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Research review paper

Giardia/giardiasis — A perspective on diagnostic and analytical tools

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ABSTRACT

Giardiasis is a gastrointestinal disease of humans and other animals caused by species of parasitic protists of the genus *Giardia*. This disease is transmitted mainly via the faecal–oral route (e.g., in water or food) and is of socioeconomic importance worldwide. The accurate detection and genetic characterisation of the different species and population variants (usually referred to as assemblages and/or sub-assemblages) of *Giardia* are central to understanding their transmission patterns and host spectra. The present article provides a background on *Giardia* and giardiasis, and reviews some key techniques employed for the identification and genetic characterisation of *Giardia* in biological samples, the diagnosis of infection and the analysis of genetic variation within and among species of *Giardia*. Advances in molecular techniques provide a solid basis for investigating the systematics, population genetics, ecology and epidemiology of *Giardia* species and genotypes as well as the prevention and control of giardiasis.

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Contents

1. Introduction . . . 281
2. Classical diagnostic methods . . . 281
 2.1. Microscopy . . . 281
 2.2. Immunological tools . . . 281
 2.3. Methods to recover or concentrate cysts . . . 282
3. Nucleic acid techniques for diagnosis or genetic analysis . . . 283
 3.1. Fluorescent in situ hybridisation (FISH) . . . 283
 3.2. Cyst viability . . . 284
 3.3. PCR-based methods for diagnosis and/or genetic analysis . . . 284
  3.3.1. DNA fingerprinting . . . 284
  3.3.2. Restriction fragment length polymorphism, specific PCR and sequencing . . . 284
  3.3.3. Mutation detection . . . 285
  3.3.4. Moderate to high throughput PCR-based approaches . . . 285
4. Conclusions . . . 286
Acknowledgements . . . 286
References . . . 286

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1. Introduction

Species of *Giardia* (Metamonada) are important parasitic protists that are transmitted by the faecal–oral route and cause gastroenteritis in vertebrates, including mammals, birds, reptiles and fishes (Adam, 2001; Filice, 1952; Thompson and Monis, 2004, 2012). In mammals, including humans, giardiasis is mainly caused by *Giardia duodenalis* (syn. *Giardia intestinalis* and *Giardia lamblia*) (Thompson, 2004). This disease is usually transmitted from human-to-human (anthroponotic) or animal-to-human (zoonotic) (Xiao and Fayer, 2008; Feng and Xiao, 2011). In agricultural animals, for instance, giardiasis can lead to morbidity and economic losses (Olson et al., 2004), although asymptomatic infections are common (Geurden et al., 2010b). In humans, giardiasis is usually a self-limiting illness, characterised by diarrhoea, colic, headache, dehydration, malabsorption, weight loss and/or wasting (Buret and Cotton, 2011). This disease particularly affects children and immuno-deficient or -compromised people (Muhsen and Levine, 2012; Stark et al., 2009; Thompson, 1994). Nonetheless, asymptomatic infections are common in apparently immuno-competent individuals, particularly in developing countries (e.g., Farthing et al., 1986; Feng and Xiao, 2011; Haque et al., 2005; Mason and Patterson, 1987). Chemotherapeutics, such as metronidazole, fenbendazole and febantel, are commonly used to treat clinical cases, while other drugs employed include albendazole, nitazoxanide, furazolidone, tinidazole, quinacrine and ornidazole (Escobedo and Cimerman, 2007; Gardner and Hill, 2001; Huang and White, 2006).

Outbreaks of human giardiasis are commonly associated with child day-care centres, contaminated drinking water or swimming pools (Baldursson and Karanis, 2011; Karanis et al., 2007). *Giardia* cysts are quite resistant to disinfectants, such as chlorine, routinely used for water treatment (Betancourt and Rose, 2004). Waterborne outbreaks of giardiasis have been reported in developed countries, including the USA, Canada and, more recently, Norway (e.g., Baldursson and Karanis, 2011; Karanis et al., 2007; Nygård et al., 2006), the most notable being the outbreak in Portland, Oregon, USA, in 1954, which resulted in ~50,000 human cases (Karanis et al., 2007). Additionally, *Giardia* is a common etiological agent of traveller's diarrhoea (Ross et al., 2013). Given the relative resilience of cysts in water and the environment (Olson et al., 1999), the cost of chemotherapeutic compounds or regimens for treatment or vaccination in animals (O'Handley and Olson, 2006) and the socioeconomic impact of giardiasis, *Giardia* is recognised as a key waterborne pathogen impairing health and development, and hindering socioeconomic improvement in developing countries (Savioli et al., 2006; WHO, 2011b).

The identification and characterisation of *Giardia* is central to investigating and understanding the epidemiology of giardiasis. However, there are significant limitations in detection or diagnosis using conventional microscopic, biochemical, immunological and serological techniques (Dixon et al., 1997; Thompson, 2004), such that there has been a need for reliable and practical molecular methods. Using genetic methods, seven recognised species and eight genotypes, called assemblages A–H, have been reported from various vertebrate host groups (Thompson and Monis, 2012), although assemblage H requires further verification. These species and genotypes cannot be distinguished based on host origin or parasite morphology (Feng and Xiao, 2011; Thompson and Monis, 2012). In the present article, we provide an account of some key microscopic and immunological methods used for the detection or identification of *Giardia*, and review nucleic acid-based approaches for the diagnosis of giardiasis and analysis of genetic variation within and among species of *Giardia*. We also describe the advantages and disadvantages of some techniques, and emphasise the benefits of using molecular tools to achieve a better understanding of the systematics, epidemiology and population genetics of members of the genus *Giardia*, underpinning the prevention and control of giardiasis in animal and human host populations. Topics that are beyond the scope of this paper can be found in key review articles of *Giardia*/giardiasis (Ankarklev et al., 2010; Appelbee et al., 2005; Feng and Xiao, 2011; Fletcher et al., 2012; Gardner and Hill, 2001; Huang and White, 2006; Monis et al., 2009; Ryan and Cacciò, 2013; Smith and Paget, 2007; Thompson, 2004; Thompson and Monis, 2012; Xiao and Fayer, 2008).

2. Classical diagnostic methods

2.1. Microscopy

Conventionally, the detection of *Giardia* cysts in duodenal, faecal, tissue, environmental and/or water (= biological) samples is achieved mainly by microscopic examination (e.g., Behr et al., 1997; de Souza et al., 2004; Dixon et al., 1997; Garcia, 2009; Goka et al., 1990; Huang and White, 2006; Mank et al., 1997; Sauch, 1985; Schuurman et al., 2007) (Fig. 1). A number of morphological features of the trophozoite, including median body shape and location, shape, ventrolateral disc length, length of caudal flagella, and number and shape of nuclei (Adam, 2001; Kulda and Nohynkova, 1978; Thompson and Monis, 2004), can be used for the identification of *Giardia*, but it is not possible to identify trophozoites or cysts to species by light microscopy (Thompson and Monis, 2004). Staining techniques can be employed to aid the detection of these stages of *Giardia*, and their differentiation from other microorganisms, protists and faecal or environmental debris (Fig. 1). The simplest stains include iodine (Smith and Paget, 2007; Wolfe, 1990) and iron-haematoxylin (Garcia, 2007), Giemsa (Ament, 1972; Wolfe, 1990) or trichrome (Thornton et al., 1983) (Fig. 1). Cysts can be concentrated using various methods which employ, for example, formalin-ether or formalin-ethyl acetate (Smith and Paget, 2007). Motile trophozoites can be detected by direct microscopic examination of fresh samples (smears prepared immediately with warm [37 °C] saline), while dead trophozoites can be detected in air-dried faecal smears stained, for instance, with Giemsa (Smith and Paget, 2007). Multiple, successive faecal samples should be taken and examined over a period of 1–2 weeks, because of the intermittent nature of cyst excretion (Garcia, 2009; Smith and Paget, 2007). Electron microscopy might be useful for the identification of some *Giardia* species (Adam, 2001), but is not applicable for routine use.

2.2. Immunological tools

The use of immunological methods (Fig. 1) can be advantageous over light microscopy for the detection of *Giardia* cysts or trophozoites in biological samples. For instance, the use of fluorescence microscopy and the direct fluorescence antibody (DFA) test using a fluorescein isothiocyanate-conjugated anti-*Giardia* monoclonal antibody (e.g., FITC-*G*-mAb), which recognises surface epitopes on cysts, has been reported to achieve relatively high specificity (99.8–100%) and sensitivity (93–100%) for the detection of cysts in faecal smears and environmental samples (Alles et al., 1995; Baig et al., 2012; Garcia and Shimizu, 1997; Geurden et al., 2008a; Grigoriew et al., 1994; Johnston et al., 2003; Lemos et al., 2005; Mekaru et al., 2007; Riggs et al., 1983; Rimhanen-Finne et al., 2007; Zimmerman and Needham, 1995).

The detection of *Giardia* antigens in faecal samples (i.e., copro-antigens) is another approach. Various enzyme-linked immunoassays (including ELISAs) have been used (Addiss et al., 1991; Aldeen et al., 1995, 1998; Aziz et al., 2001; Behr et al., 1997; Chan et al., 2000; Elsafi et al., 2013; Fedorko et al., 2000; Garcia and Shimizu, 1997; Goka et al., 1986; Green et al., 1985; Johnston et al., 2003; Katanik et al., 2001; Knisley et al., 1989; Maraha and Buiting, 2000; Mekaru et al., 2007; Nash et al., 1987; Rosenblatt et al., 1993; Rosoff et al., 1989; Scheffler and Van Etta, 1994; Schunk et al., 2001; Schuurman et al., 2007; Stibbs et al., 1988; Ungar et al., 1984; Weitzel et al., 2006; Yolken and Ungar, 1985; Zimmerman and Needham, 1995) and have achieved specificities of 87–100% and sensitivities of 63–100%; in addition, immuno-chromography (IC) tests (Costache et al., 2009;

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