Interaction forces drive the environmental transmission of pathogenic protozoa
RUNNING TITLE: Protozoa-environment interaction forces
Aurélien Dumètre, Dominique Aubert, Pierre-Henri Puech, Jeanne Hohweyer, Nadine Azas, Isabelle Villena

To cite this version:
Interaction forces drive the environmental transmission of pathogenic protozoa

RUNNING TITLE: Protozoa-environment interaction forces

Aurélien Dumètre,1* Dominique Aubert,2 Pierre-Henri Puech,3 Jeanne Hohweyer,2 Nadine Azas,1 and Isabelle Villena2

Aix-Marseille Université, UMR MD3 Relations Hôte-Parasites, Pharmacologie et Thérapeutique, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France.

Université de Reims Champagne-Ardenne, Laboratoire de Parasitologie-Mycologie, EA 143800, Faculté de Médecine, IFR 53, 51 rue Cognacq-Jay, 51096 Reims, France.

INSERM U600 / CNRS UMR6212, Laboratoire Adhésion Cellulaire et Inflammation, Faculté des Sciences, 163 Avenue de Luminy, 13288 Marseille Cedex 09, France.

* Corresponding author: Aurélien Dumètre
Telephone: +33 4 91 83 55 44 / Fax: +33 4 91 83 55 37
E-mail: aurelien.dumetre@univmed.fr
Full postal address:
UMR-MD3, Laboratoire de Parasitologie, Faculté de Pharmacie, 27 Bd Jean Moulin 13385, Marseille Cedex 05, France.
The protozoan parasites Giardia duodenalis, Cryptosporidium spp. and Toxoplasma gondii are environment-resistant pathogens that pose significant risks to public health worldwide. Their environmental transmission is closely governed by the physicochemical properties of their cysts and oocysts respectively, allowing their transport, retention and survival for months in water, soil, vegetables and mollusks, which are the main reservoirs for human infection. Importantly, the cyst/oocyst wall plays a key role in that regard by exhibiting a complex polymeric coverage that determines the charge and hydrophobic characteristics of parasites' surface. Interaction forces between parasites and other environmental particles may be, in a first approximation, evaluated following the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloidal stability. However, due to the molecular topography and nano- to micro-structure of the cyst/oocyst surface, non-DLVO hydrophobic together with additional steric attractive/repulsive forces may play a pivotal role in controlling the parasite behavior when submitted to various external conditions. Here, we review several parameters that enhance or hinder the adhesion of parasites to other particles and surfaces, and address the role of fast-emerging techniques for mapping the cyst/oocyst surface e.g. by measuring their topology and the generated interaction forces at the nano- to micro-scale. We discuss why characterizing these interactions could be a crucial step for managing the environmental matrices at risk of microbial pollution.
INTRODUCTION

The protozoan parasites Giardia duodenalis, Cryptosporidium spp. and Toxoplasma gondii are major pathogens able to survive in contrasting aquatic or terrestrial environments in order to infect a wide range of vertebrate hosts occupying very different ecological niches (34). Definitely, their environmental transmission poses significant risks to human health. Giardia cysts and Cryptosporidium and Toxoplasma oocysts are typically acquired by consuming waters or foods that are inadequately treated to kill or to remove the parasites (29, 59-61, 95, 5496). Resulting infections are among the most prevalent parasitic diseases worldwide. Giardia and Cryptosporidium are responsible for gastrointestinal diseases causing mild to severe diarrhea (11, 109), whereas Toxoplasma infections may lead to threatening birth defects, severe neurological and ocular diseases depending on the parasite and host genetic backgrounds (77, 85).

The environmental impact of these parasites greatly is closely related to their extended survival to contrasting climatic conditions and disinfection processes (5, 60, 63), and in their ability to interact with other organic or non-organic particles. This latter phenomenon governs their transport, retention/release and survival from land to sea (1, 2, 82, 98-100). The cyst/oocyst wall plays a key role by forming a highly resistant barrier to a large set of physicochemical stressors and by, at the same time, exhibiting surface properties involved in parasite-particle interactions (7, 23, 30, 101). Though the biochemical composition and molecular architecture of their respective outer wall greatly differ (14, 47, 57, 64, 76, 89, 91) (Figure 1), those three parasites could present similar surface interactions with their surrounding world due to their bio-physical features (Table 1). Such interactions may be described in a first approximation using prediction models of colloidal stability and attractive/repulsive forces (110). Importantly, interaction forces depend on the chemistry and topography of the macromolecules at the parasite surface, their hydrophobicity and electric...
charge, and on external physicochemical conditions, such as ionic composition of the surrounding media and organic contaminations, which can also contribute to promote or hinder parasite adhesion (21, 23, 56, 67-69, 74, 110). First measuring interaction forces and understanding their origin, then controlling them appear therefore critical in regulating the fate of parasites in the different aquatic and telluric environments, and consequently their transmission to animals and humans.

In this review, we describe the different parameters contributing to the interactions between the environmentally resistant stages of *Giardia*, *Cryptosporidium* and *Toxoplasma* and other particles, and point out the importance of an accurate characterization of underlying forces to better predict parasites distribution through the environment and therefore prevent their transmission to humans.

**SURFACE CHEMISTRY OF THE CYST/OOCYST WALL**

The biochemical nature of the macromolecules at the cyst/oocyst surface inherently contributes to the interactions between the parasites and their environment. Thanks to the combination of powerful imaging analysis techniques such as confocal laser-scanning microscopy and immunoelectron microscopy, and chemical methods e.g. gas chromatography-mass spectrometry, the list of described macromolecules composing the cyst/oocyst wall surface have been recently extended (57, 64, 76, 91).

The quadranucleate cysts of *Giardia duodenalis* form in the intestinal lumen of the infected host following a complex multifactorial process (70). The cyst wall is 300 to 500 nm thick and mainly consists in a surface filamentous layer (Figure 1) and is built with materials that originate from encystation-specific secretory vesicles appearing in the encysting parasites (44, 56). The biochemical composition and structural arrangement of the filamentous layer consist in a dense network of curled fibrils of N-acetylgalactosamine measuring ~10 nm in diameter.
These fibrils are closely associated with certain wall proteins called cyst wall proteins (CWP) (13). Four major proteins have been identified in the cyst wall. They include the CWP1-3 proteins, which harbor N-terminal leucine-rich repeats together with a C-terminal cysteine-rich region, and a fourth belonging to the family of cysteine-rich non-variant-specific surface proteins of Giardia (25). Aside, an epidermal growth factor-like cyst protein has been shown to be involved in the cyst wall formation, in partnership with the non-variant-specific surface protein (16). The thick filamentous outer layer of the cyst wall has been shown to be fully impermeable to water-soluble substances, enhancing the survival of cysts in water and resistance to disinfectants (5).

The four infective sporozoites of Cryptosporidium are protected by a complex multilayer wall of 50-70 nm thickness that forms while the oocyst develops in the intestinal cells of the infected hosts (47, 57, 89). The oocyst wall of Cryptosporidium is mainly built with materials released sequentially by different subsets of specific organelles found in the cytoplasm of the fertilized macrogamete, the so-called wall forming bodies (103). Current proposed data show an inner layer of glycoproteins and a central lipid-protein layer covered by an outer glucose-rich glycocalyx (12, 57, 87) (Figure 1). Large molecular weight cysteine-rich proteins, namely Cryptosporidium oocyst wall proteins (COWPs), are thought to form extensive disulphide bridges and, consequently, matrices in the inner layer that chiefly provides the overall mechanical strength of the oocyst wall (103, 108). The glycocalyx, decorating the wall structure and facing the surrounding media, provides at the same time immunogenicity and potential attachment possibilities (57, 87). The outcomes of physical and chemical treatments of the oocyst wall indicate that the glycocalyx is delicate and highly susceptible to disinfectants such as sodium hypochlorite, and to conservative agents like formalin (43, 47).

The wall of the sporulated oocyst of Toxoplasma encloses two sporocysts containing each four infectious sporozoites (36, 104). As a conserved feature among Coccidia, the double-
layered wall of the *Toxoplasma* oocyst forms a highly resistant and impermeable shell (3). Nonetheless, the outer layer can be stripped off easily in a rather easy way using chemical treatments, so the robustness of the oocyst wall appears to be mainly due to its inner layer (76). The ~100 nm thick oocyst wall forms while the oocyst is still housed by the enterocytes of cats, the definitive hosts of the parasite (37). This structure is built with materials released sequentially by different types of wall forming bodies present in the cytoplasm of the macrogamete/early stage oocyst (37). The oocyst wall of *Toxoplasma* (and of related coccidian parasites) is at more than 90% made of proteins, with two identified types so far, namely cysteine- and tyrosine-rich proteins (76) (Figure 1). In *Toxoplasma*, three cysteine-rich oocyst wall proteins (OWP), TgOWP1-3, out of the seven encoded by the parasite genome, have been recently characterized biochemically (91). They are structurally homologous to COWPs and TgOWP3 localizes specifically in the outer layer. In contrast, tyrosine-rich proteins are small molecules that form protein-protein dityrosine crosslinks responsible for the hardening and natural blue autofluorescence of oocysts under UV light (4). Such proteins have been identified in the oocyst and sporocyst walls of the closely related *Coccidia* *Eimeria* (3), but not in the *Toxoplasma* oocyst which however exhibits the same typical autofluorescence (73).

In conclusion, current models of the surface chemistry of the environmentally resistant stages of *Giardia*, *Cryptosporidium* and *Toxoplasma* strongly suggest a wall coverage made of complex polymeric matrices. This coverage determines the charge and hydrophobic characteristics of parasites' surface, which are expected to generate and modulate electrostatic attractive / repulsive interactions with the surrounding particles (Table 1).

**PARASITE-PARTICLE INTERACTIONS**
Due to their size, shape and electrical charges, it is tempting to predict parasite adhesion following the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloidal stability (26, 14872, 114). This theory takes into account the electrostatic repulsion between surface charges, which strongly depends on ionic strength of the surrounding liquid, and electro-dynamic attractions due to London-van der Walls forces. At large distances, repulsion is less important than attraction, resulting in an overall attraction, whereas a repulsive barrier due to the glycocalyx must be overcome to reach irreversible adhesion when the interparticle distance becomes small enough. However, applied to the Cryptosporidium oocyst, parasite-silica interaction models do not closely fit with the DVLO theory at separations < 35 nm because of the roughness of the oocyst surface and of the extension of the surface macromolecules from the surface into the electrical double layer (20). Thus, some other forces that are not included in the DLVO approximation may have an important contribution to parasite-particle interactions, notably hydrophobic and steric repulsion forces (19, 20, 23). At the nano scale, adhesion strongly depends on the topography (surface roughness) and on the molecular coverage of the parasite surface by macromolecules creating potential attractive / repulsive forces. Surface properties can be investigated by using several physical methods (111), among which is atomic force microscopy (AFM). AFM gives valuable information about the surface topography by directly allowing its imaging at nm-scale resolution, and allows force measurements in physiological media, with unfixed samples (40, 118). AFM uses a nano-finger, at the extremity of a very soft, several micrometers long spring, to gently delineate the surface (imaging mode), to indent the objects surface by pressing on them allowing to gain measurement of mechanical properties of the objet through its Young modulus (force mode, mechanics) (46, 80) or to probe the adhesion of surface molecules when decorated with suitable haptens and pulling the lever off the surface until all built bridges are broken, allowing to directly quantify the force that those bridges can sustain (force mode, adhesion).
Different variants of adhesion force measurements have been employed so far in cell biology (38, 65, 92-94, 107, 112). This technique is now fast-emerging to study environmental pathogens (119). To date, AFM has been employed only to observe the surface topography of the oocyst of *Cryptosporidium parvum* and measure its mechanical properties (8, 9, 18, 19). AFM images describe a rather rough landscape at the oocyst surface, while the measurements of its mechanical properties indicate that it is as hard as siliceous materials (18).

Any modification of the parasite surface chemistry may promote or hinder adhesion. In lab-scale experiments using a radial stagnation point flow system to investigate adhesion kinetics of *Cryptosporidium* oocysts and *Giardia* cysts, enzymatic treatments by proteinase K or pepsine have been found to seriously damage the outer layer of the parasites (69). While the surface glycocalyx of *Cryptosporidium* oocysts prevent their adhesion to quartz surfaces by imposing a steric repulsion, proteolytic enzymes that cause such a degradation naturally enhance their attachment to the very same surfaces (68, 69, 74). Under conditions close to natural field ones, dissolved ions and organic contaminants deeply impact the surface properties of the parasites. Dissolved calcium ions in solution tend to apparently diminish negative charges at the parasite surface, consequently abolishing repulsion, and thus enhancing attachment of *Cryptosporidium* oocysts to sand grain surface (68). Using packed-bed beads columns to investigate the behavior of *Cryptosporidium* oocysts in granular porous media (as a model for sand), Kim et al. showed that increasing ionic strength of the media promotes parasite retention in conjunction with a low velocity of the solution flow (62). It has been shown, as one may have expected, that parasites exhibit variation in their zeta potential when suspended in water-based solutions differing by their conductivity, pH and dissolved organic carbon concentrations. For instance, *Toxoplasma* oocysts are negatively charged and tend not to aggregate to other particles in freshwater solutions (ζ = -16.16 mV) while their global charge becomes near to neutral in higher-ionic-strength solutions that mimic the...
196conditions encountered in estuarine and seawater ($\zeta = -1.84$ and $-2.81$ mV respectively), thus 
197leading to efficient parasite aggregation with other particles in these environments (101).
198According to different studies, the oocyst of *Cryptosporidium parvum* has an isoelectric point 
199of $2.2-3.3$ at which electrostatic repulsion forces are abolished (6, 19, 30, 53). In contrast, 
200when placed in presence of dissolved compounds from natural organic matters, parasites 
201exhibit an absolute increase of their negative charges and of their hydrophobicity, possibly 
202due to the adsorption of clays, humic and fulvic acids onto their surface. This may thus 
203enhance transport rather than parasite sedimentation (24, 81, 82, 88, 101).

**205SURFACE INTERACTIONS DRIVE THE TRANSPORT AND SURVIVAL OF** 
**206PARASITIC PROTOZOA**

207At the field-scale, such interactions critically affect the behavior of these pathogens and their 
208distribution in terrestrial and aquatic environments. Transport of parasitic protozoa in soil 
209follows the colloid filtration theory suggesting that the size of these microorganisms control to 
210a large extend their transport in granular media (45). However, the theory does not take into 
211account the surface characteristics of the parasites, their viability state, and their reversible 
212interactions with soil grain surfaces, which promote or inhibit the terrestrial transport of 
213parasitic protozoa (97). Soil physicochemical properties e.g. mineralogy, natural organic 
214matter content, and pH critically affect the parasite-particle interactions and the mobilization 
215behavior of *Cryptosporidium* oocysts (43, 52, 79 81, 82). Spatial dissemination of excreted 
216parasites depends also on local hydrodynamic forces and occurs mainly by leaching, typically 
217following heavy rainfalls, leading to the possible entering of the parasites in waters (39, 84). It 
218has been shown that vegetated wetlands may efficiently retain parasites while degraded 
219habitats promote pathogen pollution of waters with great impacts on humans and animals 
220(100, 106).
The ability of waterborne *Giardia* cysts and *Cryptosporidium* oocysts to settle contributes to either their transport in waters or their retention in sediments. Their respective settling velocity has been determined following the Stokes law, taking into account the parasite diameter and its specific gravity (Table 1). The settling velocity of unattached parasites is relatively low (e.g. 0.35 µm.s\(^{-1}\)) for *Cryptosporidium* (78), however, when attached to particles, the very same parasites settle faster (~1.3 µm.s\(^{-1}\)) mainly due to an increase of the apparent diameter of the objects and of their specific gravity (49, 78). These parasites are likely to be associated to fecal or soil particles before entering rivers or water reservoirs, so settling may occur more or less efficiently depending on the size of particles, suggesting that some particle-attached parasites may travel along rivers (35). Also, aggregation of the parasites with particles does not preclude neither their survival in water beds for months nor their re-distribution from sediments due to local water turbulences (99). This latter phenomenon may cause recurrent parasitic contaminations as observed in certain surface waters used for drinking (85, 98, 99). Overall, one must consider that such interactions could be responsible for the amazing persistence of infective parasites in water and solid matrices, in conjunction with variations of local physical and chemical conditions (63).

If recent investigations on the molecular coverage and surface forces of *Cryptosporidium*, *Giardia* and *Toxoplasma* parasites have provided important information on the parasite behavior at different spatial and time scales, they have also enlightened some technical limits when working with protozoan cysts and oocysts. In particular, parasites used for imaging, force-based and transport experiments should be carefully purified and stored in order to prevent any modification or loss of their macromolecular coverage caused by chemical agents or by disparities in parasite’s populations (6). As a consequence, bleach-sterilized, formalin-treated or heat-inactivated parasites may exhibit modified surface properties and are therefore not suitable for such interaction experiments (9, 42, 68, 69, 88). To overcome this drawback
and because of the biohazard risks associated with the manipulation of resistant parasites, some authors have proposed to use surrogate microspheres and they have been successfully employed for transport experiments. Typically, these substrates are fluorescent glass or latex beads that are designed or decorated to mimic the size and surface properties of the targeted parasites (48). These surrogates allow a relatively good prediction of the behavior of the parasites in waters and soils, including their removal by granular porous media or their adhesion to vegetated strips (24, 81, 82, 100, 101). Interestingly, divergent behaviors have been observed between the surrogate microspheres and the parasites they mimic under particular conditions of ionic strength and organic contamination close to natural field ones. This behavior could be linked to the existence of subtle differences in their respective surface chemistry (48, 82).

**IMPLICATIONS FOR NATURAL RESOURCE MANAGEMENT**

It has been well demonstrated that the monitoring of protozoan parasites in complex matrices as well as their removal and/or inactivation may be greatly impaired due to their interactions with organic and inorganic contaminants (5, 31). For instance, these interactions critically affect the recovery rates of waterborne cysts and oocysts along the different steps of the process. Sampling surface waters may be problematic because the particle-attached parasites likely settle through gravity faster than free parasites in water bed and thus may not be sampled (98, 99). More importantly, aggregation of parasites impacts their purification when using immunomagnetic separation (IMS) techniques or floatation on dense solutions in case of high organic contamination (15, 31, 66, 71, 113). Parasite-particle complexes exhibit a greater specific gravity and cannot be readily separated by floatation (55, 90, 98) whereas particles can also mask the antigenic sites at the parasite surface, thus hampering the antigen recognition by specific antibodies one can use for IMS or immunofluorescence techniques.
The use of dispersant solutions, chelating agents, detergents or biosurfactants is not always successful in order to prevent unwanted parasite-particle interactions during sample processing (54, 75). Furthermore, even the surface of purified parasites may be still coated with divalent cations and organic substances (humic and fulvic acids) that may interfere with downstream applications such as polymerase chain reaction, which usually give useful information on the species, viability and genetic type of the detected parasites (41, 58, 102).

Interestingly, interactions have a dual role in promoting or preventing parasite removal and/or inactivation depending on treatment methods. Water industrials take advantage of these surface properties in order to remove the parasites from raw waters by using coagulant agents such as aluminum-based salts, iron-based salts or organic polymers. Such agents enhance the aggregation of parasites with other particles, allowing the flocculation of the newly formed complexes and their removal following controlled settling (5). Another significant contribution of the particular surface properties relies on interactions that may occur between parasites and sand during their transport through granular porous media in water treatments, allowing parasite retention on sand filters (62, 110). In contrast, unwanted parasite-particle interactions are clearly detrimental when using physical or chemical disinfectants. UV has been shown to inactivate Cryptosporidium and Giardia at doses commonly applied by water industrials (>40 mJ/cm²) (51), while for Toxoplasma a complete and reliable inactivation has been reported in some studies but not others (33, 116). Parasites entrapped in soft mollusk tissues or attached to sediments may not be totally inactivated at these doses (10, 105). Also, the success of chemical-based inactivation processes may also be compromised for such chlorine and ozone-resistant microorganisms (5, 33, 117). The presence of organic and inorganic compounds associated to parasites in water may require the use of higher ozone doses to achieve parasite inactivation, which may lead to the formation of potentially harmful by-products such as bromates (115). In a similar way, Cryptosporidium oocysts and Giardia
Cysts entrapped in pipe-wall biofilms survive to the concentrations of free chlorine usually used in drinking water systems (2, 50). The capacity of the *Toxoplasma* oocyst to interact with biofilms has not been investigated so far. Such investigations would be of great interest since the implication of biofilm cannot be ruled out for elucidating recurrent cases of waterborne toxoplasmosis in several areas (60).

Modeling the fate and transport of parasitic protozoa may offer a valuable tool for risk assessment. Microbial pathogen modeling basically incorporates specific information about microbial dynamic in conjunction with soil composition, hydrological dynamic, climatic conditions, vegetation, and land management (35). It has been well demonstrated that mathematical models for bacteria are not suitable for predicting the fate and transport of parasitic protozoa (17, 28, 35). In particular, fecal indicators fail to approach water contamination by *Cryptosporidium* and *Giardia* parasites because of the ability of oocysts and cysts to interact and aggregate reversibly with other particles compared to other microorganisms (35, 49). Several specific models have been successfully used for estimating protozoan loads in watersheds in some particular contexts (17, 28, 35). To our knowledge, no such models exist for *Toxoplasma* oocysts at a large scale mainly because too few information is available on their transport properties, survival and prevalence throughout the environment (31, 60, 100). Modeling parasitic protozoa incorporates therefore several major challenges, among which exact parasite surface characterization, the determination of inactivation rate, and improvements in the separation and molecular methods that are used to detect and characterize them in complex matrices. The first point is especially crucial when studying what happens following inactivation processes, in order to assess divergent aggregation and settling behaviors observed between viable and non-viable parasites.

**CONCLUSIONS AND FUTURE PERSPECTIVES**
The circulation of *Giardia*, *Cryptosporidium* and *Toxoplasma* parasites in the different environmental sources leading to potential human infections strongly depends on how the parasites interact with their surrounding media, mainly other organic and inorganic particles. Hydrophobic, steric and electrostatic attractive/repulsive forces created by the polymeric coverage of the parasite surface greatly enhance or hinder parasite adhesion following the environmental physicochemical factors. They contribute to parasite adhesion to natural organic matters, promoting thus their retention in soils or increasing their deposition kinetic in waterbeds. Consequently, such interactions make parasite fluxes hard to predict on a large scale, affecting therefore the management of resources at risk of microbial pollution.

An accurate characterization of parasite-particle interactions clearly requires additional information on the topography and on the molecular composition of the outer surface of the cyst/oocyst wall. The combination of powerful microscopic and spectroscopic techniques, such as fluorescence microscopy, AFM and/or Raman scattering microscopy, may give new insights on the biochemical nature and arrangement of surface macromolecules as well as the adhesive and mechanical properties of the robust wall (27, 86). To date, only the surface of the oocyst of *Cryptosporidium parvum* has been mapped in terms of force and mechanics, and most efforts should focused now on the surface of other medically-important *Cryptosporidium* species, *Giardia* cysts and *Toxoplasma* oocysts and on the interaction forces that might result from the micro- and nano- structures of their coats.

ACKNOWLEDGEMENTS

This work was supported by the French National Research Agency (grant ANR-09-ALIA-343009) and Aix-Marseille University (Préciput 2011 program). PHP is currently supported by ANR JCJC DissecTion program.
REFERENCES


736


740


743


748


751


755


786116.  Wainwright, K. E., M. Lagunas-Solar, M. A. Miller, B. C. Barr, I. A. Gardner, C.

790


794


798


801


804

**TABLE 1:** Physicochemical characteristics of the environmentally resistant stages of *Giardia duodenalis*, *Cryptosporidium* spp. and *Toxoplasma gondii*.

<table>
<thead>
<tr>
<th></th>
<th><em>Giardia duodenalis</em> cyst</th>
<th><em>Cryptosporidium</em> spp. oocyst</th>
<th><em>Toxoplasma gondii</em> oocyst</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (µm) Wall</td>
<td>7-10 x 5</td>
<td>3.8-6.3 x 4.6-8.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 x 12</td>
<td>11, 60, 109</td>
</tr>
<tr>
<td>Thickness (nm)</td>
<td>300-500</td>
<td>50-70</td>
<td>~100</td>
<td>14, 37, 57</td>
</tr>
<tr>
<td>Number of layers</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Outer wall thickness (nm)</td>
<td>250</td>
<td>8</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Surface biochemistry</td>
<td>Matrix of filamentous GalNAc and Cys-rich proteins</td>
<td>Glucose-rich glycocalyx</td>
<td>Possible polymeric cross-links of Cys-/Tyr-rich proteins</td>
<td>14, 37, 57, 64, 76, 103</td>
</tr>
<tr>
<td>Zeta potential (mV)</td>
<td>-33.5 in distilled H&lt;sub&gt;2&lt;/sub&gt;O at pH 6.4</td>
<td>-25.0 in deionized H&lt;sub&gt;2&lt;/sub&gt;O H&lt;sub&gt;2&lt;/sub&gt;O (conductivity 4 µS.cm&lt;sup&gt;-1&lt;/sup&gt; at pH 6.5)</td>
<td>-43.7 in ultrapure H&lt;sub&gt;2&lt;/sub&gt;O (conductivity 83.9 µS.cm&lt;sup&gt;-1&lt;/sup&gt; at pH 6.7)</td>
<td>30, 88, 101</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.013-1.117</td>
<td>1.009-1.08</td>
<td>1.050-1.100</td>
<td>22, 31, 55, 78, 83, 120</td>
</tr>
<tr>
<td>Settling velocity (µm.s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.84-1.4</td>
<td>0.35-1.31</td>
<td>Not reported</td>
<td>22, 78, 121</td>
</tr>
</tbody>
</table>

<sup>a</sup> depending on species.

**LEGEND TO FIGURE 1:** Schematic drawing of the different walls of the *Giardia* cyst, and *Cryptosporidium* and *Toxoplasma* oocysts. OW: outer wall; CW: central wall; IW: inner wall.
FIGURE 1

Giardia

Cryptosporidium

Toxoplasma

GalNac homopolymers = cysteine-rich proteins
Membranous inner wall

Glycocalyx

Lipids
Proteins
Glycoproteins (cysteine-rich proteins)

Cysteine-rich proteins (and tyrosine-rich proteins*)
Proteins