Trends in Parasitology

CellPress

Opinion

Life without a Host Cell: What is *Cryptosporidium*?

Peta L. Clode,[1,*] Wan H. Koh,[2] and R.C. Andrew Thompson[2]

***Cryptosporidium* is a parasite responsible for widespread disease in livestock and humans. Recent phylogenetic reclassification of *Cryptosporidium* from a coccidian to a gregarine dictates an urgent need to reconsider the biology and behavior of this parasite. Overwhelming data now confirm that, like its close relatives, *Cryptosporidium* is a facultatively epicellular apicomplexan that is able to multiply in a host cell-free environment. We complement the latest phylogenetic and taxonomic proposals with advances in our understanding of *Cryptosporidium*'s biology, with particular focus on *in vitro* studies that have characterized the development of *Cryptosporidium* stages in the absence of host cells. Opportunities to revisit *in vivo* infections are discussed and questions about the *Cryptosporidium* host cell-free life cycle that remain unanswered highlighted.**

Trends

Cryptosporidium is a widespread yet neglected human and livestock pathogen.

The re-emergence of *Cryptosporidium* as a life-threatening opportunistic pathogen in children in developing countries highlights an urgent need for effective anticryptosporidial therapies.

Based on recent genetic analyses, *Cryptosporidium* is no longer considered a coccidian and has been reclassified as a gregarine, within the subclass Cryptogregaria.

Like the gregarines, *Cryptosporidium* exhibits plasticity in both its life cycle and options for parasitism, including the ability to multiply without host cell encapsulation.

The ability of *Cryptosporidium* to replicate in host cell-free systems such as aquatic biofilms poses a previously unconsidered environmental risk in the spread of disease.

From Coccidian to Gregarine: A New Beginning

Cryptosporidium came into prominence in the early 1970s as a clinically significant opportunistic zoonotic pathogen of humans for which water is an important vehicle of transmission [1]. This stimulated the rapid development of discriminatory molecular tools for use in surveillance. Unfortunately, this heightened interest and associated research activity has not resulted in the discovery of effective curative drugs to treat *Cryptosporidium* infections [2]. This is particularly significant today as we see the re-emergence of *Cryptosporidium* as a life-threatening opportunistic pathogen, not in the developed world but in children in developing countries, particularly Africa, who urgently need specific anticryptosporidial therapies [3,4]. To address this unmet need we must accept that we have been 'barking up the wrong tree' for too long and view the whole biology of *Cryptosporidium* in a different light.

In addition to the advances that molecular tools have provided in terms of surveillance and biodiversity, they have also challenged our perceptions of what *Cryptosporidium* is in terms of its phylogenetic relationships. The studies of Carreno *et al.* [5] using 18S sequencing demonstrated a closer phylogenetic relationship to gregarine protozoa than to coccidians and these have since been complemented by observations of *Cryptosporidium*'s developmental biology and metabolism (Box 1) [6–20]. In particular, the initial controversial images and theories of Hijjawi *et al.* [6] and Karanis *et al.* [10], which showed that *Cryptosporidium* oocysts, like gregarines, could excyst and produce **pleomorphic** (see Glossary) stages without host cells or triggers, were widely disputed within the *Cryptosporidium* research community. Only now, more than a decade later, are these significant findings being recognized and endorsed by complementary methods and investigations. Karanis [18] later noted that our understanding of both the close affinity of *Cryptosporidium* with gregarines and the ability of *Cryptosporidium* to grow under different conditions, was the key to successfully developing new anticryptosporidial drugs.

This accumulation of genomic, biochemical, and developmental data has now culminated in a revision of gregarine higher classification on the basis of gregarine site-heterogeneous 18S rDNA

[1]Centre for Microscopy, Characterisation, and Analysis, The University of Western Australia, Crawley WA 6009, Australia
[2]School of Veterinary and Life Sciences, Murdoch University, South Street, Murdoch WA 6150, Australia

*Correspondence: peta.clode@uwa.edu.au (P.L. Clode).

Box 1. Comparison with Gregarines

Table I summarizes the features that *Cryptosporidium* shares with gregarines. There are striking similarities both structurally and in feeding behavior between *Cryptosporidium* and gregarines [11,14,20,26]. Conversely, *Cryptosporidium* is unusual compared with other gregarines with regard to its diverse and broad host range and its close association with the host cell, which is epicellular (intracellular but extracytoplasmic) [25]. This is reflected in the new classification proposed by Cavalier-Smith [21] where *Cryptosporidium*, which is the only gregarine parasitic in vertebrates, has been placed in a class of its own.

Table I. Comparison of Gregarine and *Cryptosporidium* Structural and Life Cycle Features

Feature	Gregarines	*Cryptosporidium*
Apicoplast	No apicoplast in some gregarines	No apicoplast
Site and mode of attachment	**Transmembrane** and extracellular, depending on nutrient availability: Transmembrane, through attachment to host cell surface, and epicellular: **Myzocytosis** (i.e., gregarine pierces the cell membrane of the host cell with a feeding tube, sucks out the cellular contents and digests them)	Epicellular and extracellular, depending on nutrient availability: Epicellular parasites (i.e., trophozoite plasma membrane fuses with that of host); the vacuole membrane on the trophozoite cytosol side folds extensively to construct a feeder organelle that extends into the host cell (myzocytosis)
Size range of developmental stages	1–50 μm or more	In the host: 0.1–5 μm Without host: 0.1–50 μm
Multiplication mode	Multiple fission, merogony (in some), gametogony, sporogony, binary fission, syzygy	Multiple fission, merogony, gametogony, sporogony, binary fission, syzygy
Diversity of morphology	Variable morphologies (pleiomorphic) that are highly dependent on nutrient availability and the surrounding environment	Variable morphologies (pleiomorphic) that are highly dependent on nutrient availability and the surrounding environment
Feeder organelle	Surface-mediated nutrition via epimerite	Surface-mediated nutrition via feeder organelle (epimerite)

trees [21]. This has firmly placed *Cryptosporidium* with the gregarines, demonstrating that some 'eugregarines' and all 'neogregarines' (both now abandoned as taxa, see [21]) are closely related to *Cryptosporidium* and hopefully helping to persuade the 'nonbelievers' to reconsider the biology of this ubiquitous parasite. A new subclass, the Orthogregarinia, has been established for gregarines most closely related to *Cryptosporidium* and placed in its own subclass, the Cryptogregaria [21]. This subclass is defined as comprising **epicellular** parasites of vertebrates possessing a gregarine-like **feeder organelle** but lacking an apicoplast. In addition, *Cryptosporidium* differs from other apicomplexans in that it has lost genomes for both the plastid and the mitochondrion, possesses a highly streamlined repertoire of metabolic pathways, and is largely devoid of metabolic functions beyond the core glycolytic enzymes, which is likely to be the result of its specialized parasitism of gut epithelial cells [22,23].

The recent realization that *Cryptosporidium* is a gregarine coupled with these unique characteristics should now dictate research efforts in drug discovery. In terms of control, recent evidence demonstrating that we are in reality dealing with a parasite with both environmental and endogenous stages must be considered if future endeavors are to be successful.

Life Cycle

Cavalier-Smith [21] considers that *Cryptosporidium* is most closely related to the orthogregarines, a relationship reflected in their life cycle with multiplication in extracellular or epicellular locations. Details of the life cycle involving the epicellular association with host cells has been described in detail and is now well understood [14,24,25]. In particular, recent studies have shown the dominance of the **trophozoite** stage in the life cycle, the occurrence of **syzygy**

Glossary

Epicellular: internalization of the parasite by host cell membrane with separation from the host cell cytoplasm by the formation of a vacuole and development of an apical feeding structure (feeder organelle) through which host cell contents are obtained (myzocytosis).
Epimerite: anterior part of a gregarine cell associated with attachment and containing the apical complex.
Feeder organelle: anterior vacuolar membrane of zoite, folds extensively to form an elaborate feeding structure; equivalent of epimerite.
Gamont: sexual stage in the apicomplexan life cycle resulting from gametogony initiated by a trophozoite or merozoite.
Merogony: asexual reproduction by multiple fission of the parasite nucleus followed by simultaneous cellular division producing daughter cells (merozoites); synonymous with schizogony.
Meront: asexual multinucleate stage formed during merogony; synonymous with schizont.
Mucron: anterior attachment structure of aseptate (no transverse septum) gregarines; equivalent to the epimerite in septate gregarines.
Myzocytosis: predatory mode of feeding in which a parasite cell pierces the cell wall and/or membrane of the prey (host) cell with an apical 'feeding tube' and sucks out the cellular contents (myzozoa = 'sucking life').
Pleomorphism: variation in cell size and shape or the ability to change cell size and shape in response to environmental conditions.
Syzygy: association/pairing of trophozoites or gamonts end to end or laterally before the formation of gamonts or gametes.
Transmembrane: mode of feeding in some gregarines directly through the cell surface either extracellularly from the surrounding environment or at host cell surface (i.e., cell surface nutrition).
Trophozoite: non-encysted, feeding and/or resting stage.