International Journal for Parasitology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

The affinity of magnetic microspheres for *Schistosoma* eggs

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ARTICLE INFO

Article history:
Received 5 June 2014
Received in revised form 7 August 2014
Accepted 9 August 2014
Available online xxxx

Keywords:
Schistosomiasis
Diagnosis
Low endemicity
Helmintex™
Magnetic properties

ABSTRACT

Schistosomiasis is a chronic parasitic disease of humans, with two species primarily causing the intestinal infection: *Schistosoma mansoni* and *Schistosoma japonicum*. Traditionally, diagnosis of schistosomiasis is achieved through direct visualisation of eggs in faeces using techniques that lack the sensitivity required to detect all infections, especially in areas of low endemicity. A recently developed method termed Helmintex™ is a very sensitive technique for detection of *Schistosoma* eggs and exhibits 100% sensitivity at 1.3 eggs per gram of faeces, enough to detect even low-level infections. The Helminthex™ method is based on the interaction of magnetic microspheres and schistosome eggs. Further understanding the underlying egg-microsphere interactions would enable a targeted optimisation of egg-particle binding and may thus enable a significant improvement of the Helmintex™ method and diagnostic sensitivity in areas with low infection rates. We investigated the magnetic properties of *S. mansoni* and *S. japonicum* eggs and their interactions with microspheres with different magnetic properties and surface functionalization. Eggs of both species exhibited higher binding affinity to the magnetic microspheres than the non-magnetic microspheres. Binding efficiency was further enhanced if the particles were coated with streptavidin. *Schistosoma japonicum* eggs bound more microspheres compared with *S. mansoni*. However, distinct differences within eggs of each species were also observed when the distribution of the number of microspheres bound per egg was modelled with double Poisson distributions. Using this approach, both *S. japonicum* and *S. mansoni* eggs fell into two groups, one having greater affinity for magnetic microspheres than the other, indicating that not all eggs of a species exhibit the same binding affinity. Our observations suggest that interaction between the microspheres and eggs is more likely to be related to surface charge-based electrostatic interactions between eggs and magnetic iron oxide rather than through a direct magnetic interaction.

1. Introduction

Schistosomiasis is a public health problem that affects more than 200 million people in 74 countries in Africa, South America and Asia, with 10% of the affected persons presenting the severe form of the disease, and up to 60% presenting clinical manifestations (World Health Organization (WHO), 1993). The disease is caused by helminths of the genus *Schistosoma*, with three species causing most of the infections in humans: *Schistosoma mansoni* and *Schistosoma japonicum*, responsible for the hepato-intestinal manifestations, and *Schistosoma haematobium* which causes genitourinary infection. Despite efforts to control this infection, which are based on treatment of infected people with appropriate and effective chemotherapies such as praziquantel (Davis, 1993; Savioli et al., 1997), schistosomiasis remains the second most widespread parasitic infection globally after malaria (Chitsulo et al., 2000).

Currently, schistosomiasis infections are diagnosed through direct visualisation of eggs of characteristic shape in faecal

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http://dx.doi.org/10.1016/j.ijpara.2014.08.011
0020-7519/

samples. The eggs are formed by female schistosomes in the ootype (egg mould), a bottle-neck in the reproductive tract that forms eggs one by one (DeWalick et al., 2012), with a production of 100–300 eggs per day for *S. mansoni* and 500–3500 for *S. japonicum* (Pittella, 1997). To continue the life cycle, the egg must migrate from the mesenteric vasculature across the endothelial and mucosal barriers to the lumen of the intestine with subsequent excretion. When the eggs are not detected by coproparasitological examination in areas of low transmission or after mass drug administration, the true prevalence of the infection in the studied population cannot be established. Cases of misdiagnosis in areas of low or very low transmission intensities and low worm burdens currently occur worldwide because the available diagnostic techniques are too expensive to be used on a greater scale or are not sensitive enough to detect low egg burdens in the stool, leading to a number of false-negative diagnoses (Engels et al., 1996; Enk et al., 2008), which can result from daily variations in egg excretion within an individual (Van Etten et al., 1997).

Several molecular methods (Pontes et al., 2002; Sandoval et al., 2006; Ten Hove et al., 2008) and immunological methods (Deelder et al., 1989, 2000; De Jonge et al., 1990; Doenhoff et al., 2004) and have been developed in an attempt to improve diagnostic sensitivity. Molecular methods are based on the amplification of a highly repeated parasite DNA sequence using PCR for human samples, but those techniques require a well-equipped laboratory and proper skills to perform them. Antibody detection tests provide information about whether an individual has been exposed to the parasite. However, their specificity for detection of active infections is limited since specific antibodies in the host, once developed against *Schistosoma* spp., are long-lived and therefore could often be present in individuals who have already cleared the infection (Sturrock, 2001). The most common immunological method used is the ELISA which consists of detecting host antibodies to a parasités antigens. However, use of the ELISA also requires well-trained people, and some authors have shown cross-reactivity between diagnostic antigens for schistosomiasis and antigens of other parasites (Correa-Oliveira et al., 1988; Valli et al., 1997).

Errors in assessing prevalence and intensity of infection are exaggerated due to an inherent lack of sensitivity and accuracy in common diagnostic techniques, particularly in areas with low prevalence and in individuals in the earliest or latest stages of schistosome infection. The problem will thus be further exacerbated when the prevalence and intensity of schistosomiasis is being reduced through the introduction of effective control measures (Hamilton et al., 1998). The importance of diagnosing individuals with undetected infections due to a low parasite burden is highlighted by the following considerations: (i) the degree of pathology and the egg count are not always correlated; (ii) undetected and untreated infections may be responsible for the persistence of transmission; (iii) the proportion of missed infections increases after chemotherapy, which overestimates cure rates and, (iv) persistent light infections may maintain concomitant immunity leading to acquired resistance, which interferes with vaccine trials and with conventional treatment (DeVlas and Gryseels, 1992).

The WHO currently recommends the Kato-Katz thick smear technique for diagnosing intestinal schistosomiasis infection in epidemiological studies. The Kato-Katz technique has the advantage of being a simple, low-cost procedure, and allows for quantification of egg loads (Katz et al., 1972). However, due to the relatively small amount of faecal matter observed with this method, it lacks sensitivity. This leads to an underestimated number of positive cases and, thus, an inaccurate measurement of the prevalence of the disease, especially in areas of low endemicity (Ebrahim et al., 1997; Zhanga et al., 2009).

The Helmintex™ technique is a very sensitive method for detection of *Schistosoma* eggs by isolating the eggs from a larger volume of faeces. Helmintex™ is based on the interaction of the eggs with magnetic particles and this novel method has been shown to exhibit 100% sensitivity for egg intensities of 1.3 eggs per gram of faeces (epg) (Teixeira et al., 2007).

Studies have shown that eggshells of *S. japonicum* and *S. mansoni* contain iron (Jones et al., 2007; Karl et al., 2013). The iron is believed to help stabilization of the proteins that form the eggshells (Jones et al., 2007). Recently, we provided the first magnetic characterisation of eggshells of *Schistosoma* spp., showing that, despite the shells containing paramagnetic iron compounds, the interaction between magnetic particles and the eggs is unlikely to be purely magnetic in origin (Karl et al., 2013). Mediators of the interactions were postulated to be surface elaborations of the shells, notably the microspines, demonstrated in earlier studies (Ford and Blankespoor, 1979).

In order to clarify the properties responsible for the interaction between the eggs and the microspheres and to optimise the Helmintex™ method, we characterise here the affinity of *S. mansoni* and *S. japonicum* eggs for a variety of polystyrene microspheres using direct microscopic observations and Poisson distribution analysis of the numbers of microspheres bound to eggs.

2. Materials and methods

2.1. Maintenance of the parasite life cycles

Schistosoma mansoniand *S. japonicum* were maintained at the QIMR Berghofer Medical Research Institute, Australia by passage in Swiss mice and *Biomphalaria glabrata* snails for *S. mansoni*, and *Oncomelania hupensis hupensis* snails collected in Anhui Province (China) for *S. japonicum*. The use of animals was approved by the Animal Ethics Committee of the QIMR Berghofer Medical Research Institute (Project P1289). The experiments were conducted in the School of Physics, The University of Western Australia, Perth, Australia, and in the QIMR Berghofer Medical Research Institute, Brisbane, Australia.

2.2. Acquisition of parasites' eggs

Mice infected with either *S. mansoni* or *S. japonicum* were euthanased at approximately 42 days p.i. and the livers were removed for digestion with collagenase B in PBS overnight at 37 °C. The following day, samples were sieved for isolation of the eggs and purified using Percoll density gradient centrifugation as described by Dalton and colleagues (Dalton et al., 1997). The eggs were stored in PBS at −80 °C until use.

2.3. Incubation of eggs with microspheres

We used four types of commercial polystyrene microspheres with diameters of ~4 μm, schematically represented in Fig. 1 (Spherotec Inc, USA). The microspheres comprised uncoated polystyrene microspheres (PP-40); magnetite-coated polystyrene microspheres (PM-40); streptavidin-coated polystyrene microspheres (SVP-40) and magnetite-streptavidin-coated polystyrene microspheres (SVM-40).

Streptavidin was chosen as ligand for both magnetic and non-magnetic particles due to its capability to bind schistosome eggs (Teixeira et al., 2007). The eggs and microspheres were incubated in 1.5 mL microtubes (Eppendorf, USA) and mixed by agitation using a Rotary Suspension Mixer (Ratek Lab, Australia) at pH 7 for 30 min. A custom-made filter of 42 μm pore size was cut and glued to the end tips of the microtubes in an attempt to try to remove as many unbound particles as possible. The mixture of microspheres and eggs was placed inside the microtubes with

Please cite this article in press as: Candido, R.R.F., et al. The affinity of magnetic microspheres for *Schistosoma* eggs. Int. J. Parasitol. (2014), http://dx.doi.org/10.1016/j.ijpara.2014.08.011