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3 **Interaction forces drive the environmental transmission**
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5 ***RUNNING TITLE: Protozoa-environment interaction forces***

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26ABSTRACT

27The protozoan parasites *Giardia duodenalis*, *Cryptosporidium* spp. and *Toxoplasma gondii*
28are environment-resistant pathogens that pose significant risks to public health worldwide.
29Their environmental transmission is closely governed by the physicochemical properties of
30their cysts and oocysts respectively, allowing their transport, retention and survival for
31months in water, soil, vegetables and mollusks, which are the main reservoirs for human
32infection. Importantly, the cyst/oocyst wall plays a key role in that regard by exhibiting a
33complex polymeric coverage that determines the charge and hydrophobic characteristics of
34parasites' surface. Interaction forces between parasites and other environmental particles may
35be, in a first approximation, evaluated following the Derjaguin-Landau-Verwey-Overbeek
36(DLVO) theory of colloidal stability. However, due to the molecular topography and nano- to
37micro- structure of the cyst/oocyst surface, non-DVLO hydrophobic together with additional
38steric attractive/repulsive forces may play a pivotal role in controlling the parasite behavior
39when submitted to various external conditions. Here, we review several parameters that
40enhance or hinder the adhesion of parasites to other particles and surfaces, and address the
41role of fast-emerging techniques for mapping the cyst/oocyst surface e.g. by measuring their
42topology and the generated interaction forces at the nano- to micro- scale. We discuss why
43characterizing these interactions could be a crucial step for managing the environmental
44matrices at risk of microbial pollution.

45

46

47INTRODUCTION

48The protozoan parasites *Giardia duodenalis*, *Cryptosporidium* spp. and *Toxoplasma gondii*
49are major pathogens able to survive in contrasting aquatic or terrestrial environments in order
50to infect a wide range of vertebrate hosts occupying very different ecological niches (34).
51Definitely, their environmental transmission poses significant risks to human health. *Giardia*
52cysts and *Cryptosporidium* and *Toxoplasma* oocysts are typically acquired by consuming
53waters 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*
55and *Cryptosporidium* are responsible for gastrointestinal diseases causing mild to severe
56diarrhea (11, 109), whereas *Toxoplasma* infections may lead to threatening birth defects,
57severe neurological and ocular diseases depending on the parasite and host genetic
58backgrounds (77, 85).

59The environmental impact of these parasites greatly is closely related to their extended
60survival to contrasting climatic conditions and disinfection processes (5, 60, 63), and in their
61ability to interact with other organic or non-organic particles. This latter phenomenon governs
62their transport, retention/release and survival from land to sea (1, 2, 82, 98-100). The
63cyst/oocyst wall plays a key role by forming a highly resistant barrier to a large set of
64physicochemical stressors and by, at the same time, exhibiting surface properties involved in
65parasite-particle interactions (7, 23, 30, 101). Though the biochemical composition and
66molecular architecture of their respective outer wall greatly differ (14, 47, 57, 64, 76, 89, 91)
67(Figure 1), those three parasites could present similar surface interactions with their
68surrounding world due to their bio-physical features (Table 1). Such interactions may be
69described in a first approximation using prediction models of colloidal stability and
70attractive/repulsive forces (110). Importantly, interaction forces depend on the chemistry and
71topography of the macromolecules at the parasite surface, their hydrophobicity and electric

72charge, and on external physicochemical conditions, such as ionic composition of the
73surrounding media and organic contaminations, which can also contribute to promote or
74hinder parasite adhesion (21, 23, 56, 67-69, 74, 110). First measuring interaction forces and
75understanding their origin, then controlling them appear therefore critical in regulating the
76fate of parasites in the different aquatic and telluric environments, and consequently their
77transmission to animals and humans.

78In this review, we describe the different parameters contributing to the interactions between
79the environmentally resistant stages of *Giardia*, *Cryptosporidium* and *Toxoplasma* and other
80particles, and point out the importance of an accurate characterization of underlying forces to
81better predict parasites distribution through the environment and therefore prevent their
82transmission to humans.

83

84SURFACE CHEMISTRY OF THE CYST/OOCYST WALL

85The biochemical nature of the macromolecules at the cyst/oocyst surface inherently
86contributes to the interactions between the parasites and their environment. Thanks to the
87combination of powerful imaging analysis techniques such as confocal laser-scanning
88microscopy and immunoelectron microscopy, and chemical methods e.g. gas
89chromatography-mass spectrometry, the list of described macromolecules composing the
90cyst/oocyst wall surface have been recently extended (57, 64, 76, 91).

91The quadrinucleate cysts of *Giardia duodenalis* form in the intestinal lumen of the infected
92host following a complex multifactorial process (70). The cyst wall is 300 to 500 nm thick
93and mainly consists in a surface filamentous layer (Figure 1) and is built with materials that
94originate from encystation-specific secretory vesicles appearing in the encysting parasites (44,
9564). The biochemical composition and structural arrangement of the filamentous layer consist
96in a dense network of curled fibrils of *N*-acetylgalactosamine measuring ~10 nm in diameter

97(13). These fibrils are closely associated with certain wall proteins called cyst wall proteins
98(CWP) (13). Four major proteins have been identified in the cyst wall. They include the
99CWP1-3 proteins, which harbor *N*-terminal leucine-rich repeats together with a C-terminal
100cysteine-rich region, and a fourth belonging to the family of cysteine-rich non-variant-specific
101surface proteins of *Giardia* (25). Aside, an epidermal growth factor-like cyst protein has been
102shown to be involved in the cyst wall formation, in partnership with the non-variant-specific
103surface protein (16). The thick filamentous outer layer of the cyst wall has been shown to be
104fully impermeable to water-soluble substances, enhancing the survival of cysts in water and
105resistance to disinfectants (5).

106The four infective sporozoites of *Cryptosporidium* are protected by a complex multilayer wall
107of 50-70 nm thickness that forms while the oocyst develops in the intestinal cells of the
108infected hosts (47, 57, 89). The oocyst wall of *Cryptosporidium* is mainly built with materials
109released sequentially by different subsets of specific organelles found in the cytoplasm of the
110fertilized macrogamete, the so-called wall forming bodies (103). Current proposed data show
111an inner layer of glycoproteins and a central lipid-protein layer covered by an outer glucose-
112rich glycocalyx (12, 57, 87) (Figure 1). Large molecular weight cysteine-rich proteins, namely
113*Cryptosporidium* oocyst wall proteins (COWPs), are thought to form extensive disulphide
114bridges and, consequently, matrices in the inner layer that chiefly provides the overall
115mechanical strength of the oocyst wall (103, 108). The glycocalyx, decorating the wall
116structure and facing the surrounding media, provides at the same time immunogenicity and
117potential attachment possibilities (57, 87). The outcomes of physical and chemical treatments
118of the oocyst wall indicate that the glycocalyx is delicate and highly susceptible to
119disinfectants such as sodium hypochlorite, and to conservative agents like formalin (43, 47).
120The wall of the sporulated oocyst of *Toxoplasma* encloses two sporocysts containing each
121four infectious sporozoites (36, 104). As a conserved feature among Coccidia, the double-

122layered wall of the *Toxoplasma* oocyst forms a highly resistant and impermeable shell (3).
123Nonetheless, the outer layer can be stripped off easily in a rather easy way using chemical
124treatments, so the robustness of the oocyst wall appears to be mainly due to its inner layer
125(76). The ~100 nm thick oocyst wall forms while the oocyst is still housed by the enterocytes
126of cats, the definitive hosts of the parasite (37). This structure is built with materials released
127sequentially by different types of wall forming bodies present in the cytoplasm of the
128macrogamete/early stage oocyst (37). The oocyst wall of *Toxoplasma* (and of related
129coccidian parasites) is at more than 90% made of proteins, with two identified types so far,
130namely cysteine- and tyrosine-rich proteins (76) (Figure 1). In *Toxoplasma*, three cysteine-
131rich oocyst wall proteins (OWP), TgOWP1-3, out of the seven encoded by the parasite
132genome, have been recently characterized biochemically (91). They are structurally
133homologous to COWPs and TgOWP3 localizes specifically in the outer layer. In contrast,
134tyrosine-rich proteins are small molecules that form protein-protein dityrosine crosslinks
135responsible for the hardening and natural blue autofluorescence of oocysts under UV light (4).
136Such proteins have been identified in the oocyst and sporocyst walls of the closely related
137Coccidia *Eimeria* (3), but not in the *Toxoplasma* oocyst which however exhibits the same
138typical autofluorescence (73).

139In conclusion, current models of the surface chemistry of the environmentally resistant stages
140of *Giardia*, *Cryptosporidium* and *Toxoplasma* strongly suggest a wall coverage made of
141complex polymeric matrices. This coverage determines the charge and hydrophobic
142characteristics of parasites' surface, which are expected to generate and modulate electrostatic
143attractive / repulsive interactions with the surrounding particles (Table 1).

144

145PARASITE-PARTICLE INTERACTIONS

146 Due to their size, shape and electrical charges, it is tempting to predict parasite adhesion
147 following the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloidal stability (26,
148 72, 114). This theory takes into account the electrostatic repulsion between surface charges,
149 which strongly depends on ionic strength of the surrounding liquid, and electro-dynamic
150 attractions due to London-van der Waals forces. At large distances, repulsion is less important
151 than attraction, resulting in an overall attraction, whereas a repulsive barrier due to the
152 glycocalyx must be overcome to reach irreversible adhesion when the interparticle distance
153 becomes small enough. However, applied to the *Cryptosporidium* oocyst, parasite-silica
154 interaction models do not closely fit with the DLVO theory at separations < 35 nm because of
155 the roughness of the oocyst surface and of the extension of the surface macromolecules from
156 the surface into the electrical double layer (20). Thus, some other forces that are not included
157 in the DLVO approximation may have an important contribution to parasite-particle
158 interactions, notably hydrophobic and steric repulsion forces (19, 20, 23). At the nano scale,
159 adhesion strongly depends on the topography (surface roughness) and on the molecular
160 coverage of the parasite surface by macromolecules creating potential attractive / repulsive
161 forces. Surface properties can be investigated by using several physical methods (111), among
162 which is atomic force microscopy (AFM). AFM gives valuable information about the surface
163 topography by directly allowing its imaging at nm-scale resolution, and allows force
164 measurements in physiological media, with unfixed samples (40, 118). AFM uses a nano-
165 finger, at the extremity of a very soft, several micrometers long spring, to gently delineate the
166 surface (imaging mode), to indent the object's surface by pressing on them allowing to gain
167 measurement of mechanical properties of the object through its Young modulus (force mode,
168 mechanics) (46, 80) or to probe the adhesion of surface molecules when decorated with
169 suitable haptens and pulling the lever off the surface until all built bridges are broken,
170 allowing to directly quantify the force that those bridges can sustain (force mode, adhesion).

171 Different variants of adhesion force measurements have been employed so far in cell biology
172 (38, 65, 92-94, 107, 112). This technique is now fast-emerging to study environmental
173 pathogens (119). To date, AFM has been employed only to observe the surface topography of
174 the oocyst of *Cryptosporidium parvum* and measure its mechanical properties (8, 9, 18, 19).
175 AFM images describe a rather rough landscape at the oocyst surface, while the measurements
176 of its mechanical properties indicate that it is as hard as siliceous materials (18).

177 Any modification of the parasite surface chemistry may promote or hinder adhesion. In lab-
178 scale experiments using a radial stagnation point flow system to investigate adhesion kinetics
179 of *Cryptosporidium* oocysts and *Giardia* cysts, enzymatic treatments by proteinase K or
180 pepsine have been found to seriously damage the outer layer of the parasites (69). While the
181 surface glycocalyx of *Cryptosporidium* oocysts prevent their adhesion to quartz surfaces by
182 imposing a steric repulsion, proteolytic enzymes that cause such a degradation naturally
183 enhance their attachment to the very same surfaces (68, 69, 74). Under conditions close to
184 natural field ones, dissolved ions and organic contaminants deeply impact the surface
185 properties of the parasites. Dissolved calcium ions in solution tend to apparently diminish
186 negative charges at the parasite surface, consequently abolishing repulsion, and thus
187 enhancing attachment of *Cryptosporidium* oocysts to sand grain surface (68). Using packed-
188 bed beads columns to investigate the behavior of *Cryptosporidium* oocysts in granular porous
189 media (as a model for sand), Kim et al. showed that increasing ionic strength of the media
190 promotes parasite retention in conjunction with a low velocity of the solution flow (62). It has
191 been shown, as one may have expected, that parasites exhibit variation in their zeta potential
192 when suspended in water-based solutions differing by their conductivity, pH and dissolved
193 organic carbon concentrations. For instance, *Toxoplasma* oocysts are negatively charged and
194 tend not to aggregate to other particles in freshwater solutions ($\zeta = -16.16$ mV) while their
195 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).

204

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 extent 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).

221The ability of waterborne *Giardia* cysts and *Cryptosporidium* oocysts to settle contributes to
222either their transport in waters or their retention in sediments. Their respective settling
223velocity has been determined following the Stokes law, taking into account the parasite
224diameter and its specific gravity (Table 1). The settling velocity of unattached parasites is
225relatively low (e.g. $0.35 \mu\text{m}\cdot\text{s}^{-1}$ for *Cryptosporidium*) (78), however, when attached to
226particles, the very same parasites settle faster ($\sim 1.3 \mu\text{m}\cdot\text{s}^{-1}$) mainly due to an increase of the
227apparent diameter of the objects and of their specific gravity (49, 78). These parasites are
228likely to be associated to fecal or soil particles before entering rivers or water reservoirs, so
229settling may occur more or less efficiently depending on the size of particles, suggesting that
230some particle-attached parasites may travel along rivers (35). Also, aggregation of the
231parasites with particles does not preclude neither their survival in water beds for months nor
232their re-distribution from sediments due to local water turbulences (99). This latter
233phenomenon may cause recurrent parasitic contaminations as observed in certain surface
234waters used for drinking (85, 98, 99). Overall, one must consider that such interactions could
235be responsible for the amazing persistence of infective parasites in water and solid matrices,
236in conjunction with variations of local physical and chemical conditions (63).

237If recent investigations on the molecular coverage and surface forces of *Cryptosporidium*,
238*Giardia* and *Toxoplasma* parasites have provided important information on the parasite
239behavior at different spatial and time scales, they have also enlightened some technical limits
240when working with protozoan cysts and oocysts. In particular, parasites used for imaging,
241force-based and transport experiments should be carefully purified and stored in order to
242prevent any modification or loss of their macromolecular coverage caused by chemical agents
243or by disparities in parasite's populations (6). As a consequence, bleach-sterilized, formalin-
244treated or heat-inactivated parasites may exhibit modified surface properties and are therefore
245not suitable for such interaction experiments (9, 42, 68, 69, 88). To overcome this drawback

246and because of the biohazard risks associated with the manipulation of resistant parasites,
247some authors have proposed to use surrogate microspheres and they have been successfully
248employed for transport experiments. Typically, these substrates are fluorescent glass or latex
249beads that are designed or decorated to mimic the size and surface properties of the targeted
250parasites (48). These surrogates allow a relatively good prediction of the behavior of the
251parasites in waters and soils, including their removal by granular porous media or their
252adhesion to vegetated strips (24, 81, 82, 100, 101). Interestingly, divergent behaviors have
253been observed between the surrogate microspheres and the parasites they mimic under
254particular conditions of ionic strength and organic contamination close to natural field ones.
255This behavior could be linked to the existence of subtle differences in their respective surface
256chemistry (48, 82).

257

258IMPLICATIONS FOR NATURAL RESOURCE MANAGEMENT

259It has been well demonstrated that the monitoring of protozoan parasites in complex matrices
260as well as their removal and/or inactivation may be greatly impaired due to their interactions
261with organic and inorganic contaminants (5, 31). For instance, these interactions critically
262affect the recovery rates of waterborne cysts and oocysts along the different steps of the
263process. Sampling surface waters may be problematic because the particle-attached parasites
264likely settle through gravity faster than free parasites in water bed and thus may not be
265sampled (98, 99). More importantly, aggregation of parasites impacts their purification when
266using immunomagnetic separation (IMS) techniques or floatation on dense solutions in case
267of high organic contamination (15, 31, 66, 71, 113). Parasite-particle complexes exhibit a
268greater specific gravity and cannot be readily separated by floatation (55, 90, 98) whereas
269particles can also mask the antigenic sites at the parasite surface, thus hampering the antigen
270recognition by specific antibodies one can use for IMS or immunofluorescence techniques.

271The use of dispersant solutions, chelating agents, detergents or biosurfactants is not always
272successful in order to prevent unwanted parasite-particle interactions during sample
273processing (54, 75). Furthermore, even the surface of purified parasites may be still coated
274with divalent cations and organic substances (humic and fulvic acids) that may interfere with
275downstream applications such as polymerase chain reaction, which usually give useful
276information on the species, viability and genetic type of the detected parasites (41, 58, 102).
277Interestingly, interactions have a dual role in promoting or preventing parasite removal and/or
278inactivation depending on treatment methods. Water industrials take advantage of these
279surface properties in order to remove the parasites from raw waters by using coagulant agents
280such as aluminum-based salts, iron-based salts or organic polymers. Such agents enhance the
281aggregation of parasites with other particles, allowing the flocculation of the newly formed
282complexes and their removal following controlled settling (5). Another significant
283contribution of the particular surface properties relies on interactions that may occur between
284parasites and sand during their transport through granular porous media in water treatments,
285allowing parasite retention on sand filters (62, 110). In contrast, unwanted parasite-particle
286interactions are clearly detrimental when using physical or chemical disinfectants. UV has
287been shown to inactivate *Cryptosporidium* and *Giardia* at doses commonly applied by water
288industrials ($>40 \text{ mJ/cm}^2$) (51), while for *Toxoplasma* a complete and reliable inactivation has
289been reported in some studies but not others (33, 116). Parasites entrapped in soft mollusk
290tissues or attached to sediments may not be totally inactivated at these doses (10, 105). Also,
291the success of chemical-based inactivation processes may also be compromised for such
292chlorine and ozone-resistant microorganisms (5, 33, 117). The presence of organic and
293inorganic compounds associated to parasites in water may require the use of higher ozone
294doses to achieve parasite inactivation, which may lead to the formation of potentially harmful
295by-products such as bromates (115). In a similar way, *Cryptosporidium* oocysts and *Giardia*

296cysts entrapped in pipe-wall biofilms survive to the concentrations of free chlorine usually
297used in drinking water systems (2, 50). The capacity of the *Toxoplasma* oocyst to interact with
298biofilms has not been investigated so far. Such investigations would be of great interest since
299the implication of biofilm cannot be ruled out for elucidating recurrent cases of waterborne
300toxoplasmosis in several areas (60).

301Modeling the fate and transport of parasitic protozoa may offer a valuable tool for risk
302assessment. Microbial pathogen modeling basically incorporates specific information about
303microbial dynamic in conjunction with soil composition, hydrological dynamic, climatic
304conditions, vegetation, and land management (35). It has been well demonstrated that
305mathematical models for bacteria are not suitable for predicting the fate and transport of
306parasitic protozoa (17, 28, 35). In particular, fecal indicators fail to approach water
307contamination by *Cryptosporidium* and *Giardia* parasites because of the ability of oocysts and
308cysts to interact and aggregate reversibly with other particles compared to other
309microorganisms (35, 49). Several specific models have been successfully used for estimating
310protozoan loads in watersheds in some particular contexts (17, 28, 35). To our knowledge, no
311such models exist for *Toxoplasma* oocysts at a large scale mainly because too few information
312is available on their transport properties, survival and prevalence throughout the environment
313(31, 60, 100). Modeling parasitic protozoa incorporates therefore several major challenges,
314among which exact parasite surface characterization, the determination of inactivation rate,
315and improvements in the separation and molecular methods that are used to detect and
316characterize them in complex matrices. The first point is especially crucial when studying
317what happens following inactivation processes, in order to assess divergent aggregation and
318settling behaviors observed between viable and non-viable parasites.

319

320CONCLUSIONS AND FUTURE PERSPECTIVES

321The circulation of *Giardia*, *Cryptosporidium* and *Toxoplasma* parasites in the different
322environmental sources leading to potential human infections strongly depends on how the
323parasites interact with their surrounding media, mainly other organic and inorganic particles.
324Hydrophobic, steric and electrostatic attractive/repulsive forces created by the polymeric
325coverage of the parasite surface greatly enhance or hinder parasite adhesion following the
326environmental physicochemical factors. They contribute to parasite adhesion to natural
327organic matters, promoting thus their retention in soils or increasing their deposition kinetic in
328waterbeds. Consequently, such interactions make parasite fluxes hard to predict on a large
329scale, affecting therefore the management of resources at risk of microbial pollution.

330An accurate characterization of parasite-particle interactions clearly requires additional
331information on the topography and on the molecular composition of the outer surface of the
332cyst/oocyst wall. The combination of powerful microscopic and spectroscopic techniques,
333such as fluorescence microscopy, AFM and/or Raman scattering microscopy, may give new
334insights on the biochemical nature and arrangement of surface macromolecules as well as the
335adhesive and mechanical properties of the robust wall (27, 86). To date, only the surface of
336the oocyst of *Cryptosporidium parvum* has been mapped in terms of force and mechanics, and
337most efforts should be focused now on the surface of other medically-important
338*Cryptosporidium* species, *Giardia* cysts and *Toxoplasma* oocysts and on the interaction forces
339that might result from the micro- and nano- structures of their coats.

340

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

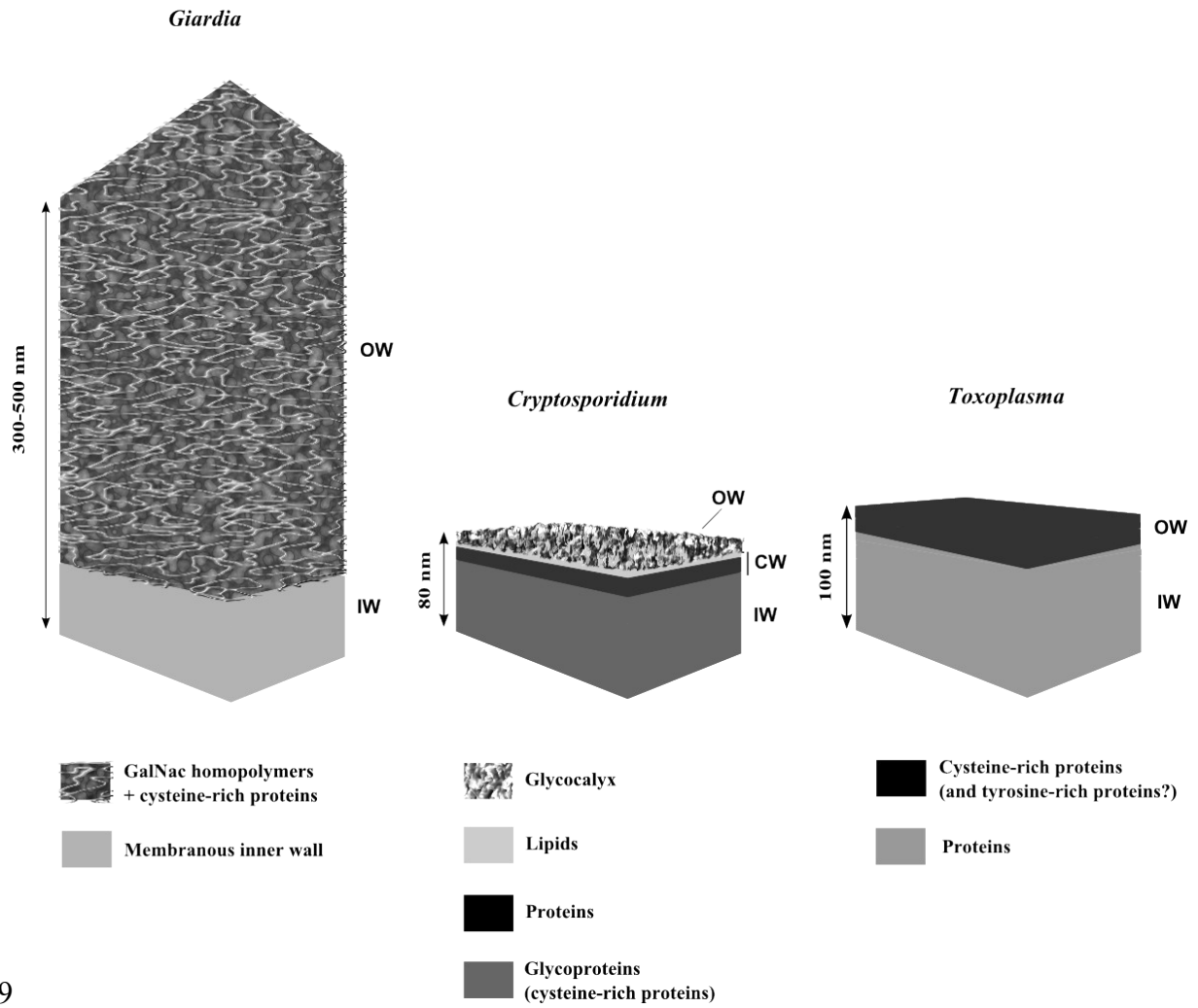
	<i>Giardia</i>	<i>Cryptosporidium</i>	<i>Toxoplasma gondii</i>	References
	<i>duodenalis</i> cyst	spp. oocyst	oocyst	
Size (µm) Wall	7-10 x 5	3.8-6.3 x 4.6-8.4 ^a	10 x 12	11, 60, 109
Thickness (nm)	300-500	50-70	~100	14, 37, 57
Number of layers	2	3	2	
Outer wall thickness (nm)	250	8	30	
Surface biochemistry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

813^a depending on species..

814**LEGEND TO FIGURE 1:** Schematic drawing of the different walls of the *Giardia* cyst, and
815*Cryptosporidium* and *Toxoplasma* oocysts. OW: outer wall; CW: central wall; IW : inner
816wall.

817FIGURE 1

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