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Apicomplexan Parasites: Environmental Contamination and Transmission

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Abstract

The Apicomplexa are a diverse group of intracellular parasitic protists. The majority of species from the classes Coccidea, Haemosporea and Piroplasmea are responsible for widespread diseases of humans and domestic animals. Oocysts of these parasites can persist for long periods of time in the environment (*i.e.* in water, soil, on vegetation and other food resources), maintaining their infectivity even under harsh environmental conditions and therefore are important for dispersal and transmission to hosts. This review will address the biology, transmission patterns and survival in the environment of *Cryptosporidium*, *Cyclospora* and *Toxoplasma* species, the most common causes of human diseases.

Key words: Intracellular parasites, *Cryptosporidium*, *Cyclospora*, *Toxoplasma*, transmission, environmental survival

The phylum Apicomplexa contains over 4600 named species of protists, many of which are of significant medical and economic importance. Major species responsible for widespread diseases of humans and domestic animals are presented in Table I. All are unicellular and obligatory intracellular parasites occupying a variety of cells within the body of the host. Apicomplexa are characterized by lacking obvious locomotory structures and presence of an apical complex in their infective life cycle stage (zoities). The apical complex comprises polar rings and associated cortical microtubules, rhoptries, micronemes, and usually a conoid. Life cycles of the apicomplexans include both successive sexual and asexual phases of development: gamogony, the sexual phase with production of gametes and fertilisation (syngamy); sporogony, the asexual production of numerous sporozoites from the zygote within oocyst; and schizogony (merogony), a phase of asexual multiplication characterized by multiple fission (Fig. 1). The Protists of the Apicomplexa are currently divided into seven classes (Marquardt *at al.*, 2000), including Coccidiea, which form oocysts and have direct, contaminative life cycle, Haemosporea and Piroplasmea which do not form oocysts and have an indirect life cycle with an arthropod vector. However, the essential features of the life cycles of all these classes are very similar, differing mainly in aspects relating to transmission.

Table I
Major apicomplexan parasites of humans and domestic animals

Parasite	Cell	Disease	Host
Plasmodium spp.	red blood cells	malaria	humans
Babesia spp.	red blood cells	piroplasmosis	humans
Cryptosporidium spp.	enterocytes	cryptosporidiosis	humans
Cyclospora cayetanensis	enterocytes	cyclosporosis	humans
Sarcocystis hominis (*Isospora* spp.)	enterocytes	sarcocystosis	humans
Toxoplasma gondii	macrophages and many others	toxoplasmosis	humans

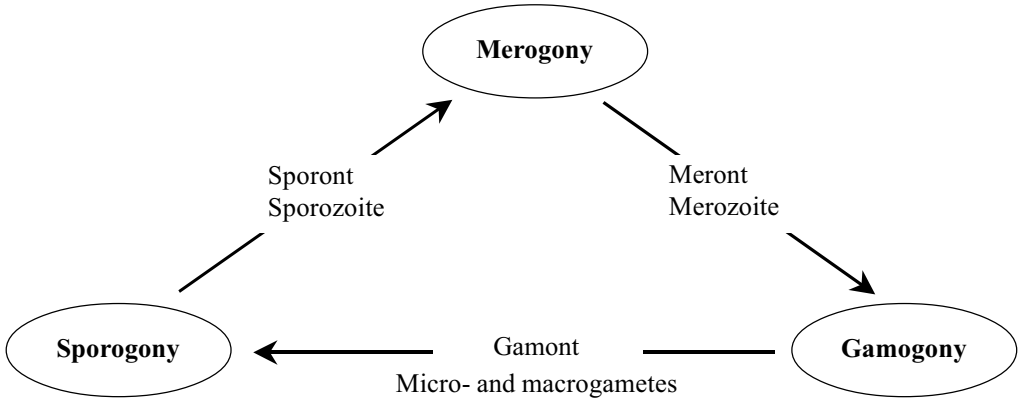

Fig. 1. Life cycle of the Apicomplexa.
The sporont, meront and gamont multiply by an asexual reproductive process called schizogony

Class: Coccidea

In the families of Cryptosporidae and Eimeriidae the normal mode of transmission is *via* a resistant oocyst, including:

1. direct faecal-oral, host-to-host transmission and indirect transmission following contamination of food or water resources (*e.g. Cryptosporidium* sp., *Cyclospora cayetanensis*)
2. direct faecal-oral transmission in definitive hosts and indirect transmission by food, characteristic for extra-intestinal or tissue coccidians (*e.g. Toxoplasma gondii*). They have so-called isosporoid oocysts with 2 sporocysts, each of which has 4 sporozoite, similar to those of the genus *Isospora*. The life cycles of the tissue coccidia involve two hosts, usually a carnivore and a herbivore. The life cycle of *Toxoplasma* can be completed in one host, the cat, and its intermediate hosts are not mandatory.

Classes: Haemosporea and Piroplasmea

Indirect transmission between hosts is achieved by blood-sucking arthropod vectors. Maturation of the gamonts, fertilization, and sporogony take place in the vector, the definitive host, merogony and gamogony take place in the vertebrate host, the intermediate host (*e.g. Plasmodium*, *Babesia*).

For the majority of species in the phylum the oocyst stage is of primary importance for dispersal, survival, and infectivity of the parasites. It is also the stage of major importance for detection, identification of the parasite and clarification of host specificity. Table II summarizes the biological characteristics, combined with the unique size and shape of the oocysts and their internal structures consisting of sporocysts and

Table II
Characteristic features of oocysts in the environment for genera of *Cryptosporidium*, *Cyclospora*, *Isospora* and *Toxoplasma*

Genera of parasite	Shape of oocyst*	Environmental form	Time of sporulation (days)	Mean number of oocysts excreted during 24 h	Duration of infectivity under optimal condition (months)
Cryptosporidium	III	thick walled oocyst, measure 4–5 µm, immediately infectious upon excretion	0	over 10 mil.	up to 4, humidity over 90%, temp. 1°C –15°C
Cyclospora	II II	oocyst measure 8–10 µm requires sporulation, not immediately infectious upon excretion	12–14	nd	up to 1, humidity over 80%, temp. 22°C–32°C
Isospora *Toxoplasma* *Sarcocystis*	III III	oocyst measure 12×11 µm requires sporulation, not immediately infectious upon excretion	3–4	over 1 mil.	from 4 to 24, humidity over 75%, temp. 5°C–12°C

* diagrammatic representation of relationship between oocysts, sporocysts and sporozoites
nd – no data

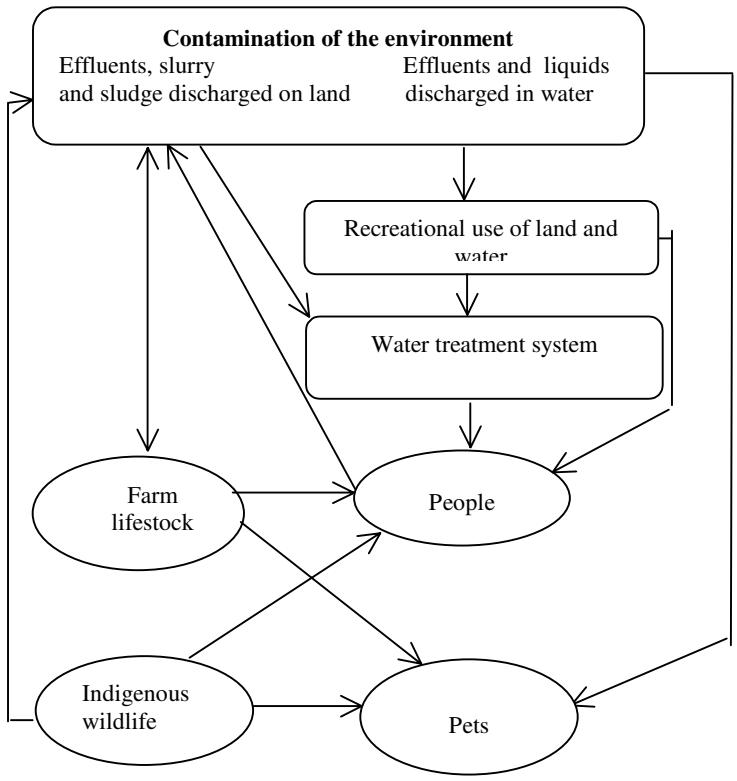

Fig. 2. Environmental reservoirs and potential patterns of transmission of *Cryptosporidium*, *Cyclospora* and *Toxoplasma* species

sporozoites, for each of the genera *Cryptosporidium*, *Cyclospora*, *Isospora*, *Toxoplasma*. The environmental reservoirs and potential patterns of transmission of these parasites are shown in Fig. 2.

The reasons for emergence of infectious diseases, due to *Cryptosporidium parvum* and *Toxoplasma gondii* have been attributed mainly: (1) to zoonotic transmission, *e.g.* more animals and changes in agricultural practices have led to a higher probability of successful parasite transmission and spread from animals to humans; (2) sensitive or susceptible populations, *e.g.* there is an increasing number of older and immunocompromised individuals (AIDS and transplant patients), diabetics, infants, and pregnant women, all of whom may be more susceptible to infection with both parasites.

Cryptosporidium spp.

Cryptosporidiosis has rapidly emerged as a worldwide disease of man since the first cases were identified in 1976. Approximately 6000 cases of infection are reported in England and Wales each year, and the likely number of infections in the population is probably greatly underestimated.

Cryptosporidum spp. are obligate intracellular parasites in the phylum Apicomplexa, order Eucoccidiida, family Cryptosporidae. *C. parvum* an intestinal parasite of mammals, which causes life-threatening diarrhoea in immunosuppressed humans and in young livestock animals is one of 13 valid species of *Cryptosporidium*: 7 in mammals, 3 in birds, 2 in reptiles and 1 species in fish, which are currently recognized and accepted according to the International Code of Zoological Nomenclature (Xiao *et al.*, 2004). Table III shows the main characteristics of these species.

Cryptosporidium is currently classified as an eimeriid coccidian, however there is mounting phenotypic and molecular phylogenetic evidence for a closer relationship with the gregarines and reclassification has been proposed but not yet adopted (Tenter *et al*., 2002). Also the complete genome sequence of *C. parvum*, type II isolate has been reported recently. Genomic analysis has identified extremely streamlined metabolic pathways and a reliance on the host for nutrients. In contrast to *Plasmodium* and *Toxoplasma*, the parasite lacks an apicoplast and its associated genome, and possesses a degenerate mitochondrion that has also lost its genomic components (Abrahamsen *et al.*, 2004). However, it is quite clear that use of conventional taxonomic approaches can be informative with respect to understanding the biology of *Cryptosporidium*

Table III
Characteristics of currently accepted species of *Cryptosporidium**

Species	Orginal host	Location**	Mean oocyst size (μm)	Status of infected host	
				immuno competent	immuno compromised
C. hominis	man	SI	4.9×5.2	+	+
C. parvum	mice	SI	5.0×4.5	+	+
C. felis	cat	SI	4.6×4.0	+	+
C. canis	dog	SI	5.0×4.7	+	+
C. wrairi	quinea-pig	SI	5.4×4.6	–	–
C. muris	mice	ST	8.4×6.3	–	+
C. andersoni	cattle	A	7.4×5.5	–	–
C. meleagridis	turkey	SI	5.2×4.6	+	+
C. baileyi	chicken	BF, CL	6.2×4.6	–	–
C. galli	birds	CL	6.0×4.5	–	–
C. serpentis	snakes	ST	6.2 × 5.3	–	–
C. saurophilum	lizard	SI	5.0×4.7	–	–
C. molnari	fish	SI, ST	4.3×3.3	–	–

* after Fayer *et al.*, 2000; Chalmers *et al.*, 2002 and Xiao *et al.*, 2004, with some modifications
** A – abomasum, BF – bursa of Fabricius, CL – cloaca, ST – stomach, SI – small intestine

species, characterizing transmission dynamics, tracking infections and identifying sources of contamination and hence assessing the public health significance for humans, animals as well as for the environment,

An essential stage in the life cycle of *Cryptosporidium* is the formation in the gastrointestinal tract of two types of oocysts, each containing four infectious sporozoites. Thin walled oocysts remain in the gut to prolong the infection. Thick walled oocysts are shed in apparently normal or diarrhoeic faeces to contaminate soil and water. *C. parvum* causes symptomatic illnesses mainly in young animals, although older animals may be carriers, and it is thought to be readily passed from animals to humans by the faecal-oral rout. The infective dose is relatively low, with an LD_{50} of 132 oocysts reported for healthy adults (Du Pont *et al*., 1995). *Cryptosporidium* is resistant to disinfectants used in the water industry and has been implicated in over 20 waterborne outbreakes (Smith and Rose, 1998; Girdwood and Smith, 1999). The Milwaukee outbreak resulted in the death of 104 of 403 000 cases (Mac Kenzie *et al.*, 1994).

A number of biological features of *Cryptosporidium* affect its transmission and epidemiology:
- the life cycle does not require dual or multiple hosts
- the oocyst stage is shed in a fully sporulated state, so direct transmission can occur between hosts
- autoinfection enables persistant disease in immunocompromised hosts
- there is a large zoonotic (rodents, livestock) and human reservoir
- the thick-walled oocysts are resistant to a wide range of pressures and can survive for long periods in the environment
- the infectious dose is low and so small numbers of contaminating organisms are significant
- hosts can shed large numbers of oocysts
- there is a lack of specific drug therapy to clear infections efficiently.

Cyclospora cayetanensis

As with other coccodian parasites, *C. cayetanesis* (previously termed cyanobacterium like body) is an obligate intracellular parasite. It infects the cells of the upper portion of the small intestine causing recurring diarrhoeic disease – cyclosporosis in both immunocompetent and immunocompromised persons (Guerrant *et al*., 2001). Infections are endemic in many developing countries in South and Central America, Africa, India as well as in parts of Asia, and through trade have become a problem for many developed countries, *e.g.* USA, Canada. However, the true prevalence of the parasite in any population is unknown (Soave and Johnson 1995). Cyclosporosis appears to be a seasonal disease with periodicity linked to spring and early summer and seems to be both food and water borne. Moreover, *C. cayetanesis* is an important agent of

traveler's diarrhoea, most reported cases of cyclosporosis in Europe and Australia having been associated with international travel to endemic areas (Shields and Olson 2003).

Toxoplasma gondii

Toxoplasma gondii is an opportunistic intracellular parasite that, in the tachyzoite stage in the life cycle, is capable of infecting a wide range of nucleated cells of vertebrate hosts. The mode of *T. gondii* transmission remained unclear until 1970, when its life cycle was elucidated. This tissue-cyst forming coccidium (order Eucoccidiorida, family Eimeriidae) has a heterogeneous life cycle comprising an asexual phase in a variety of warm-blooded intermediate hosts and a sexual phase in the intestines of felines definitive hosts (Frenkel, 1973). In the intestinal cells of domestic cats the parasite undergoes both schizogony (asexual proliferation) and gametogony (sexual phase of the cycle), the latter resulting in the formation of immature oocysts. Cats can shed oocysts for 1–2 weeks in extremely large numbers, peaking at over a million a day, and these oocysts can remain viable for 1 year or even longer. Mature oocysts measured 12×11 μm, contain eight sporozoites and are infective to over 300 species of warm-blooded intermediate hosts, such as birds and mammals, including humans. When oocysts are ingested, the parasites undergo an asexual cycle with two stages: the tachyzoites (2 μm×6 μm) is the intracellular proliferative form which is present during the acute phase of infection, and the bradyzoite, the slowly dividing encysted tissue form (cyst 12 μm to 100 μm), is characteristic of the chronic phase of infection. Bradyzoites may persist throughout the life of intermediate hosts.

Human infection with *Toxoplasma* is widespread, *e.g.* in the USA and UK estimates vary between 16 and 40% of the population being infected, while in Europe, Central and South America prevalence of infection ranges from 50 to 80% (Dubey and Beattie 1988). However, toxoplasmosis is generally asymptomatic in approximately 85% of immunocompetent persons, and for them there is no significant health risk. In immunodeficient individuals, including AIDS patients, organ transplant recipients and patients undergoing chemotherapy, central nervous system disease is common and chorioretinities or pneumonities may develop (Renold *et al*., 1992).

T. gondii horizontal transmission *via* tissue cysts in humans is generally acquired by the oral route, after ingestion of raw or undercooked meat containing parasite cysts (lamb, pork and beef), and of vegetables or water contaminated with oocysts from infected cat faeces.

Another route of transmission (vertical) is *via* transplacental passage of parasites from infected mothers to fetuses (congenital toxoplasmosis). Infection acquired during pregnancy, in the absence of prior immunity, may cause abortion, or congenital disease resulting in mental retardation or blindness in the infant (Remington *et al*., 2001).

Contamination and survival in the environment of *Cryptosporidium*, *Cyclospora* and *Toxoplasma* oocysts

The potential for environmental contamination depends upon a variety of factors including the geographic distribution of the parasite, number of infected human and non-human hosts, seasonal influence and duration of infection, the number of transmission stages excreted, agricultural practices, host behaviour and activity, socioeconomic and ethnic differences in human behaviour, and sanitation.

Cryptosporidium oocysts have been detected in a variety of environmental matrices including farmyard manure, leachate, slurry, and soil. Also various water sources and foodstuffs (Rose and Slifco, 1999). The thick, two layered wall of *Cryptosporidium* oocysts ensures that oocysts are robust and resistant to a variety of environmental pressures particularly under cool, moist conditions. For example extremes of temperature and dehydration including freeze-drying, exposure to temperatures above 60°C and below –20°C for 30 minutes will all kill *Cryptosporidium* (Anderson, 1985), as will brief pasteurization (Harp *et al.*, 1996). According to Blewett (1989) oocysts are killed by five minutes of exposure to moist heat of at least 60°C. Survival in manure heaps and slurry stores is adversely affected by the pH, temperature and ammonia characteristic of these environments (Jenkins *et al.*, 1998). Although many common disinfectants used on farms, in hospitals or veterinary surgeries have little effect on *Cryptosporidium*, both hydrogen peroxide and ammonia inactivate oocysts (Casemore *et al.*, 1989).

Cyclospora oocysts have been detected in environmental samples, including water, wastewater and foods. Oocysts leave their host in an unsporulated non-infective form. In order to become infective they must

sporulate for at least 12–14 days. Under appropriate conditions oocysts differentiate into two sporocysts, each containing two sporozoites and the rate of sporulation is probably influenced by environmental factors. However, it appears that sporulation dose not occur following exposure to –20°C for 24 hours or to 60°C for 1 hour thus rendering oocysts non-infective (Smith *et al.*, 1997).

Heating to 67°C kills toxoplasma tissue stages (tachyzoites and bradyzoites), but bradyzoites can survive refrigeration and some can survive freezing, maintaining infectivity after storage at –5°C. Oocysts of *Toxoplasma* are shed unsporulated in felid faeces, and after sporulation which requires some 1 to 5 days become infective. Oocysts of *T. gondii* have been detected in soil naturally contaminated with cat faeces (Ruiz *et al.*, 1973) and in soil from gardens (Coutinho *et al.*, 1982). According to studies conducted by Frenkel *et al.* (1975) oocysts may survive in soil for as long as 18 months. There is also evidence that oocysts can become distributed in the environment by mechanical spreading through the activities of invertebrate animals. However, there are only a few studies indicating that oocysts of *Toxoplasma* can survival in water; *e.g.* laboratory studies have shown they can survive for up to 4.5 years at 4°C (Dubey 1998). Survival for several months has been demonstrated in water, as has resistance to many disinfectants *e.g.* chlorine, freezing at –10°C, and drying, but they are killed by heating to 55–60°C (Kuticic and Wikerhauser 1996). Morever, infections with *T. gondii* have been detected even in aquatic mammals-southern sea otters (*Enhydra lutris nereis*) and these imply that oocysts contaminating seawater can survive long enough for transmission to take place (Cole *et al*., 2000).

The efficient transmission of cryptosporidiosis and cyclosporiosis is to a large extent dependent on the biological characteristics and especially the robust, and environmentally resistant oocysts of these parasites, which evolved specifically for this purpose. The potential risk of outbreaks of infection in human communities is dependent on precise local environmental and climatic conditions, and on farming practices (*e.g.* use of effluents to fertilise farm land) that facilitate access of oocysts to drinking water or result in contamination of food. Waterborne transmission is exacerbated particularly when climatic conditions affect the water resources of human communities (*e.g.* during periods of flooding) or when water treatment systems fail (*e.g.* employ inappropriate disinfectants or simply cannot cope with increases in human populations).

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