

Chapter 64

Advancements in Parasite Diagnosis and Challenges in the Management of Parasitic Infections: A Mini Review



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Abstract Intestinal parasitic infections (IPIs) remain a widespread public health concern causing severe implications in both developed and developing countries. Globally, numerous studies have been carried out ranging from various communities to schoolchildren as well as indigenous communities. The infections are commonly caused by helminths (e.g. *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) and protozoa (e.g. *Blastocystis hominis*, *Cryptosporidium* sp., *Entamoeba histolytica* and *Giardia duodenalis*). Poor sanitation and poverty are some of the factors associated with IPIs. With the ever-increasing impact of IPIs, newer detection approaches have been developed and studied. The efficacy of diagnostic method is crucial to give an accurate identification of these parasites. Recent developments of diagnostic tools such as serology- and molecular-based assays are assisting the conventional method of microscopy in detecting and further confirming current or past infections and the specific species of parasites. Ongoing investigations in parasitic infections using these advanced tools will provide useful information that will enable the evaluation of the effectiveness of the current control program and thus, assist future planning for improved strategies in eradicating these parasitic infections.

Keywords Diagnostic tools · Helminths · Intestinal parasitic infections Management · Protozoa

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1 Introduction

The world's population has long been threatened by infectious diseases throughout the centuries. Currently, intestinal parasites are one of the major contributors to the global disease burden with a wide range of parasites that are reported to be prevalent around the world (Mehraj et al. 2008; Pullan et al. 2014; Mama and Alemu 2016), especially in sub-Saharan Africa, USA and Asia. Although these parasites are highly reported in underdeveloped countries, the emergence of intestinal parasitic infections (IPIs) has continued to compromise the quality of human life in developed nations. Infections are commonly caused by helminths (e.g. *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) and protozoa (e.g. *Blastocystis hominis*, *Cryptosporidium* sp., *Entamoeba histolytica* and *Giardia duodenalis*) resulting in significant morbidity and mortality, especially in endemic countries (Haque 2007). An estimation of 2 billion people are infected with intestinal parasites (Chan 1997) and the numbers have rapidly increased each year with 4 billion reported to be at risk in acquiring infections (Hotez et al. 2014). Extreme poverty, poor sanitation and social stigma as well as lack of education on the prevention and treatment are some of the factors contributing to these diseases (Liese et al. 2010). Furthermore, Basuni et al. (2012) had stated that the effects caused by IPIs depended on the species of parasites, the affected organ and the host immunological status. Although IPIs rarely cause death, the infection can impair the physical and mental growth, particularly among children (Varkey et al. 2007).

The challenges that arise due to the elevation of parasitic diseases have propelled newer, advance approaches and opportunities towards parasites diagnosis (Elsheikha 2014). In addition, latest techniques should be less time-consuming without compromising the quality of results. Therefore, rapid diagnosis is crucial and remained a top priority in determining the accurate identification of the parasites and eventually providing appropriate treatment as well as preventing fatalities among patients (Tavares et al. 2011). This mini review paper will briefly discuss the techniques commonly used in laboratory diagnosis with each method having its own advantages and disadvantages. The methods that are used for the diagnosis of several parasites that caused IPIs are further summarized in Table 1 as tabulated by Ndao (2009) and Ricciardi and Ndao (2015) with additional data by Wang et al. (2016).

1.1 Advancements in Parasite Diagnosis

For the past decades, various tests had been developed to increase the specificity and sensitivity in identifying parasites. The advancement of knowledge and technology had catapulted the diagnosing of parasitic infections to a new level. These techniques have been employed in numerous studies throughout the world, hence, enabling disease-combating efforts (Ricciardi and Ndao 2015).

Table 1 Diagnostic approaches for the detection of intestinal parasites

Helminth	Diagnostic approaches				References
	Microscopy-based	Serology-based	Molecular-based	Proteomics	
<i>Schistosoma</i> species	Using Kato-Katz technique	IHA, ELISA, dipstick	PCR, real-time PCR, multiplex PCR	LC-MS/MS	Katz et al. (1972); van Gool et al. (2002); ten Hove et al. (2008); Cnops et al. (2013); Sousa-Figueiredo et al. (2013); Lোধ et al. (2014); Wang et al. (2016)
Soil-transmitted helminths	Using sedimentation or concentration techniques	ELISA	Multiplex real-time PCR	–	Bungiro et al. (2005); Basuni et al. (2011)
<i>Taenia solium</i>	Using sedimentation or concentration techniques	ELISA, Immunoblot	Nested PCR	Mass Spectrometry	Del Brutto et al. (2001); Bueno et al. (2005); Deckers et al. (2008); Mayta et al. (2008)
<i>Protozoa</i>					
<i>C. parvum</i> , <i>C. hominis</i>	Using modified acid-fast staining	DFA, ICT assay kit	PCR, real-time PCR, multiplex real-time PCR, LAMP, Luminex	LC-MS/MS	Weber et al. (1991); Johnson et al. (1995); Johnston et al. (2003); Bandyopadhyah et al. (2007); Karanis et al. (2007); ten Hove et al. (2008); Jothikumar et al. (2008)
<i>Giardia lamblia</i>	Using trichrome or iron hematoxylin staining; Using concentration or sedimentation techniques	DFA, ICT assay kit	Multiplex real-time PCR	–	Danciger et al. (1975); Young et al. (1979); Garcia et al. (1997); ten Hove et al. (2007); Siddiki (2012)
<i>Entamoeba histolytica</i>	Using staining methods	IHA, IIF, ELISA, ICT assay	Multiplex real-time PCR, LAMP	LC-MS/MS	Hira et al. (2001); Gonin and Trudel (2003); Fotedar et al. (2007); Liang et al. (2009); Ali et al. (2012); Luca-Nacar et al. (2016)

C. Cryptosporidium, *IHA* indirect hemagglutination, *ELISA* enzyme-linked immunosorbent assay, *PCR* polymerase chain reaction, *LAMP* loop-mediated isothermal amplification, *DFA* direct fluorescent antibody, *ICT* immunochromatographic, *LC-MS/MS* liquid chromatography-tandem mass spectrometry

1.2 *Microscopy-Based Approach*

Routine laboratory diagnosis that includes conventional microscopy technique has been widely used for morphological identification of parasites and was then the only tool for the detection of parasites obtained from cerebrospinal fluid, faeces, blood smears and tissue specimens (Tavares et al. 2011). This method is commonly employed as it requires inexpensive reagents or dyes and using only the microscope alone. However, throughout the years, although microscopy examination is considered as a gold standard, it is rather difficult to determine or distinguish the species through naked eye as it is largely dependent on an experienced microscopist to ensure quality results and it consumes time to process starting from sample collection to concentration of the parasite's identification (Jamil et al. 2016). This situation can be further proven on the inability to distinguish *E. histolytica* and *E. dispar* through only morphological observation. Despite the disadvantage, dual techniques such as formalin-ether sedimentation, trichrome and Ziehl-Neelsen staining are usually applied together as conducted by Ngui et al. (2011) and Shahrul Anuar et al. (2013).

In addition, Kato-katz and McMaster counting methods are also common nowadays and regarded as a standard technique for the detection and quantification of IPIs for nearly forty years as reported by Komiya and Kobayashi (1966), Uga et al. (2002) and Belizario et al. (2015) and had since been recommended by WHO (1991). Meanwhile, McMaster counting method is extensively used to assess soil-transmitted helminths or STHs. It is also rather usual to include McMaster and Kato-katz technique in the same study as stated by previous studies (Pullan et al. 2010; Geiger et al. 2011; Periago et al. 2015). A study done by Levecke et al. (2011) revealed Kato-katz was more sensitive in detecting *A. lumbricoides* but not for *T. trichiura* and hookworm. Both methods were reported to have a considerable variation in sensitivity between different trials as Kato-Katz method covers larger quantity of stool but its drawback is when the infection intensity is rather low (Kongs et al. 2001) while McMaster technique is based on eggs flotation. Furthermore, both techniques are valid for diagnosis of IPIs with the latter being more suitable for further standardization due to its robust factor (Levecke et al. 2011).

1.3 *Serology-Based Approach*

Indirect identification of parasites using serology-based technique is employed if the parasite density is low or is unable to be directly demonstrated due to its life cycle in the host such as *Toxoplasma gondii* (Ambrosio and Waal 1990). The development of serology-based approach allows for faster and more practical diagnosis of IPIs that further provides an additional insight together with microscopic observation of the parasites. Serology-based diagnosis is further divided into

two categories namely antigen detection assays and antibody detection assays that include enzyme-linked immunosorbent assay (ELISA), hemagglutination (HA) test, indirect or direct immunofluorescent antibody (IFA or DFA), complement fixation (CF) test and immunoblotting and rapid diagnostic tests (RDTs) (Ndao 2009). ELISA test is the most popular antibody detection assay in laboratory diagnosis while dipstick assays have also considered to be a more practical choice due to its simplicity and achieved higher sensitivity as compared to microscopy in detecting intestinal schistosomiasis (Sousa-Figueiredo et al. 2013).

Other serology-based assays namely indirect hemagglutination (IHA) and indirect immunofluorescence (IIF) are commonly performed in laboratories due to its sensitivity but limited studies had been conducted to analyse their reproducibility (Lescure et al. 2010). Furthermore, immunoassays have also become a main tool in diagnosing parasites (Castelino 1986; Okangba et al. 2010). For the detection of *Giardia* and *Cryptosporidium*, several commercial kits available in the market use immunoassay-based technique to test the parasites using FITC-monoclonal antibodies that target cell wall antigens (Ricciardi and Ndao 2015). The results from the assay is easier to interpret and consume less time to perform the test. However, the disadvantage of serology-based approach is that the diagnosis is retrospective due to the presence of antibodies that varies in different periods after infection occurred (Ndao 2009; Ricciardi and Ndao 2015).

1.4 Molecular-Based Approach

Polymerase chain reaction (PCR) method has become an important tool in the quantification of parasites as well as determining the efficacy of treatment process. This approach offers greater sensitivity and specificity in comparison to the current diagnostic examinations. With the advancement of technology, traditional PCR has evolved to nested, multiplexed and real-time PCR. For protozoan infections, PCR assay has successfully detected *Cryptosporidium* from the environmental samples by targeting the 18S rRNA (Johnson et al. 1995). In addition, multiplex real-time PCR assay used to detect *E. histolytica*, *G. lamblia* and *C. parvum/C. hominis* was reported to be comparable to microscopy as mentioned by ten Hove et al. (2007) and allows to detect multiple sequences simultaneously within the same reaction tube. Previous study carried out by Basuni et al. (2011) has successfully detected four species of soil-transmitted helminths namely *Ancylostoma*, *N. americanus*, *A. lumbricoides* and *Strongyloides stercoralis* through a pentaplex real-time PCR method. Meanwhile, nested PCR has revealed 100% of sensitivity and specificity for the detection of *Taenia solium* DNA by targeting the *TSO31* gene (Mayta et al. 2008). Other previous findings showed that real-time PCR has proven to be sensitive in detecting *Giardia* and *Cryptosporidium* (oo)cysts (Guy et al. 2004; Gasser 2006). Conventional PCR-based method is rather time-consuming and does not provide quantitative data (Lin et al. 2000). However, although cost is a problem for multiplex PCR and real-time PCR, both have given rapid response as compared to

the conventional method (Tavares et al. 2011). Furthermore, restriction fragment length polymorphism (RFLP) is also one of the most commonly used approaches in diagnosing parasites such as *Toxoplasma gondii* (Quan et al. 2008; Tavares et al. 2011). This technique is based on the digestion of PCR products by restriction enzymes and proven to be suitable for environmental samples as it is able to detect multiple genotypes from the same sample (Monis and Andrews 1998).

Besides PCR-based method, several other amplification techniques have also been developed. Notomi et al. (2000) have introduced a novel gene amplification technique, loop-mediated isothermal amplification (LAMP) that has been used in numerous studies. The advantages of LAMP technique are in its ability to amplify DNA with high efficiency under isothermal conditions and highly specific for the target sequence (Notomi et al. 2000). The method is also deemed simple and easy to perform as it only requires four primers, DNA polymerase and a regular laboratory water bath or heat block for reaction (Tavares et al. 2011). In addition, with the combination of reverse transcription, LAMP is able to amplify RNA sequences with high efficiency. Moreover, reagents can be kept at room temperature without any post-PCR steps as mentioned in Ricciardi and Ndao (2015). LAMP has been applied for the detection of both DNA and RNA viruses such as West Nile and SARS viruses as stated from previous study by Parida et al. (2004) and Poon et al. (2005). Previous study was also carried out to compare LAMP with multiplex PCR from stool samples of patients with taeniasis (Nkouawa et al. 2010). Meanwhile, a recent study by Imai et al. (2017) reported a novel diagnostic approach in identifying human *Plasmodium* species by combining LAMP and MinION sequencer method.

In addition, proteomics work has also rapidly expanded in recent years in analysing proteins expressed by the parasites (Boersema et al. 2015). The current interest in proteomics had led researchers to overcome limitations of early diagnosis and treatment (Petricoin et al. 2002) and has since evolved in the need for sensitivity. The identification of proteins involved two approaches namely bottom-up and top-down. The top-down strategy involves a two-dimensional polyacrylamide gel electrophoresis (Ndao 2009). Several other diseases such as malaria (Nyunt et al. 2005), taeniasis (Deckers et al. 2008) and Chagas disease (Santamaria et al. 2014) have incorporated the study of proteomics. Another new approach named microsatellites consisted of simple sequence tandem repeats and have also been described in reports on parasites obtained from both humans and animals (Temperley et al. 2009) with the ability to mutate rapidly (Johnson et al. 2006). Microsatellites are considered useful genetic markers as it is highly polymorphic (Abdul-Muneer 2014). Meanwhile, Luminex-based assays have also emerged as a possible approach in diagnosing parasitic infections that combine flow cytometry, fluorescent beads, lasers and digital signal processing (Tavares et al. 2011; Chen et al. 2016). Luminex was performed in a study conducted by Bandyopadhyay et al. (2007) that is able to differentiate *C. hominis* and *C. parvum* species by a single nucleotide. The differentiation of both species cannot be distinguished by using antigen detection or other serology tests. The study of the assay improves speed, accuracy and reliability of other PCR methods (Tavares et al. 2011).

1.5 Challenges in Management of Parasitic Infections

Our human body is constantly exposed to parasites daily from our surroundings causing diseases to occur. Intestinal parasites which were once considered as harmless commensals are now shown to be potential pathogens (Lukes et al. 2015). Nowadays, it is quite a trend among researches to focus on improving the current diagnostic techniques rather than inventing a new method, hence, with further improvement of the procedures, more parasites can be detected simultaneously. The useful feature of mass screening and rapid diagnostic will improve the understanding of the parasites as well as to reduce transmission of disease (Yansouni et al. 2014). Besides, the development of field-based diagnosis is also necessary to avoid critical delays. However, sensitivity and specificity as well as cost are still an issue. Renewed and sustainable intervention must be carried out especially in endemic regions. There are various ways that can be implemented to enhance the status of public health, notably in the field of medical parasitology, throughout the world such as by incorporating proper guidelines or policies, monitoring, evaluating and strengthening parasitic disease surveillance (Colley 2000; CDC CDC 2012). There is a crucial need for the monitoring of anti-parasite drugs resistance and other alternatives in developing better treatment for patients. Improved awareness such as regular deworming (Traversa 2012) and other preventive measures need to be carried out consistently especially in targeted areas. Finally, increasing the funding towards parasitological research and interventions is also needed to improve and eradicate potential pathogens (Zilungile et al. 2012).

2 Conclusion

Microscopy-based technique still remains as a useful tool in diagnosing patients with parasitic infections, despite the overwhelming development of new approaches. However, serology- and molecular-based methods are considered as excellent alternatives especially in low range of parasitic infections. The ongoing investigations and current available techniques in detecting the diseases provide a better platform in developing more efficient, reliable and inexpensive methods, hence, improving the quality of life as well as future reductions in global disease burden. The implementation of these recommendations requires full commitment from higher authorities that includes public health and healthcare agencies, medical professionals and funding providers. The support from all stakeholders will boost the efforts in combating IPis. Future studies need to be carried out to further narrow the major gaps in science.

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