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Surface binding properties of aged and fresh (recently excreted) *Toxoplasma gondii* oocysts

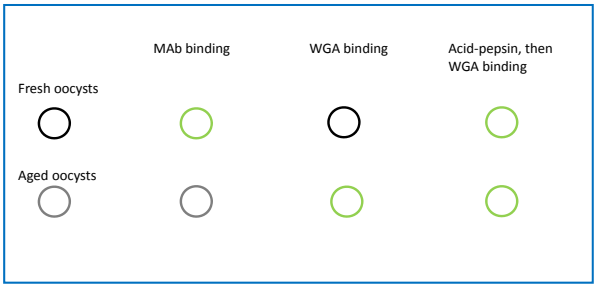
Jemere Bekele Harito [a], Andrew T. Campbell [b], Kristin W. Prestrud [c], J.P. Dubey [d], Lucy J. Robertson [a, *]

[a] Parasitology Laboratory, Section for Microbiology, Immunology and Parasitology, Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences, P.O. Box 8146 Dep, N-0033 Oslo, Norway
[b] Alere A.S., Oslo, Norway
[c] Norwegian Kennel Club and Norwegian University of Life Sciences, Faculty of Veterinary Medicine and Biosciences, Department of Companion Animal Clinical Sciences, P.O. Box 8146 Dep, N-0033 Oslo, Norway
[d] United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Building 1001, BARC-East, Beltsville, MD 20705-2350, USA

HIGHLIGHTS

- Age of Toxoplasma oocysts affects surface-binding properties.
- Fresh oocysts bound monoclonal antibody, but not old oocysts.
- Old oocysts bound wheat germ agglutinin (WGA), but not fresh oocysts.
- Treating fresh oocysts with detergent or trypsin did not affect WGA binding.
- Fresh oocysts bound WGA after treatment with acidified pepsin.

GRAPHICAL ABSTRACT



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ABSTRACT

The surfaces of aged (10 years) and fresh (recently excreted) oocysts of *Toxoplasma gondii* were investigated using monoclonal antibody (mAb) and lectin-binding assays. Fresh oocysts bound a wall-specific mAb labelled with fluorescein isothiocyanate while aged oocysts did not. In contrast, the walls of aged oocysts bound a lectin (wheat germ agglutinin, WGA), but not the walls of fresh oocysts. Exposure of oocysts to detergent solutions or trypsin did not affect the binding properties of the walls of the oocysts. However, exposure of fresh oocysts to acidified pepsin enabled labelling of the walls with WGA, presumably due to the relevant moieties on the oocyst walls becoming exposed. WGA binding, but not mAb binding, was partially abrogated with periodate exposure. These findings reveal a significant difference in the binding properties of oocyst walls from "aged" and "fresh" oocysts. The results are of relevance when considering technologies for isolating or detecting *T. gondii* oocysts in environmental samples based on oocyst surface properties, as used for other protozoan parasites. Our results suggest the possibility of developing a WGA-based separation procedure for isolating *Toxoplasma* oocysts from environmental matrices, in which pepsin pre-treatment would be included to ensure that both fresh and aged oocysts were isolated.

* Corresponding author.
E-mail address: lucy.robertson@nmbu.no (L.J. Robertson).

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1. Introduction

Toxoplasma gondii is a protozoan parasite that infects humans and other warm-blooded animals, including birds and marine mammals worldwide (Dubey, 2010). The life cycle of the parasite consists of asexual reproduction in the intermediate hosts and sexual reproduction in the intestinal mucosa of the definitive host. Felids (wild and domestic) are the only known definitive hosts and therefore serve as the main reservoir of infection shedding millions of oocysts in their faeces (Frenkel et al., 1970; Dubey et al., 1970). There are three infectious stages of the parasite for all hosts: tachyzoites (the rapidly dividing form) in tissues, bradyzoites (the slowly dividing form) inside cysts in tissues, and sporozoites in the oocyst (Jones and Dubey, 2010; Petersen and Dubey, 2001).

Postnatal transmission occurs by eating raw or undercooked meat containing viable tissue cysts, or ingestion of water, soil, or food contaminated with sporulated oocysts derived from the environment (Jones and Dubey, 2010; Dumetre and Darde, 2003; Dubey, 2004). A foetus may be infected by tachyzoites crossing the placental barrier, possibly leading to congenital defects (Sullivan and Jeffers, 2012). *T. gondii* oocysts are highly resistant to the environment and can remain viable in water sources for years. Some published reports suggest that *T. gondii* oocysts can survive both chemical and physical inactivation steps often employed in drinking water treatment processes (Dumetre and Darde, 2003; Kuticic and Wikerhauser, 1996; Wainwright et al., 2007a, 2007b) and sporulated *T. gondii* oocysts survived in seawater for up to 2 years (Lindsay and Dubey, 2009).

Toxoplasmosis resulting from ingestion of oocysts can be clinically severe, and epidemics of waterborne toxoplasmosis have been reported from several countries (Dardé et al., 1998; Benenson et al., 1982; Bahia-Oliveira et al., 2003; Keenihan et al., 2002; de Moura et al., 2006; Gattás et al., 2000; Heukelbach et al., 2007; Dubey et al., 2012; Bowie et al., 1997; Aramini et al., 1999; Burnett et al., 1998. Balasundaram et al., 2010; Hall et al., 1999; Palanisamy et al., 2006). Despite the substantial risks and health implications of waterborne toxoplasmosis in humans and animals, detection of *T. gondii* oocysts in environmental samples (water, soil, fruits or vegetables) is a considerable challenge due to the lack of sensitive and specific methods (Jones and Dubey, 2010; Dumetre and Darde, 2003; Shapiro et al., 2009, 2010). Standard methods for detection of other protozoan parasites, such as *Cryptosporidium* oocysts and *Giardia* cysts, in environmental matrices have been developed, standardized, and adopted globally (Anonymous, 2006, 2012, 2015). These methods rely on specific isolation techniques, such as immunomagnetic separation (IMS), and similarly specific detection techniques such as immunofluorescent antibody tests (IFAT). Other approaches, including fluorescence-activated cell sorting and polymerase chain reaction, have shown some promise in detecting protozoa from complex environmental matrices (Dumetre and Darde, 2003), but do not compete with IMS and IFAT regarding sensitivity. However, neither IMS nor IFAT are currently commercially available for *T. gondii* oocysts (Jones and Dubey, 2010.).

Improved knowledge on the antigenic and biochemical composition of the oocyst wall will provide valuable information for developing similar techniques for *T. gondii* oocysts. It should be noted that although the oocysts of *Toxoplasma* are very robust, like *Cryptosporidium* oocysts and *Giardia* cysts, an important difference is that *Toxoplasma* oocysts need time to sporulate in the environment. This implies that metabolic processes are ongoing within the *Toxoplasma* oocyst, and therefore may not be as inert as the transmission stages of *Cryptosporidium* and *Giardia*.

The aim of this study was to investigate the surface binding properties of *T. gondii* oocysts using antibody-binding and lectin-binding studies, and to investigate whether the surfaces altered with ages of oocysts and following different treatments. The information obtained could be of value for developing methods based on the oocyst surfaces for concentrating and detecting them in environmental matrices.

2. Materials and methods

2.1. T. gondii oocysts

Two batches of oocysts were included in this study. Both batches of oocysts were genotype II and obtained from infection of *T. gondii* free cats at the USDA facility in Beltsville, Maryland. The procedures for collection and purification of oocysts have been described previously (Dubey, 1995, 2010).

Batch 1 oocysts, derived from an Arctic fox strain, were collected in 2005 (Prestrud et al., 2008). They were sporulated in 2% sulphuric acid at room temperature for 1 week and then stored at 4 °C. Based on characteristic UV autofluorescence, this population of oocysts was heterogeneous, with autofluorescence that ranged from an extremely faint, ghost-like appearance to a dense blue UV emission. They contain two ellipsoidal sporocysts and appear subspherical or ellipsoidal in shape. Throughout the text, these oocysts are referred to as “aged oocysts”.

Batch 2 oocysts, derived from the ME 49 strain, were collected on 13 January 2015, suspended in water, and kept cold to prevent sporulation. The oocysts were unsporulated when received in Norway within 2 days of shipment from USA. The characteristic UV autofluorescence was less intense than that of the aged oocysts, but there was greater homogeneity within the population, with the majority (>99%) of oocysts showing similar characteristic autofluorescence. Throughout the text, these oocysts are referred to as “fresh oocysts”.

2.2. Storage of oocysts

Upon arrival at the parasitology laboratory in Oslo, the stock of fresh oocysts was subdivided into 500 μl suspensions each containing approximately 5×10^7 oocysts. These were re-suspended in either 4.5 ml laboratory-grade water, 4.5 ml 2% sulphuric acid, or 4.5 ml 1% potassium dichromate solutions. All suspensions were kept refrigerated in the dark at 4 °C. The aged oocysts were held refrigerated in 2% sulphuric acid.

Oocysts were washed (tubes centrifuged at 4000 rpm (~1560 *g*) for 3 min and the supernatant removed) at least four times using laboratory-grade water before being used in the various experiments. During and following experiments and washing procedures all potentially contaminated liquids were collected into 50 ml tubes that were subsequently sealed and disposed of by incineration. Oocyst enumeration was performed both using KOVA GLASSTIC Slide 10 with grids (Fisher Scientific).

2.3. Pre-treatments of oocysts prior to binding assays

2.3.1. Detergent treatments

Oocyst suspensions were exposed to the following detergent solutions: sodium dodecyl sulphate (SDS; 1%, 5%), and 1% alkaline SDS (dissolved in 0.2 N NaOH rather than water), Triton X-100 (1%), sodium deoxycholate (0.5%), Tween 80 (1%), and Tween 20 (1%).

Briefly, 100 μl of oocyst suspension pre-stored in water, sulphuric acid, or potassium dichromate solutions and washed in laboratory grade water was added to a 900 μl detergent solution in a microfuge tube. The sample was then incubated for 18–24 h at 37 °C then washed three times as described above using laboratory grade water. Treated, washed oocysts were stored in distilled water at 4 °C and then used for mAb and lectin-binding assays (see