysis (12,24–26,41,43,52–56).

Real time PCR is a method that allows for the logarithmic amplification of short strands of DNA and detection in a "real-time" by the reporting of fluorescent probes. This tool can be the alternative method for the microscopic based detection oocysts and cysts, this is because unlike Nested PCR, real time PCR can offer quantitative results. This tool can be the method of choice for many developing countries as it is cost effective and less labor intensive than using EPA method 1623. Many works have been done to develop and validate these methods so that it can be used for monitoring, detection and enumeration of Cryptosporidium spp and Giardia spp in different environmental samples (50,57–60).

Real Time PCR makes use of primer and fluorescent labeled TaqMan probe which target ß-giardia gene and COWP gene (Cryptosporidium oocysts wall protein gene). The real time PCR products are then sequenced and compared with available sequences in the Gene Bank database for genotyping analysis (41,57)

Real time PCR, on top of detecting Cryptosporidium oocysts and Giardia cysts, the method can be used to identify sources of fecal contamination in water bodies. The process is recognized as Microbial Source Tracking (MST). The method can detect the presence of oocysts and cysts and the sources of that fecal contamination to water bodies.

MST makes use of several host specific markers to distinguish human verses non human fecal pollution in water samples. MST gives a more complete picture of the land uses and environmental health risks associated with fecal pollution and thus for effective resources management. This method makes amplification of 16S rRNA marker sequence of the order Bacteriodales (2,61–65).

Reverse Transcription-PCR: This method has been developed to detect viable oocysts and cysts from environmental samples. Many developmental studies show this method to be more sensitive than a widely used IF microscope procedure, but their some variation from one laboratory to another. PCR inhibitors from environmental are the potential drawback for many molecular based works. The use of Oligo (dT) magnetic beads helps to overcome all potential RT-PCR inhibitors. RT-PCR detect mRNA from Cryptosporidium heat stable protein (hsp) to determine the presence and viability of the oocysts as only viable cells produce mRNA (66–68).

The method manages to detect even a single viable *C. purvam* oocyst from river water sample. Therefore this method can be a very useful tool for molecular epidemiology of *Cryptosporidium spp* and *Giardia spp* (68,69)

Situation in Tanzania

Tanzania like other developing countries has a major problem of having no access to basic water supply and sanitation both in rural and urban. Nationwide, about 93% of the population do not get water piped directly into their homes, and only 49% has access to improved water sources (for example, piped water nearby, public taps or borehole). Water sources can produce clean and safe water for drinking, but it commonly re-contaminated during collection, storage and use at home. In 2004 national surveys, estimated that in Tanzanian diarrhea is the leading cause of morbidity and mortality for children less than five years. The survey shows also children under five suffer between 3-4 episodes of diarrhea per year (70)

Cryptosporidium spp and *Giardia* spp are among enteric parasites in Tanzania, prevalence of cryptosporidiosis cases range from 5.4% to 35% and 7% to 20% for animal(calves aged <3months) and human(in HIV patients) respectively. (71–74). For giardiasis range from 6.9% to 16.4% in human, no clear data on giardiasis in animals (75,76).

Distribution of these parasites mainly associated with fecal pollution from both point and non-point sources which introduce waste directly into water sources or diffuse land base from human and livestock (2)

Studies in Tanzania reported the occurrence of these two parasites targeting humans and animals in different groups (71–76). There is a limited or not well documented data on the prevalence of *Cryptosporidium spp* and *Giardia spp* in water as a zoonotic diseases or it is not well known how a lack of access to clean water, sanitation and hygiene drives the transmission of this waterborne protozoan. It's important to note that *Cryptosporidium* and *Giardia* can exist in a body of water treated with chlorine (i.e., it is chlorine tolerant), and they can be transmitted through water in dormant, resistant forms known as oocysts (*Cryptosporidium*) and cysts (*Giardia*), which poses challenges for traditional chemical treatment of drinking and recreational water and for environmental surface cleaning (77)

On top of that increase in the population density around water sources in Tanzania and other developing countries, amount of extract from humans and animals which enter water source increased dramatically. This has negative

impacts of widespread contamination of water sources by humans and livestock is the spread of enteric zoonotic pathogens like *Cryptosporidium* spp and *Giardia* spp. Therefore, it is very important to carry out both prevalence and microbial source tracking studies targeting the two protozoa in diverse water sources, on top of routine fecal bacteria monitoring. By targeting different water sources will help to reveal how contaminated water contributes to the transmission of *Cryptosporidium* spp and *Giardia* between humans and livestock and other animals like wildlife or how humans and livestock contribute to the contamination of water sources. The gene-based qPCR TaqMan method or other related molecular technique can be validated and used to identify the *Cryptosporidium* spp and *Giardia* spp simultaneous and track their sources(23)

Conclusion

Cryptosporidium spp and *Giardia* spp remain one of causes of severe diarrhea in young, old, and immuno-compromised human and cattle populations both in developed and developing countries, and most of an outbreak associated with contaminated water. Occurrence of these enteric parasite on humans and animals and finally in the water sources impose serious concern in both animals and humans health. The developing countries which most of them have inadequate sewage system, many people lack access to clean and safe water. In other setting humans and animals including wildlife share the same water sources which might increase the likelihood of water fecal contamination. Genetic methods to track sources of fecal contamination should be incorporating in the routine fecal monitoring assays.

In spite of advances in the detection technology of *Cryptosporidium* and *Giardia* in water, these methods are not used in the routine monitoring assays.

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