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Molecular epidemiology of *Giardia* and *Cryptosporidium* infections

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ABSTRACT

Giardia and *Cryptosporidium* are ubiquitous enteric protozoan pathogens of vertebrates. Although recognised as the aetiological agents of disease in humans and domestic animals for many years, fundamental questions concerning their ecology have been unresolved. Molecular tools have helped to better understand their genetic diversity and in so doing have helped to resolve questions about their transmission patterns and associated impacts on public health. However, the value of molecular tools is often complicated by questions concerning their applications, interpretation of results and terminology. Taxonomic issues have, until recently, made it difficult to determine the epidemiology of infections with both *Giardia* and *Cryptosporidium*. Similarly, improved understanding of their respective phylogenetic relationships has helped to resolve questions about zoonotic potential and distribution in wildlife. In the case of *Cryptosporidium*, imaging technologies have complemented phylogenetic studies in demonstrating the parasite's affinities with gregarine protozoa and have further supported its extracellular developmental capability and potential role as an environmental pathogen.

1. Introduction

Giardia and *Cryptosporidium* are ubiquitous protozoan parasites of the small intestine and stomach of vertebrates (Checkley et al., 2014; Fletcher et al., 2012; Thompson, 2011;). Their host range is broad and diverse, including all vertebrate groups. They have direct life cycles comprising an environmentally resistant infective stage, cyst or oocyst, which initiates infection following ingestion. The cyst and oocyst (Fig. 1) play essential roles in the plasticity of transmission routes available to both parasites and are the stages most frequently used in molecular epidemiological studies (Fletcher et al., 2012; Thompson, 2003, 2004), although trophozoites are expelled in the faeces in acute infections.

Asexual multiplication is the dominant means of proliferation in the gut and both *Giardia* and *Cryptosporidium* have clonal population structures (Tibayrenc and Ayala, 2014). Sexual reproduction is not a feature in the life cycle of *Giardia* and no mechanisms of genetic exchange have been identified. However, epidemiological evidence indicates that occasional bouts of genetic exchange may occur, particularly in circumstances where the frequency of transmission is high (Caccio and Sprong, 2010; Thompson and Monis, 2012). A sexual phase of gametogony does occur in the life cycle of *Cryptosporidium*, as in other apicomplexans.

Asexual multiplication allows rapid multiplication in the gut leading to acute, often asymptomatic infection, although chronic infections can occur. Clinically, the most significant impact of *Giardia* and *Cryptosporidium* is in the very young, particularly children and domestic animals (Checkley et al., 2014; FAO, 2014). Their importance as parasites of children in the developing world and disadvantaged communities has resulted in both giardiasis and cryptosporidiosis being considered neglected diseases (Hotez et al., 2015; Savioli et al., 2006). As such, *Giardia* and *Cryptosporidium* are common in areas that support the transmission of other parasites, particularly enteric protozoa and soil-transmitted helminths (Lymbery and Thompson, 2011). Thus *Giardia* and *Cryptosporidium* are rarely present as mono-infections in developing countries and the resultant polyparasitic scenarios exacerbate the clinical impact of individual parasites, and complicate diagnosis, treatment and control (Thompson and Smith, 2011; Thompson, 2015).

With both parasites, the host plays an important role in the clinical impact of infections and expression of disease. With *Giardia*, the nutritional status of the host is very important, particularly in young children with poor nutrition who may suffer failure to thrive (FAO, 2014; Thompson, 2015). In individuals with a compromised or deficient immune system, *Cryptosporidium* infections persist leading to intractable diarrhoea and potentially death (Checkley et al., 2014).

Drug treatment is inadequate for infections with both parasites, and does not provide a reliable strategy for control (Checkley et al., 2014; Fletcher et al. 2012; Leitsch, n.a). The few available drugs to treat *Giardia* require multiple doses and in the case of the most widely used drugs, the nitroimidazoles, there is often poor patient compliance, toxicity issues and adverse effects on the normal gut microflora (Thompson, 2011). There are no curative drugs to treat infections with *Cryptosporidium* (Checkley et al., 2014; Thompson et al., 2005).

In terms of control, there are different priorities in developed and developing countries. In the former, the need is for effective treatment for individuals, and the prevention of food and waterborne transmission. The latter is a significant issue for water utilities and the relevant

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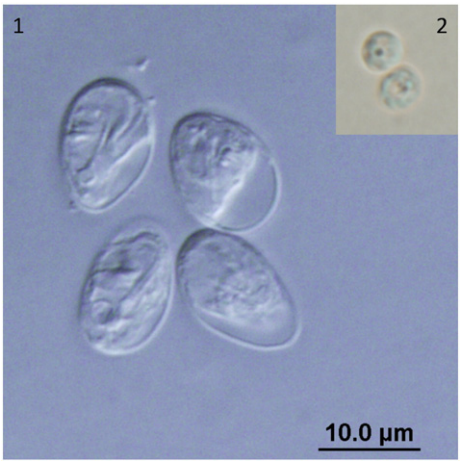

Fig. 1. Light microscopy images of *Giardia canis* cysts (1) from the faeces of a dog, and oocysts of *Cryptosporidium pestis* (2) from the faeces of an experimentally infected mouse.

authorities in terms of both economic and legislative aspects. In the developing world, the need is to lessen the burden of disease in those most at risk of infection, particularly children (Hotez et al. 2015).

The taxonomy of both *Giardia* and *Cryptosporidium* has been controversial since the early 20th century, and remains so today. This has principally been due to the broad host range of both parasites, and a paucity of reliable morphological features on which to define species. These problems have been resolved to some extent with the application of molecular tools. However, the most valuable contribution of these tools has been a better understanding of the epidemiology of infections with both parasites.

2. Molecular detection

The development and use of molecular tools to detect species of *Giardia* and *Cryptosporidium* has undergone great expansion in recent times and as a result so has the understanding of host ranges and transmission dynamics of these two protozoan parasites. Indeed, these morphologically indistinguishable parasites have been found to consist of numerous additional species solely through the use of molecular tools (Hopkins et al., 1997; Lalle et al., 2007; Monis et al., 1998; Reid et al., 2010; Ryan et al., 2003; Xiao et al., 1999; Yang et al., 2011).

Commonly targeted genes used for characterising species of *Giardia* include the small subunit ribosomal DNA (SSU-rDNA), the closely situated internal transcriber regions (ITS1-2), the *Giardia* specific ß-giardin, the triosephosphate isomerase (TPI) and the glutamate dehydrogenase (GDH) genes (Caccio et al., 2002; Hopkins et al., 1997; Lalle et al., 2005; Read et al., 2004; Sulaiman et al., 2003) (Table 1). The SSU-rDNA was one of the first genes commonly used for genotyping *Giardia* and gave rise to the realisation that *Giardia duodenalis* contained several assemblages (A–G) (Andrews et al., 1989; Hopkins et al., 1997; Monis et al., 1999) and more recently have been assigned species names according to host specificity (Monis et al., 2009; Thompson and Monis, 2004) (Table 2). Additional research using multiple genes in various combinations has consolidated this understanding and through the identification of intra-specific genetic variation has also highlighted the existence of sub-genotypes, particularly within *G. duodenalis* (Assemblage A) and *Giardia enterica* (Assemblage B) (Adam et al., 2013; Caccio et al., 2008; Sprong et al., 2009; Weilinga and Thompson, 2007; Wielinga et al., 2015). The significance of these sub-genotypes has gained importance as the question of zoonotic transmission continues to be unravelled.

As seen with *Giardia* the most commonly targeted gene used for characterising species of *Cryptosporidium* is the SSU-rDNA (Xiao, 2010) and has largely been responsible for the proliferation of new species and host ranges identified (Slapeta, 2013; Xiao and Fayer, 2008). A major research area has been concerned with those species commonly infecting humans (*Cryptosporidium hominis*, *Cryptosporidium pestis*; Fig. 3) and understanding the possible transmission routes from the environment and co-habiting animals such as companion animals and livestock (Fayer et al., 2000; Hunter and Thompson, 2005). Invariably this requires genotyping at additional genes which commonly include the 70 kDA heat-shock protein (HSP70), the *Cryptosporidium* oocyst wall protein (COWP) and the internal transcriber region 1 (ITS-1)

Table 1
Commonly targeted genes for the molecular characterisation of *Giardia* and *Cryptosporidium* species.

Gene/locus	Gene copy number	Reliable differentiation of species and sub-genotyping	Reported use and benefits of specific genes
Giardia sp.			
SSU-rDNA	Multiple	Species information	Commonly used
			Often provides greatest amplification success
ITS1-5.8S-ITS2	Multiple	Species information	Recently reintroduced to the literature
		Some sub-genotypic information obtained	Good amplification success
TPI	Single	Species information	Commonly used
		Sub-genotypic information	Variable amplification success
		Species specific primers designed	Useful for suspected mixed infection
ß-giardin	Single	Species information	Commonly used
		Sub-genotypic information	Variable amplification success
			Specific to *Giardia*
GDH	Single	Species information	Commonly used
		Sub-genotypic information	Variable amplification success
ef1-α	Single	Species information	Not commonly used
		Sub-genotypic information	Variable amplification success
Cryptosporidium sp.			
SSU-rDNA	Multiple	Species information	Commonly used
		Genotype information	Often provides greatest amplification success
ITS-1	Multiple	Species information	Not commonly used
		Genotype information	Good amplification success
HSP70	Single	Species information	Commonly used
		Genotype information	Good amplification success
GP60	Single	Species information	Commonly used
		Sub-genotypic information	Variable amplification success
COWP	Single	Species information	Commonly used
		Genotype information	Variable amplification success

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