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Lectin-magnetic separation (LMS) for isolation of *Toxoplasma gondii* oocysts from concentrated water samples prior to detection by microscopy or qPCR

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ABSTRACT

Although standard methods for analyzing water samples for the protozoan parasites *Cryptosporidium* spp. and *Giardia duodenalis* are available and widely used, equivalent methods for analyzing water samples for *Toxoplasma gondii* oocysts are lacking. This is partly due to the lack of a readily available, reliable immunomagnetic separation technique (IMS). Here we investigated the use of lectin-magnetic separation (LMS) for isolating *T. gondii* oocysts from water sample concentrates, with subsequent detection by microscopy or molecular methods. Four different types of magnetic beads coated with wheat germ agglutinin (WGA) were tested for capture of oocysts from clean or dirty water samples. Dynabeads (Myone T1 and M-280) consistently provided mean capture efficiencies from 1 ml clean water in excess of 97%. High recoveries were also found with Tamavidin beads (in excess of 90%) when LMS was used for capture from a small (1 ml) volume. Dissociation (required for detection by microscopy) using 0.1N hydrochloric acid (HCl), as standard in IMS, was not successful, but could be achieved using a combination of acidified pepsin (AP) and *N*-acetyl D-glucosamine. Although simple centrifugation was as effective as LMS when concentrating high numbers of oocysts from clean water, LMS provided superior results when oocysts numbers were low or the water sample was dirty. Application of LMS integrated with qPCR enabled detection of 10 oocysts per 10 ml dirty water sample concentrate. These findings indicate that LMS with WGA coupled to magnetic beads could be an efficient isolation step in the analysis of water sample concentrates for *T. gondii* oocysts, with detection either by microscopy or by qPCR.

1. Introduction

The protozoan parasite *Toxoplasma gondii* has a worldwide distribution and is one of the most frequent parasitic infections (Dubey, 2004; Robert-Gangneux et al., 2015). Felids serve as definitive hosts and the environmentally resistant oocysts are excreted in their feces (Hutchison et al., 1969; Frenkel et al., 1970). Oocysts become infectious after sporulation in the environment and may survive for months in soil and water, thereby enhancing the probability of transmission to intermediate hosts. Almost all warm-blooded animals, including humans, can serve as intermediate hosts harboring *T. gondii* in the form of tissue cysts (Dubey, 2010).

Toxoplasmosis is a significant public health concern as infection can lead to serious consequences (Aramini et al., 1999), especially when the infection is acquired prenatally due to infection of naïve pregnant women shortly before or after conception. Such infection can lead to congenital toxoplasmosis, with consequences ranging from gross fetal abnormalities and spontaneous abortion to neonates being asymptomatic at birth but manifesting problems such

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as mental retardation and ocular disease much later in life (Gajadhar et al., 2006). *Toxoplasma* infections can also be acquired postnatally through various routes, including direct contact with cat feces containing sporulated oocysts, ingestion of sporulated oocysts from contaminated food or water or ingestion of tissue cysts in raw or undercooked meat (Dubey, 2010; Fayer et al., 2004). Infection in immunocompromised individuals can be life threatening, with encephalitis and brain abscess among the most serious manifestations (Hill et al., 2005). Although most infections in healthy individuals are asymptomatic, certain *T. gondii* strains (genotype 1) may cause symptoms in otherwise immunocompetent humans (Grigg et al., 2001).

Waterborne toxoplasmosis has emerged as a significant public health concern following the occurrences of large human outbreaks linked to oocyst contamination of water supplies as a source of infection in several countries (Jones and Dubey, 2010; Shapiro et al., 2010). Circumstantial evidence suggests that oocyst-induced infections in humans are clinically more severe than tissue cyst-acquired infections (Jones and Dubey, 2010). Oocysts can remain viable for longer periods in water and resist both freezing and moderately high water temperatures (Dubey, 1998; Frenkel and Dubey, 1973). In addition, oocysts are able to survive the effects of chemical and physical treatments currently applied in water treatment plants, including chlorination and ozone treatment (Dumetre et al., 2008).

Detecting contamination of water supplies with *T. gondii* oocysts is difficult because no standardized methods are available. For other protozoan parasites associated with waterborne transmission, *Cryptosporidium* spp. and *Giardia duodenalis*, standardized methods have been developed and are applied widely (e.g., ISO, 2006; US EPA, 2012). These methods were developed from relatively crude techniques involving high volume filtration, elution, concentration, and detection by light microscopy to the development and use of immunofluorescent antibody tests for detection in the late 1980s (e.g., Hayes et al., 1989) to the more recent use of molecular assays for genotyping. One important improvement in the analytical procedure was the development of immunomagnetic separation (IMS) for isolation of *Cryptosporidium* oocysts from water in the late 1990s (Campbell et al., 1997). Use of IMS, enabled oocysts to be separated from background debris, improving detection efficiency. However, there are currently no commercially available IMS techniques or immunofluorescent staining reagents for *T. gondii* oocysts (Dumetre and Darde, 2005).

Recent investigations on the surface-binding properties of *T. gondii* oocysts have indicated that they can bind to the lectin wheat germ agglutinin (WGA), and it has been suggested that this binding could be utilized to develop a lectin-magnetic separation (LMS) technique for improving the analysis of water samples, and other environmental samples, for *T. gondii* oocysts (Harito et al., 2016).

The current study investigates the performance of different magnetic beads coupled to WGA in the capture and separation of *Toxoplasma* oocysts from water samples (LMS) and detection using both microscopy and real-time PCR.

2. Materials and methods

2.1. Oocyst spiking and recovery

2.1.1. T. gondii oocysts

Oocysts used in this study were from the ME 49 strain, as described previously, and were stored as described earlier (Harito et al., 2016).

Sporulation was induced by incubating aliquots of oocyst suspension at room temperature (15–25 °C). After confirming oocyst sporulation, all suspensions were kept refrigerated in the dark at 4 °C until the current study. Before use, aliquots of oocyst suspension (100 μl) were resuspended in 900 μl of 0.5% acidified pepsin, incubated at 37 °C for 24 h and washed four times in 0.1% BSA in PBS. Treated oocysts were then resuspended in 1 ml 0.1% BSA in PBS.

Oocyst enumeration was performed using KOVA GLASSTIC Slide 10 with grids (Fisher Scientific).

2.1.2. Magnetic beads and their preparation

Four different magnetic beads were investigated (Table 1). Three different sizes of beads coated with streptavidin or avidin (Dynabeads™ M-280 Streptavidin and Myone™ Streptavidin T1; ThermoFisher Scientific, Oslo, Norway and SPHERO™ Avidin magnetic particles; Spherotech Inc, Lake Forest, IL, USA) were used to investigate whether dissociation could be improved by the increased shear sources with larger beads. In addition Tamavidin-coated beads (Tamavidin® 2-REV magnetic beads (Wako Pure Chemical Industries, Ltd., Japan)) were also investigated due to the lower biotin dissociation constant being hypothesized to improve dissociation (streptavidin and avidin have $K_D \approx 10^{-15}$ M; tamavidin $K_D \approx 10^{-7}$ M).

2.1.3. Lectins

Wheat germ agglutinin lectin from *Triticum vulgaris* (wheat) conjugated to biotin (Sigma-Aldrich Norway AS), suspended in phosphate buffered saline (PBS, pH 7.4) at a concentration of 1 mg/ml was used for coupling with magnetic beads pre-coated by one of the biotin-binding proteins (Streptavidin or Avidin or Tamavidin 2-REV).

All beads (Table 1) were prepared according to the manufacturers' protocols, and then bound to biotin-WGA, by incubating for 1 h on a rotating mixer at 15 r.p.m. as follows: for the Myone Streptavidin T1 beads, 40 μl of Biotin-WGA was added to 200 μl 10 mg/ml stock beads; for the other three beads (M-280 Streptavidin beads, the avidin beads and the Tamavidin beads) 20 μl of Biotin-WGA was added to 200 μl 10 mg/ml stock beads. After coating, the beads were washed four times in 1 ml of washing buffer (PBS, pH 7.4) using a magnet (Dynal MPC-S, ThermoFisher Scientific, Oslo, Norway) to separate beads from the supernatant and then re-suspended back to a concentration of 10 mg/ml.

2.1.4. Isolation of bead-bound oocysts (LMS)

2.1.4.1. Capture in clean water. This experiment was evaluated in small (1 ml) and large (10 ml) volumes. All tests were performed in independent triplicates. Tests using 1 ml volume (clean water) were performed to assess all beads (Table 1) using different incubation buffers (0.1% BSA in PBS and SL Buffer, Dynal GC Combo kit). In order to investigate capture and recovery, 20 μl aliquots of the suspension containing an estimated 1000 oocysts was spiked into 970 μl incubation buffer (0.1% BSA in PBS or SL buffer Dynal GC Combo kit) in a 1.5 ml microfuge tube and 10 μl of prepared beads (Dynabeads or SPHERO Avidin) were added. The mixture was incubated for 1 h at room temperature on a rotating mixer at 15 r.p.m. For the Tamavidin 2-REV magnetic beads, the amount

Table 1
Characteristics of the beads tested in this study for LMS following coating with WGA.

Beads	Bead diameter (μm)	Concentration used in LMS
Dynabeads™ M-280 Streptavidin	2.8	100 μg/ml
Dynabeads™ Myone Streptavidin T1	1.0	100 μg/ml
SPHERO™ Avidin beads	4.0–4.5	100 μg/ml
Tamavidin® 2-REV beads	2.8	250 μg/ml