Cryptosporidium and Giardia detection: Microscopy to genetic signature based methods

Cryptosporidium and *Giardia* are the most commonly occurring enteric protozoan parasites. These pathogens can cause gastrointestinal diseases that may lead to nutritional imbalances and severe health problems. The severity of disease is most often seen among children and patients with weakened immune system. *Cryptosporidium* has been the cause of multiple diarrheal outbreaks in the United States, Sweden, UK and in other developed and developing countries. In South Africa, it is estimated that 11 out of 110 children are infected with cryptosporidiosis. These parasites are protected by an outer shell that allow them to survive outside the body of infected host for long periods of time. Oocysts of *Cryptosporidium* and cysts of *Giardia* (thick-walled stage of the life cycle of protozoan parasites) can be found in different environments like surface and waste-water and are tolerant to chlorine disinfection. It is very important to detect these parasites and their different variants present in environment, to better protect human health.

The frequently used methods for *Cryptosporidium and Giardia* are based on the detection of typical oocysts/cysts in stool specimens. These methods are mainly based up on microscopy, antibody or enzyme-based methods. However, these traditional methods are labor-intensive, costly and/or often lacks specificity. The concerns related to conventional methods have escalated the need for the wide application and in-depth understanding of high-throughput molecular methods globally, especially in developing countries where sanitation and hygiene is a prime issue.

Genetic signature-based methods increase the specificity of detection methods, for examples, many genes detected in different environments represent species or genotype that are not virulent and cause no harm to humans. The in-depth knowledge of advanced molecular methods used to detect and quantify *Cryptosporidium* and *Giardia* genotypes will provide prospect to design and develop novel, quick and economical methods to reduce the risk to human health. In addition, the viability of pathogens is a crucial factor to assess potential health risk because it is only the viable population that can cause risk to human health. Many studies have reported the use of nucleic acid binding dyes based methods, for example, use of propidium monoazide (PMA) and ethidium monoazide (EMA) dyes can be used in combination with qPCR to address viability of protozoan parasites. Another simplified version of PCR termed as loop mediated isothermal amplification (LAMP) can also be tested for developing onsite detection system for these parasites. Genome wide sequencing, that is becoming more popular nowadays, and nanotechnology based biosensors will further increase our understanding in designing of appropriate genetic markers/methods for protozoan parasites. Some relatively new techniques are also emerging, like digital PCR and nano-biosensors which are useful and adding to our current knowledge in the field of detection and diagnosis but still require critical evaluation. Moreover, efforts should be made to expand the use of molecular tools in less privileged countries, where cryptosporidiosis and giardiasis are prevalent in human and animal populations and there are high chances of zoonotic transmission of these parasitic protozoans.

The use of advance methods in developing countries is still not very common and detection of parasites is mainly based on traditional methods. This is mainly due to the lack of research funding and expertise in molecular methods. Global research programs involving experts in this field from different countries, should be promoted to disseminate the knowledge and skills in developing countries. Different intensive program of scientific visits, students exchange, and technical workshops will be highly useful and will help students and researchers from under-privileged countries to gain necessary skills in advanced methodologies and find answer to the research questions in the field of detection of *Cryptosporidium* and *Giardia.*

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