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Review

It's official – *Cryptosporidium* is a gregarine: What are the implications for the water industry?

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ABSTRACT

Parasites of the genus *Cryptosporidium* are a major cause of diarrhoea and ill-health in humans and animals and are frequent causes of waterborne outbreaks. Until recently, it was thought that *Cryptosporidium* was an obligate intracellular parasite that only replicated within a suitable host, and that faecally shed oocysts could survive in the environment but could not multiply. In light of extensive biological and molecular data, including the ability of *Cryptosporidium* to complete its life cycle in the absence of a host and the production of novel extracellular stages, *Cryptosporidium* has been formally transferred from the Coccidia, to a new subclass, Cryptogregaria, with gregarine parasites. In this review, we discuss the close relationship between *Cryptosporidium* and gregarines and discuss the implications for the water industry.

Contents

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1. Introduction

The Apicomplexan parasite *Cryptosporidium* is a major cause of severe diarrhoea, developmental problems and death in young children and chronic, life-threatening disease in immunocompromised and malnourished individuals (Guerrant et al., 1999; Snelling et al., 2007; Costa et al., 2011; Kotloff et al., 2013; Striepen, 2013). No vaccines are available for *Cryptosporidium* (Mead, 2014) and current treatment options for cryptosporidiosis are limited, with only one drug, nitazoxanide (NTZ), exhibiting moderate clinical efficacy in children and immunocompetent people, and none in people with HIV (Abubakar et al., 2007; Amadi et al., 2009). Of the 31 valid species (Costa et al., 2016; Li et al., 2015; Ryan et al., 2015; Holubová et al., 2016; Kváč et al., 2016; Zahedi et al., 2016), *Cryptosporidium parvum* and *Cryptosporidium hominis* are responsible for the majority of human infections, although in some countries, *C. meleagridis* is as prevalent as *C. parvum* in human populations (Xiao, 2010).

Transmission of the parasite occurs via the faecal-oral route, either by ingestion of contaminated water or food, or by human-to-human or animal-to-human transmission (Xiao, 2010). The World Health Organization has categorized *Cryptosporidium* as a reference pathogen for the assessment of drinking water quality (Medema et al., 2006). This is because oocysts produced by *Cryptosporidium* are extremely hardy, easily spread via water, resistant to inactivation by chlorine and are difficult to remove from drinking water, without the use of expensive and lengthy filtration (Jakubowski, 1995; Striepen and Kissinger, 2004).

Waterborne transmission is a major mode of transmission and *Cryptosporidium* was the etiological agent in 60.3% (120) of the waterborne protozoan parasitic outbreaks that have been reported worldwide between 2004 and 2010 (Baldursson and Karanis, 2011). The severity of infections vary, depending on the species involved, but for zoonotic species, the dose required to cause an infection in 50% of subjects (ID 50) is estimated to be 10–83 oocysts for *C. hominis* and 132 for *C. parvum* (DuPont et al., 1995; Okhuysen et al., 1998; Chappell et al., 2006). The minimum infectious dose for *C. meleagridis* has yet to be determined (Chappell et al., 2011). Although the lowest infectious dose for *C. hominis* has been calculated to be 10 oocysts, in reality, one oocyst could be sufficient to cause infection in humans through direct or indirect routes of transmission (Chappell et al., 2006).

In addition to the apical complex, one main and unique feature of the phylum Apicomplexa, to which *Cryptosporidium* belongs, is the widespread presence of the apicoplast. This four-membrane-encased relict plastid (35 kb genome) of secondary endosymbiotic origin is thought to have originated by engulfment of a chloroplast-containing alga by the primitive eukaryotic ancestor of the Apicomplexa (Lim and McFadden, 2010). Microscopic, molecular, genomic and biochemical data indicate that *Cryptosporidium* differs from other apicomplexans in that it has lost the apicoplast (like the colpodellids and other gregarines) (rev. in Lim and McFadden, 2010), as well as the genomes for both the plastid and the mitochondrion (Zhu et al., 2000; Abrahamsen et al., 2004; Xu et al., 2004). *Cryptosporidium* also differs from other apicomplexans in fundamental features such as motility and invasion (Wetzel et al., 2005).

Until recently, *Cryptosporidium* was classified as a coccidian parasite. However, it has long been speculated that *Cryptosporidium* represents a 'missing link' between the more primitive gregarine parasites and coccidians. The similarities between *Cryptosporidium* and gregarines have been supported by extensive microscopic, molecular, genomic and biochemical data (Pohlenz et al., 1978; Bull et al., 1998; Carreno et al., 1999; Beyer et al., 2000; Hijjawi et al., 2002, 2004; Leander et al., 2003a; Rosales et al., 2005; Barta and Thompson, 2006; Butaeva et al., 2006; Valigurová et al., 2007; Boxell et al., 2008; Karanis et al., 2008; Zhang et al., 2009; Borowski et al., 2008, 2010; Hijjawi, 2010; Hijjawi et al., 2010; Templeton et al., 2010; Karanis and Aldeyarbi, 2011; Boxell, 2012; Koh et al., 2013, 2014; Huang et al., 2014; Clode et al., 2015; Valigurová et al., 2015; Aldeyarbi and Karanis, 2016a, 2016b; 2016c; Edwinson et al., 2016; Paziewska-Harris et al., 2016), which have served as the basis for the formal transfer of *Cryptosporidium* from subclass Coccidia, class Coccidiomorphea to a new subclass, Cryptogregaria, within class Gregarinomorphea (Cavalier-Smith, 2014). The genus *Cryptosporidium* is currently the sole member of Cryptogregaria and is described as comprising epicellular parasites of vertebrates possessing a gregarine-like feeder organelle but lacking an apicoplast (Cavalier-Smith, 2014). According to the International Code of Zoological Nomenclature (ICZN) (http://www.iczn.org/iczn/index.jsp), once a species has been formally re-classified in a peer-reviewed publically available journal, then that re-classification stands (unless challenged in the literature). As this re-classification has not been challenged, *Cryptosporidium* is now officially a gregarine.

2. What are gregarines?

Gregarines (phylum Apicomplexa; class Gregarinomorphea) are a very diverse group of large, single-celled "primitive" apicomplexan parasites that primarily infect the intestines and other extracellular spaces of invertebrates and lower vertebrates (mainly arthropods, molluscs and annelids), which are abundant in natural water sources (Leander et al., 2003a, 2003b; Barta and Thompson, 2006; Leander, 2007; Valigurová et al., 2007). The transmission of gregarines to new hosts usually takes place by oral ingestion of oocysts in both aquatic and terrestrial environments. Four or more sporozoites (depending on the species) escape from the oocysts, find their way to the appropriate body cavity and attach to, or penetrate, the host cells. The sporozoites emerge from a host cell, begin to feed and develop into large trophozoites (Rueckert and Leander, 2008).

Many gregarines do not exhibit intracellular stages and are mostly epicellular parasites. The gregarine life cycle typically only consists of gametogony and sporogony and only a few species exhibit merogony. The sporozoites will generally develop into large trophozoites and attach to the host cell with a specialized attachment apparatus (epimerite, mucron, modified protomerite) (MacMillan, 1973). These specialized structures are derived from the conoid at the apical end. This attachment to the host cell also functions in feeding in that the cytoplasm of the host is taken up by the attached parasite (i.e., myzocytosis) (Valigurová et al., 2007). Two mature trophozoites eventually pair up in a process called syzygy and develop into gamonts. The orientation of gamonts during syzygy differs depending on the species (e.g. side-to-side and head-to-tail). A gametocyst wall forms around each pair of gamonts, which then begins to divide into hundreds of gametes (gametogeny). Pairs of gametes fuse and form zygotes, each of which becomes surrounded by an oocyst wall. Within the oocyst, meiosis occurs to yield four or more spindle-shaped sporozoites (sporogony). Hundreds of oocysts accumulate within each gametocyst, and are usually released via host faeces or via host death and decay (Vivier and Desportes, 1990; Kuriyama et al., 2005; Rueckert and Leander, 2008).

The gregarines are thought to be the earliest lineage of apicomplexans (Rueckert and Leander, 2008) and were previously subdivided into three orders; Archigregarinida, Eugregarinida and Neogregarinida (Adl et al., 2012; Grassé, 1953). However, the taxonomy has recently been revised (Cavalier-Smith, 2014), on the basis that it was phylogenetically unsound (Rueckert et al., 2011). In