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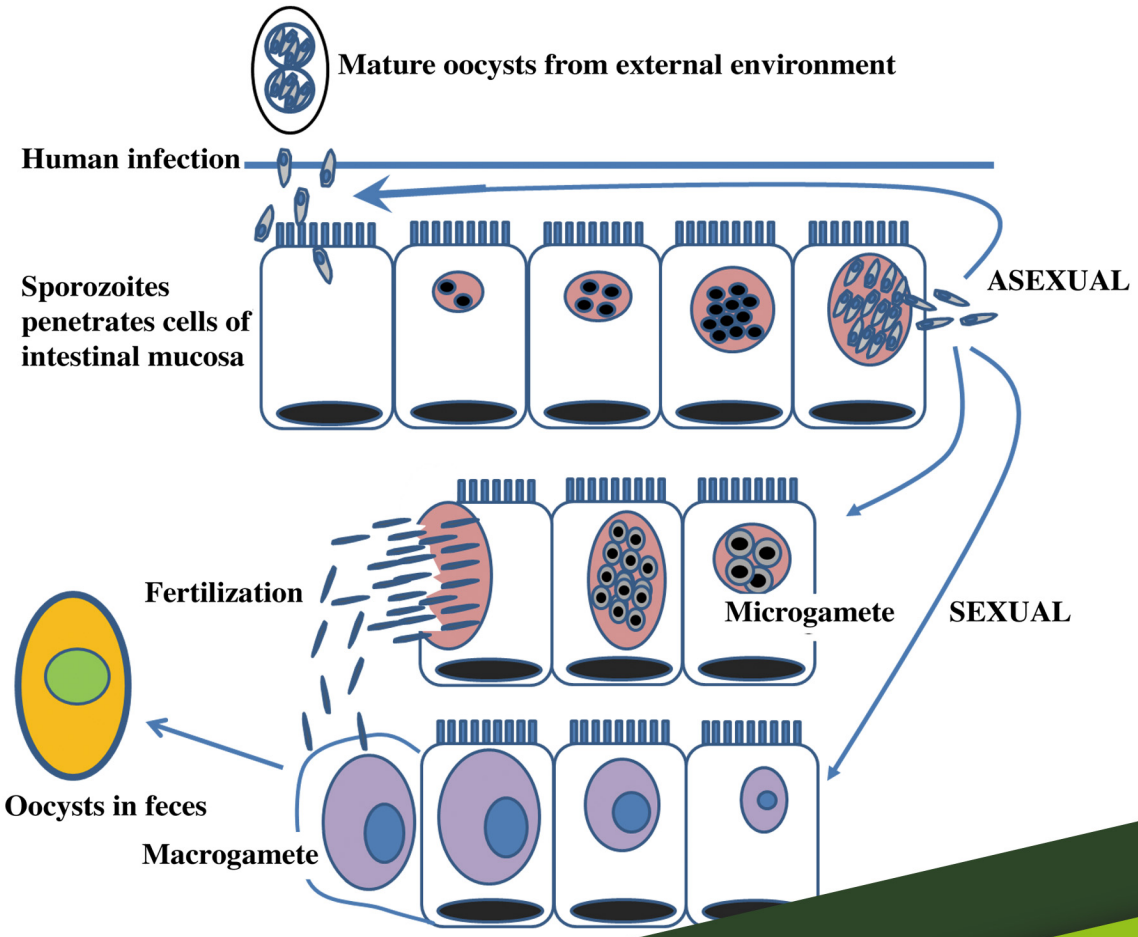
WATER-BORNE PROTOZOA IN HUMANS



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Frontiers in Parasitology

(Volume 2)

(Water-borne Protozoa in Humans)

Edited by

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Frontiers in Parasitology

Volume # 2

Water-borne Protozoa in Humans

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The figure shown on the front cover of this book is kindly supplied by Dr. Massimiliano Galdiero, Department of Experimental Medicine, Division of Microbiology and Clinical Microbiology, Second University of Naples, Italy. The cover figure shows the sexual and asexual replication of Cystoisospora belli (see chapter 4 of this book).

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FOREWORD

Parasitology continues to be a very exciting and stimulating field of study and has regained global attention and interest especially due to the data obtained from the recent 2010 and 2013 Global Burden of Disease studies that highlight how parasitic infections continue to be amongst the leading causes for the highest disabilities worldwide. In addition, even though amazing strides have been achieved towards the Millennium Development Goals, the UN member states and the world are now faced with much broader and more audacious post-2015 goals as part of the new Sustainable Development Goals to be achieved by 2030. For these objectives to be achieved it is very important not only to focus and monitor available and ongoing control implementation programs globally, but also complement these efforts with novel research leading to new and affordable health innovations.

Therefore, when Professor Fabrizio Bruschi invited me to write the foreword for this book titled **"Frontiers in Parasitology: Water-borne Protozoa in Humans"** it immediately captured my interest and excitement to know that with this book physicians, health professionals and scientists around the world will rapidly gain access to the most up to date advances and innovation about the epidemiology, pathobiology, laboratory and clinical diagnosis, and available prevention and treatment measures for this very important group of infectious and tropical diseases. It is well known that protozoan infections are important causes of diarrheal and other gastrointestinal diseases in humans including the major causes for travellers' diarrhea. In addition, some of the protozoans can affect the central nervous system and even cause keratitis.

After reading this book the hope is that it will raise awareness of the importance of water-borne protozoan infections as global public health problems, while at the same time generate new ideas, new networks and partnerships and bring new translational discoveries from the bench into the clinic ultimately leading to an improvement in health delivery mechanisms benefiting global strategies.

Indeed, and of no surprise if one were to search for a good compilation of recent articles in the last 10 years that summarizes the global distribution and burden in this field of study, there are very limited publications that provide a comprehensive review of the global health impact of waterborne parasitic protozoan infections worldwide. Furthermore, for most if not all the water-borne diseases, effective drug treatments and vaccines are not yet available and diagnostic tools are not highly sensitive or reliable especially for point of care diagnostics in resource-poor settings. One of the most recent reviews on water-borne protozoa was written by Baldursson and Karanis and published in Water Research in 2011, and shows that in Australia, Europe and North America at least 199 outbreaks of these diseases occurred and were reported between 2004-2010. However, the authors also highlight that the countries that most likely have the greatest populations afflicted by water-borne parasitic infectious and other tropical diseases lack strong surveillance systems, leading to a chronic under-reporting or even no reporting of their public health burden.

This book, then, aims to fill this gap in knowledge as well as provide a comprehensive

overview of major parasitic water-borne protozoan diseases. It includes an impressive list of authors and co-authors internationally recognized in the field of parasitology, covering in a compilation of 8 chapters the new and recent insights for waterborne-protozoa including *Blastocystis*, *Cryptosporidium, Cyclospora, Cystoisospora, Entamoeba and other pathogenic intestinal amoebae, free living amoebae, Giardia and Microsporidia.*

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PREFACE

Water is essential for living organisms on Earth, but it can also spread toxic substances and pathogens for humans and animals, particularly in low income Countries. At global level, waterborne pathogen exposure in fact correlates significantly with low socio-economical conditions.

During the recent past, human development, population growth, extreme weather events as well as natural calamities due to climate changes have affected both the quality and quantity of water resources, increasing the occurrence of water-borne infectious diseases. These are a major cause of morbidity and mortality, with an estimate of 4.0% of global deaths and 5.7% of the global disease burden (in terms of Disability Adjusted Life Years) attributable to a complex of factors like water, sanitation, and hygiene conditions. Water-borne pathogens such as viruses, bacteria and protozoa are responsible for diarrheal diseases which affect, sometimes in a serious manner, in particular children, worldwide.

This E-book is designed to inform the reader on water-borne protozoa infections in humans.

The audience is represented by medical students, parasitologists, clinical microbiologists, infectivologists, researchers, environmental technicians, people who are engaged in water purification and control and public health personnel. Each chapter addresses the history, morphology and life cycle, global epidemiology and risk factors, immunology and immunopathology, symptoms, diagnosis and detection methods, treatment and perspectives of control for major waterborne parasites transmissible with water, either used for drinking or for recreational activities.

In addition to the parasites considered in this book, others can be transmitted by water, like *Toxoplasma gondii* for example, but were not considered in this book.

The Editor is grateful to David Di Cave, Marco Lalle, Simonetta Mattiucci, Walhul Khan, Una Ryan, Chuck Sterling, Magda Azab, Nicola Coppola for valuable reviewing process.

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CHAPTER 1

Blastocystis spp.

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Abstract: *Blastocystis* spp. are intestinal parasitic protozoa of humans and many other organisms Using molecular approaches the taxonomy of *Blastocystis* was determined. At present, this parasite is believed to belong to the subclass of Stramenopiles (or Heterokonts), subgroup Opalinata. *Blastocystis* is the only Stramenopile known to be a human parasite. For this reason it was classified first as *Blastocystis* hominis, but in the light of new knowledge, the term of *Blastocystis* spp. is more appropriate In this chapter epidemiology, life cycle and transmission, morphology and structure, laboratory diagnosis including microscopy, culture and molecular techniques are reviewed. Finally, clinical aspects, including the association with irritable bowel syndrome and therapeutic approaches are considered.

Keywords: *Blastocystis* spp., Diagnostic method, Epidemiology, Human infection, Immunology, Molecular epidemiology, Molecular subtypes, Pathogenesis, Treatment.

INTRODUCTION

Blastocystis spp. are single-celled protozoa, intestinal parasites of humans and numerous other organisms such as mammals, birds, amphibians, reptiles, fish, arthropods, annelids, insects and mollusks [1 - 5]. For years, the taxonomy of *Blastocystis* was very controversial and through the use of molecular methods it has been possible to define the phylogenetic characteristics of the parasite. Today it is believed to belong to the subclass of Stramenopiles (or Heterokonts), subgroup Opalinata [6 - 8]. *Blastocystis* is the only Stramenopile known to be a human parasite, for this reason Brumpt suggested the name of *Blastocystis* hominis [9], which today has been substituted, in the light of new acquisitions, by *Blastocystis* spp.

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Fabrizio Bruschi (Ed.)

EPIDEMIOLOGY

The geographic distribution of *Blastocystis* spp. is very wide, above all in developing countries, and it is estimated that about 1 billion subjects are colonized [10, 11]. Even if the epidemiology of this parasite has not yet been well defined [12, 13] it is probably the most widespread eukaryote organism in humans [14].

Table 1. Prevalence of *Blastocystis* infections in different countries.

Prevalence (%)	Country	Reference
2.1	Turkey	[15]
3.7	China	[16]
7	Mexico	[10]
13.5	Thailand	[11]
13.6	Italy	[17]
14.2	Turkey	[18]
14.7	India	[19]
15	Germany	[20]
20	USA	[21]
22.9	Argentina	[22]
26.5	Brazil	[23]
26.6	Libya	[24]
36.4	Colombia	[25]
40.9	Brazil	[26]
52.3	Malaysa	[27]

The prevalence of *Blastocystis* spp. is clearly higher in developing countries, approximately between 30% and 50% [28, 29]. Immigrants, adopted children and people who work in close contact with animals, have a greater probability of contracting an infection caused by *Blastocystis* spp [30, 31]. Scientific evidence suggests that patients with AIDS and severe immunosuppression, have higher risk of infection by *Blastocystis* spp [32 - 35].

The prevalence of *Blastocystis* spp. in HIV/AIDS patients is estimated at 8% in India [35], and 38% in Germany [33]. A recent study evaluated it at 72.4% in Jakarta, Indonesia [34].

Fig. (1) **Fig. (2)** **Fig. (3)**

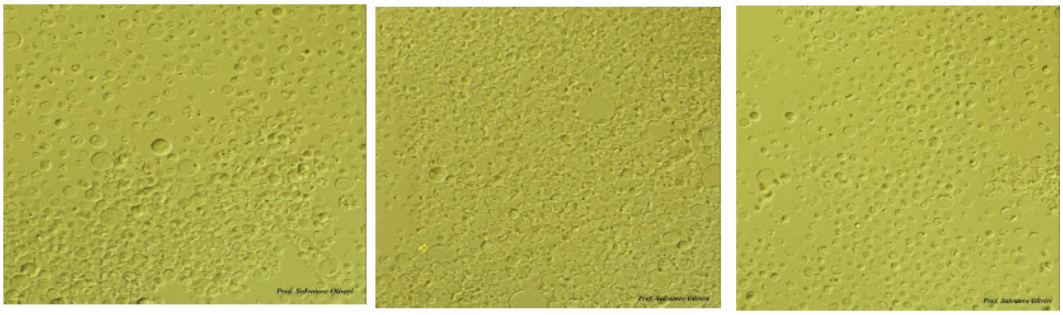

Fig. (1-3). Cystic form of *Blastocystis spp.* from laboratory culture on Boeck and Drbohlav's egg enriched medium. Optical microscopy, x400 magnification.

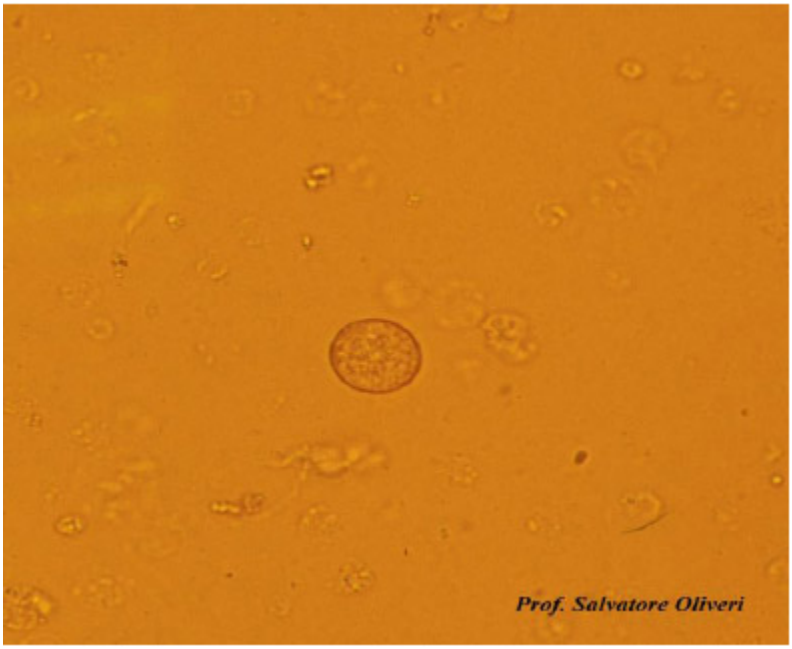

Fig. (4). Detail of a Cystic form of *Blastocystis spp.* from culture. Optical microscopy, x1000 magnification.

MORPHOLOGY AND STRUCTURE

Blastocystis spp. is an extremely polymorphic organism of which four principle forms have been described, in fecal samples or in *in vitro* cultures: vacuolar, granular, amoeboid and cystic [1, 35 - 37].

Vacuolar Form

The vacuolar form is the most frequently identified in axenic cultures and fresh feces of infected patients. Under brightfield illumination this form is seen as a roundish mass between 2 and 200 μm in size, with an average diameter of 4-45

CHAPTER 2

Cryptosporidium and Cryptosporidiosis

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Abstract: *Cryptosporidium* spp. has emerged as an important cause of diarrheal disease in humans and animals, with a global distribution. The epidemiology of cryptosporidiosis is complex, and transmission occurs through both direct (human-to-human, animal-to-human) and indirect (through water and food) routes. In humans, the majority of infections are caused by *C. hominis* and *C. parvum*, but a number of other species have been recognized as human pathogens. The burden of disease is particularly high among young children in developing regions of the world, where cryptosporidiosis is one of the major causes of moderate-to-severe diarrhea and is associated with an increased risk of death. In developed countries, contamination of drinking water has caused several large waterborne outbreaks. Effective drug treatments and vaccines are not yet available, but the partial immunity after exposure suggests the potential for developing vaccines. Routine diagnostic methods for *Cryptosporidium* often have a low sensitivity, and those based on antigen or DNA detection, that greatly improve sensitivity, are underused. Recent advances in next-generation sequencing techniques will significantly improve our knowledge of the transmission of *Cryptosporidium*. However, increased funding will be essential to combat this important pathogen.

Keywords: Cryptosporidiosis, *Cryptosporidium*, Diagnostic methods, Epidemiology, Genomics, Genotyping, Human infection, Immunology, Intestinal disease, Molecular epidemiology, Pathogenesis, Risk factors, Transmission routes, Treatment.

INTRODUCTION

History

The first description of the parasite dates back to more than a century ago, with two publications by Ernest Edward Tyzzer, a medical parasitologist at Harvard University in Boston. Tyzzer gave the name *Cryptosporidium* because of the

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absence of sporocysts within the oocysts, a characteristic of other coccidians. He first described the type species, *C. muris*, from the gastric glands of laboratory mice [1] and provided a more complete description of the life cycle in a subsequent publication [2]. Soon thereafter, Tyzzer described a second species, *C. parvum*, also from the laboratory mice, which differed from the type species because infection occurred in the small intestine and not in the stomach, and because of the smaller size of the oocysts [3]. Remarkably, these first three publications have defined most of what we currently know about the biology and life history of *C. muris* and *C. parvum* [4].

Research on *Cryptosporidium* then entered a long period of oblivion, and it was not until 1955 that its pathogenicity was demonstrated in turkeys [5], and, some twenty years later, in livestock [6]. In 1976, the first cases of human cryptosporidiosis were reported by two groups, and involved either children or immunosuppressed adults [7, 8].

Two events stand out for having significantly impacted our awareness of these parasites and the disease they cause. First, the HIV epidemic and the emergence of cryptosporidiosis as a potentially severe opportunistic infection in people living with AIDS was significant, not only because of the clinical implications, but because it motivated a substantial research effort and a desire to better understand these parasites [9]. A second notable event was the waterborne cryptosporidiosis outbreak in Milwaukee in 1993, which is still the largest waterborne outbreak ever recorded [10]. The importance of this outbreak is illustrated by the fact that the article reporting on the epidemiological investigation of the outbreak was cited over 600 times, more than any other paper dealing with this parasite.

Taxonomy

The taxonomy of the genus *Cryptosporidium*, like is the case for many other protozoa, is still in a state of flux. The genus *Cryptosporidium* belongs to the phylum Apicomplexa, but the exact position within the phylum has been a matter of debate. Traditionally, *Cryptosporidium* has been placed within the coccidia, since it shares with *Eimeria*, *Isospora* and *Cyclospora* the ability to develop in the gastrointestinal tract and to complete the entire life cycle within a single host. However, *Cryptosporidium* shows several peculiarities that separate it from any other coccidian, including its location within the host cell (intracellular, but extracytoplasmic), its ability to form a feeder organelle that facilitates the uptake of nutrients from the host cell, the presence of two morpho-functional types of oocysts (thick-walled and thin-walled), and its insensitivity to all anti-coccidial agents tested so far.

Besides these observations, molecular phylogenetic studies have challenged the inclusion of *Cryptosporidium* within the coccidia. Indeed, analysis of ribosomal and tubulin genes showed that *Cryptosporidium* is more closely related to the gregarines, a basal group within the phylum Apicomplexa [11], and this phylogenetic association was supported by genome comparison of *Cryptosporidium* and the gregarine *Ascogregarina taiwanensis* [12]. A clearer understanding of the correct taxonomic placement of the genus *Cryptosporidium* will probably require further genomic studies of gregarines.

At the species level, inherent difficulties have hampered the definition of an accurate taxonomy, not least the lack of consensus as to which definition of species can be applied to organisms like *Cryptosporidium*. However, it has been proposed that the naming of new species must be based on: a) morphometric studies of oocysts; b) genetic characterization, with sequences deposited in public databases; c) demonstration of natural and, whenever feasible, at least some experimental host specificity; and d) compliance with International Code of Zoological Nomenclature [13].

As a result of intense research, at present 27 species are regarded as valid; of these, 19 are from mammals, 3 from birds, 1 from amphibians, 2 from reptiles, and two from fish [14]. Besides species, there are at least 40 genotypes of still undefined taxonomic status. The majority of human infections are caused by two species, *C. hominis* and *C. parvum*, but many other species and genotypes have been associated with human cryptosporidiosis, although with a lower prevalence [15]. Table **1** lists the species/genotypes currently regarded as human pathogens.

Table 1. List of the *Cryptosporidium* species and genotypes currently recognized as human pathogens. Original references of species description can be found in [15].

Species or Genotypes	Main Hosts	Public Health Relevance
C. hominis	Human	High
C. parvum	Cattle, human	High
C. meleagridis	Turkey	Moderate
C. felis	Cat	Moderate
C. canis	Dog	Moderate
C. cuniculus	Rabbit	Moderate
C. ubiquitum	Sheep, wildlife	Moderate
C. viatorum	Human	Moderate
C. muris	Rodent	Minor
C. scrofarum	Pig	Minor

CHAPTER 3

Cyclospora cayetanensis

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Abstract: The coccidian parasite *Cyclospora cayetanensis* can cause gastrointestinal illness. It has been associated with consumption of contaminated foods, particularly those eaten raw or minimally processed. First considered a cyanobacteria or coccidian-like organism, in the early 1990s it was characterized as a member of the *Cyclospora* genus based on morphological properties. This parasite has been associated with travelers' diarrhea. To date, there is no animal reservoir identified for *C. cayetanensis*. Waterborne outbreaks have also been reported. In July of 1990, 21 household staff physicians developed diarrheal illness and analysis of their stool specimens showed organisms consistent with *Cyclospora* oocysts. Epidemiological investigations implicated tap water as the likely source of the outbreak. In another instance, in 1994, British soldiers and their dependents stationed in Nepal developed cyclosporiasis. Local drinking water with chlorine concentrations of 0.3-0.8 ppm was associated with this outbreak and *Cyclospora* oocysts were isolated from the water. Additionally, imported berries, salad greens, and herbs were associated with foodborne outbreaks of cyclosporiasis in the U.S., Canada, and European countries in the late 1990s. In 2013, 2014, and 2015 *C. cayetanensis* caused significant outbreaks in the U.S. and were epidemiologically associated with imported salad greens and cilantro. *Cyclospora* is resistant to many commonly used sanitizers and certain environmental conditions which are found in many locations used to process/package produce. Oocysts can be inactivated by either freezing or heating. Tools for traceback studies and *in vitro* or *in vivo* models to propagate this parasite are currently not available.

Keywords: Coccidia, Control, *Cyclospora cayetanensis*, Eimeriidae, Epidemiology, Foodborne, Transmission, Waterborne.

INTRODUCTION

Cyclospora cayetanensis is a coccidian parasite that causes gastrointestinal illness in humans, and it is a frequent cause of traveler's diarrhea in people visiting endemic areas such as Nepal, Haiti, Mexico, Guatemala, and Peru. The seasonality of this parasite makes the risk of acquiring this infection higher during

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particular months of the year of each endemic location. Since 1995, *Cyclospora* outbreaks have been reported in the U.S. and Canada. Foodborne transmission has been confirmed and in most instances, linked to imported food commodities such as salad greens, herbs, and fruits. In this chapter the biology, epidemiology, treatment, diagnosis, prevention, the effectiveness of water treatment processes and recommendations for *Cyclospora cayetanensis* will be described.

History

Reports of travelers' diarrhea caused by coccidian/cyanobacterian-like bodies were perhaps the first reports of *Cyclospora*. Ashford reported three cases in Papua New Guinea which he thought were caused by *Isospora* spp. (now denominated *Cystoisospora*) [1]. Later in 1986, Soave described 4 cases of patients with organisms resembling *Cyclospora* in their stool samples. In 1989, 55 immunocompetent travellers and expatriates in Nepal presented with gastrointestinal illness, particularly diarrhea, and had algae-like organisms in their stools. At that time, it was thought that the outbreak had occurred during the months of May-June, but epidemiological studies associated with this outbreak were not performed [2]. We now know that these months correspond to the annual high season of *Cyclospora* in Nepal and that *Cyclospora* is endemic in that country. In the early 1990's, a team of scientists in Peru working on diarrheal illnesses in children noticed the presence of cyst-like structures twice the size of *Cryptosporidium.* Further investigation showed that if these structures were left in dichromate, they would differentiate from a morula-like form to two sporocysts within a round oocyst. These organisms or CLBs, at that time called "big *Cryptosporidium*", were in fact oocysts of a new coccidian parasite. This new parasite was classified into the genus *Cyclospora* based on morphologic characteristics and the parasite species was later named '*cayetanensis*' in recognition of the work performed at the Cayetano Heredia University in Lima, Peru [3, 4]. At the same time, treatments were being explored for this illness and the same Peruvian team identified trimethoprim sulfamethoxazole as the drug of choice to effectively control cyclosporiasis [5, 6]. These results were later validated by other groups of investigators [7 - 9].

MORPHOLOGY AND LIFE CYCLE

Cyclospora has three stages in its life cycle. The first stage occurs outside the host. The unsporulated oocysts are not differentiated or infectious when excreted and have a morula-like form. In about 7 to 15 days outside the host and under favorable environmental conditions, the unsporulated oocyst will differentiate to form the sporulated oocyst that consists of two sporocysts, each with two

sporozoites. The second phase starts when the oocysts are ingested *via* contaminated water or foods; these oocysts will excyst and the sporozoites will infect intestinal epithelial cells. The first asexual multiplication results on the formation of a type I meront containing 8 merozoites. This is followed by the formation of a type II meront with 4 merozoites. The meronts are localized in the cytoplasm of intestinal epithelial cells in the jejunum. The third phase occurs when sexual multiplication takes place and merozoites differentiate to form gametocytes. A microgametocyte will fertilize a macrogametocyte to form a zygote which in turn will develop into an unsporulated oocyst that will be excreted in the feces of an infected individual (Fig. **1**).

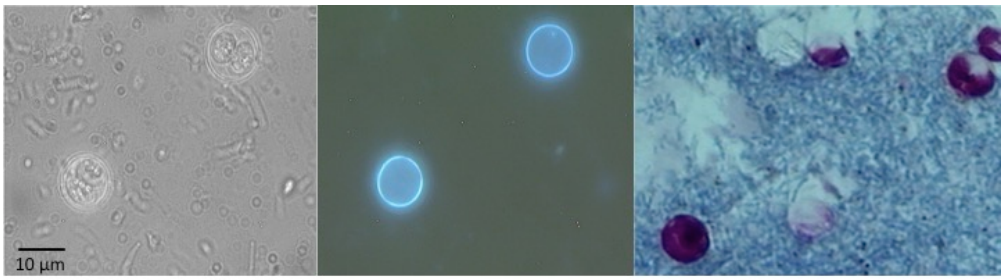

Fig. (1). *Cyclospora cayetanensis* oocysts observed using Differential Interference Contrast (DIC) microscopy, autofluorescence, and acid fast staining.

GLOBAL EPIDEMIOLOGY AND RISK FACTORS

Cyclospora is endemic in various locations worldwide and in-depth epidemiological studies have been performed in Nepal, Peru, Haiti, Guatemala, and Mexico [2, 3, 6, 7, 10 - 13]. Sporadic cases of *Cyclospora* have also been reported in Canada, Germany, Australia, and the U.S. and several outbreaks have been reported in countries considered not endemic for *C. cayetanensis* [14 - 17]. This infection can be acquired by ingestion of contaminated water and foods containing *Cyclospora* oocysts. Person to person transmission is unlikely since oocysts require at least 7 days to sporulate in the environment. Reservoirs and mechanical vectors have also been suggested to play a role in the life cycle of this parasite [18 - 20], but it is currently considered exclusively anthroponotic. *Cyclospora* oocysts have been found in feces of chickens, ducks, and dogs in several studies [18 - 20], but attempts to reproduce infection in laboratory animals have been unsuccessful and surveys in some endemic locations have come up negative in these and other animals. It has been suggested that coprophagic activities of these animals may have led to the reported identifications in those studies [21].

CHAPTER 4

Cystoisospora belli

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Abstract: Cystoisosporiasis is a human intestinal disease caused by the protozoan parasite *Cystoisospora belli.* It is an obligate intracellular parasite that invades intestinal epithelial cells. It causes a self-limited or prolonged diarrhea, depending on the host's immune status. *C. belli* is cosmopolitan, but more common in the tropics and subtropics. The parasite can be spread by ingesting contaminated food or water. *C. belli* oocysts are ellipsoid and approximately 25 μm to 33 μm by 10 μm to 20 μm and contain two sporocysts, which contain 4 sporozoites each. Feces are the common way of immature form of the parasite release. The parasite, usually needs between 1 or 2 days in the environment (outside of host) to performe the adequate maturation steps to be able infect further subjects. Watery diarrhea is the most common symptom. Other symptoms can include cramps, abdominal pain, dehydration, loss of appetite, nausea, fever and vomiting. Diagnosis is achieved by oocysts observation *via* microscopic stool examination. Enhanced detection is obtained by staining stool samples *via* modified acid-fast stain, modified safranin stain or by fluorescence microscopy. Molecular diagnostic methods have the advantage of high sensibility for an early detection of these coccidian parasites in stool samples. If untreated, immunocompromised individuals may increase the risk for prolonged and severe illness. The usual treatment is with trimethoprim-sulfamethoxazole. Cystoisosporiasis can be prevented with adequate sanitation, measures to protect food and water supplies, and increased public awareness of the means of transmission.

Keywords: *C. belli* life cycle, Coccidia, *Cystoisospora belli*, Intracellular parasite, Parasitic infection, Protozoa.

INTRODUCTION

Cystoisospora belli (previously named *Isospora belli*) is a coccidian protozoan parasite that inhabits the gastrointestinal tract and causes cystoisosporiasis, a

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human intestinal disease. It belonged to the genus Isospora until 2005; since then, it has been included in the genus Cystoisospora [1] (Table **1**). Morphological and molecular phylogenetic analysis [2] were fundamental to demonstrate the difference and divergence between the genus *Isospora* belonging to the Eimeriidae family and the genus *Cystoisospora* belonging to the Sarcocystidae. Both families belong to the suborder Eimeriorina and are included in the Coccidia class within the Apicomplexa phylum. *Isospora* are characterized by coccidia with tetrasporozoic, diplosporocystic oocyst with sporocysts containing stiedae bodies (an ultrastructural organelle located at the polar region of the sporocyst with a knoblike structure occluding a hole in the sporocyst), while *Cystoisospora* are coccidian with a similar structural organization but lacking stiedae bodies in their sporocysts.

Table 1. *Cystoisospora belli* Taxonomy Classification.

***Cystoisospora belli* Taxonomy Classification from the NCBI Taxonomy Browser (2015) (http://www.ncbi.nlm.nih.gov/Taxonomy)**	
Domain	Eukaryota
Kingdom	Chromalveolata
Superphylum	Alveolata
Phylum	Apicomplexa
Class	Conoidasida
Subclass	Coccidiasina
Order	Eucoccidiorida
Suborder	Eimeriorina
Family	Sarcocystidae
Genus	*Cystoisospora*
Species	*Cystoisospora belli* *Cystoisospora felis* *Cystoisospora ohioensis* *Cystoisospora suis* *Cystoisospora timoni* *Cystoisospora ef. ohioensis* *Cystoisospora sp. 1-MM* *Cystoisospora sp. 2-MM* *Cystoisospora sp. Ex-vulpes lagor*

Studies using phylogenetic analysis of the rRNA 18S gene and of the rRNA internal transcribed spacer 1 (ITS1) have shown that *Cystoisospora* is more closely related to *Toxoplasma*, *Neospora* and *Sarcocystis* spp. than *Eimeria* [3 - 5].

Humans are the only known hosts for *C. belli*. Several mammalian species are infected by other *Cystoisopora* species, such as *C. felis* and *C. rivolta* in cats, *C. ohioensis* in dogs, *C. suis* in pigs and *C. timoni* in meerkats.

History

In 1860 Virchow described a species of Isospora isolated from human samples. In 1915 Dr. H.M. Woodcock reported again this species when he observed coccidian oocysts in the stools of European soldiers stationed in the Middle East, therefore, the parasite was named belli (from latin: bellum = war). Also during World War II it frequently diagnosed as a coccidial pathogen among troops serving in the Pacific [6]. In the Americas, isosporiasis was first reported only in the 1960s [7]. However, relatively few cases of disease were registered until the parasite was recognized as an opportunistic infection in immunocompromised patients [8]. Actually, both names (*isospora* or *cystoisospora*) are used in the medical field, often intended as synonymous by clinicians, but for the rest of the chapter we prefer to refer to the most appropriate taxonomic name as earlier defined, that is *Cystoisospora belli*. The parasite is one of the most commonly identified causes of severe and prolonged diarrhoea in immunodeficient individuals, particularly in AIDS patients. The effects of cystoiosporiasis may lead to increases in morbidity and mortality in these patients [9]. This parasite is principally known to cause the traveler's diarrhea in immunocompetent hosts and is an opportunistic pathogen in immunocompromised hosts.

Morphology and Life Cycle

Cystoisospora oocysts measure approximately between 25 μm to 30 μm in length and between 10 μm to 20 μm wide. The sporocysts measuring 12 μm x 7 to 9 μm. The *Cystoisospora* oocysts are relatively delicate and colourless, elongated and ellipsoid. The smooth cyst wall is composed of 2 layers. The outer layer is robust, impermeable to fluids, and environmentally resistant; the inner layer is membranous and the protoplasm is finely grained. Oocysts eliminated in the environment are not yet sporulated. After maturation in the external environment, the oocysts contains two sporocysts, which contain 4 sporozoites each (Fig. **1**). Sporozoites are slender and crescent-shaped. The inner structure of sporozoites and merozoites is similar to that of other coccidians and includes polar rings, rhoptries, micronemes, conoids, microtubules, and amylopectin granules. Macrogamonts contain a single large, centrally located nucleus and wall-forming bodies. Microgamonts contain multiple nuclei that migrate to the periphery, elongate and protrude from the surface, and bud into mature flagellated microgametes 5 μm to 6 μm long [10, 11]. The intracellular development of *C.*

CHAPTER 5

Entamoeba histolytica and other Pathogenic Intestinal Amoebea

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Abstract: *Entamoeba histolytica* is a parasitic enteric protozoan and the etiologic agent of amebic colitis and liver abscess in humans. Amebiasis is commonly acquired through water- and food-borne transmissions and highly prevalent in tropical areas of the world where sanitation is poor. Symptoms can be mild, including loose stools and abdominal cramping but if the parasite invades the colon it can advance to amebic dysentery characterized by frequent watery and bloody stools. Following colonic invasion, trophozoites can enter the bloodstream and lymphatics and disseminate to the liver, heart, lungs, brain, or other organs, to cause abscesses, which if left untreated can lead to death. As treatment for amebiasis with metronidazole has adverse side effects research into novel therapeutics and vaccines is still actively being pursued. The present chapter summarizes the characteristics of amebiasis, their treatments and control and the importance of water transmission.

Keywords: Amebiasis, Colitis, Cysteine proteinases, *Entamoeba dispar*, *Entamoeba histolytica*, Liver abscess, Protozoa.

INTRODUCTION

Entamoeba histolytica is an extracellular anaerobic parasitic enteric protozoan and the etiologic agent of amebic colitis and liver abscesses through water- and food-borne transmissions. Infection with *E. histolytica*, named amebiasis, affects up to 10% of the world's population (approximately 500 million people worldwide) and is a major cause of symptomatic illnesses (50 million of persons/year) and death (100,000 persons/year). Although the incidence of amebiasis may be over-

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estimated by misdiagnosing pathogenic *E. histolytica* with non-pathogenic amoebas (*e.g.*, *E. dispar* and *E. moshkovskii*), amebiasis is considered one of the major causes of death due to parasitic infections in developing countries, after malaria and schistosomiasis [1].

History

Reports of people with bloody and mucous diarrhoea (probably amebiasis) were written 1000-600BC when Assyrian and Babylonian texts mentioned cases of dysentery in the Tigris-Euphrates basin [2]. By the 16th century, amebiasis was commonly described in developed Europe, mostly due to the growth of European colonies and increased world trade. At that time, there was a distinction of hepatic and intestinal forms of amebiasis. The first accurate description of both forms of the disease came from the book "*Researches into the Causes, Nature and Treatment of the More Prevalent Diseases of India and of Warm Climates Generally"* written by James Annersley in the 19th century [2]. With advances in microscopy, Friedrich Losch was the first to describe *E. histolytica* in Russia in 1873 from a young farmer who had been suffering from chronic dysentery [2]. In 1925, Emile Brumpt suggested that there must be two species: one that is invasive while the other is not (later named *E. dispar*) to explain why some people who were infected with *E. histolytica* (*i.e.* cysts in their stool) could never develop symptoms of the disease and spontaneously clear the infection [3]. However, it was not until the 1990's, due to biochemical, immunological and genetic tools that *E. histolytica* was reclassified as an invasive species named *E. histolytica* and the non-invasive species named *E. dispar* [3].

Morphology and Life Cycle

The life cycle of *E. histolytica* has 2 stages: the infectious cyst and the disease-inducing motile trophozoite stage. This life cycle begins when viable quadrinucleate cysts of *E. histolytica* are ingested from fecally contaminated food or water [4]. This amebic cyst has a protective wall formed of proteins and polysaccharides that make it highly resistant to most disinfectants and antiseptics commonly used in water treatments. Among those proteins, a proteomic study indicated the *E. histolytica* cysts contain specific glycoproteins Jacob, Jessie and chitinase and protein kinase, small GTPase signaling molecules, DNA repair proteins, epigenetic regulators and surface associated proteins [5]. Moreover, transcriptomic data showed that *E. histolytica* possesses at least 672 genes specific to cysteine proteases, putative DNA-binding and transcription factor-related proteins of the cyst wall [6]. The resistance of *E. histolytica* cysts is likely due to the cell walls are made of chitin fibrins, formed in small vesicles and

regulated by chitin synthase and chitinase [7]. Therefore, ingested cysts transit intact through the acid stomach until the terminal ileum of the small intestine. In the intestine, cysts excyst and mature into trophozoites that migrate and colonize the colon. *E. histolytica* trophozoites in the colonic lumen feed bacteria, cellular debris and mucin and begin the process of aggregation and eventually encystation forming cysts that are then excreted in stool [4].

The presence of other *Entamoeba* spp. may not be associated with amebiasis but represents an epidemiological problem in public health. The other species that belong to the genus *Entamoeba* isolated from humans, including *E. dispar*, *E. moshkovskii*, *E. poleki* (also called *E. chattoni*), *E. coli* and *E. hartmanii*. Among the non-pathogenic amoebas, *E. dispar* is usually isolated from most individuals infected with *Entamoeba* spp. (about 90%) and patients remain as asymptomatic carriers (*i.e.*, healthy individuals without diarrhea [8], and the parasite is considered non-pathogenic (Table **1**).

Table 1. Biological characterization between pathogenic *Entamoeba histolytica* and non-pathogenic *E. dispar*.

Characteristics	*Entamoeba Histolytica*	*Entamoeba Dispar*
Trophozoite (µM)	12-30	12-40
Cyst (µM)	10-30	10-30
Localization	Colon and systemic organs (liver, pericardium, brain)	Colon
Transmission	Fecal-oral *via* contaminated food and water	Fecal-oral *via* contaminated food and water
Life Cycle-Cyst Stage	Cysts pass through the stomach and small intestine and excyst to motile trophozoites in the large intestine	Cysts pass through the stomach and small intestine and excyst to motile trophozoites in the large intestine
Life Cycle- Trophozoite Stage	Trophozoites inhabit the large intestine and encyst to cysts that are excreted with the feces. Trophozoites penetrate the intestinal mucosa and disseminate systemically to the liver and brain	Trophozoites inhabit the large intestine and encyst to cysts that are excreted with the feces
Epidemiology	Worldwide (predominantly in developing areas), human-human transmission	Worldwide, human-human transmission
Clinical Features	Amebic diarrheic colitis and extra-intestinal signs, including amebic liver abscess	None
Clinical Specimen	Intestinal stool	Intestinal stool
Treatment	Metronidazole and luminal microbicidals	None

CHAPTER 6

Free-living Amoebae

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Abstract: *Acanthamoeba Species* is the most common free living amoeba present in environment. It is isolated from soil, water, contact lens solutions, transplant units and various other hospital environment. There are many species of Acanthamoeba such as *A.astronyxis, A.castellani, A.culbertsoni, A.hatchetti, A.keratitis etc.* which are known to cause opportunistic infection in both immunocompetent as well as immunocompromised host. Transmission mainly occurs through direct contact. There are two described morphological forms; a trophozoite form and a cyst form. The trophozoites have characteristics pointed thorn like acanthapodias, containing one nucleus with central dense large nucleolus. The cytoplasm measures between 15-50μm, granular and contains various organelles. Clinically patients usually presents with granulomatous amoebic encephalitis (GAE) which is characterized by focal neurological deficit, headache, visual disturbances, seizures and behavioral abnormalities which develops over months to years. Laboratory diagnosis of Acanthamoeba spp. is done by examining CSF which generally shows predominant lymphocytes, elevated proteins and low glucose levels. Histopathological samples of the brain generally reveals cerebral edema, multiple necrotic and hemorrhage lesions. Acanthamoeba can easily be cultivated on non-nutrient agar with overlay of *Escherichia coli* or *Entrobacter spp.* Amoeba feeds on bacteria's and confluent growth is seen in 4-5 days of culturing. The combination therapy is advisable in proven cases of Acanthamoeba infection. Combining Amphotericin B plus Trimethoprim-Sulphamethoxazole plus rifampicin has successfully used in few cases.

Naegleria Fowleri: The organism was first reported in Australia in 1965. It is an environmental ameboflagellate parasite found in variety of water bodies such as ponds, swimming pools; aquarium *etc.* prefers temperature of 30-45ºC. There are three stages seen in Naegleria life cycle: the infective trophozoites, transient flagellated and the resistant cystic stage. The portal of entity of trophozoites is *via* olfactory neuroepithelial cell lining covering the cribriform plate to reach olfactory bulb. Demyelination and myelinoclasis are observed in gray matter due to vascular blockage. These pathological changes are attributed to release of phospholipolytic enzymes which causes breaks in the lipid membrane of neuronal cells. Clinically, patients of primary amoebic meningoencephalitis (PAM) usually presented with high grade fever,

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headache, photophobia and features of raised intracranial pressure. Laboratory diagnosis is done using peripheral smear, CSF examination, culture, histopathological examination and imaging modalities. Hematological findings are leukocytosis with predominant neutrophils. The CSF shows low glucose and high protein levels. Centrifugation of fresh CSF sample up to 500 RPM may reveal motile trophozoites. Morphologically, the size of trophozoites ranges between 12-25µm, with a single nucleus and centrally placed nucleolus in the absence of peripheral chromatin. Liquid culture media such as such as Nelson's medium containing ox liver digest and glucose are used with serum for growing amoebae. Mammalian cell lines can be employed to demonstrate cytopathic effect. Multiplex PCR detects free living amoeba within 6 hours but routine use in diagnostic laboratory is limited due to rarity of finding these organisms and having high cost of PCR. Brain imaging is easy to perform but restricted by nonspecific findings such as cerebral edema. Specifically, infraction involving frontal, orbital and cerebellum area can be observed in few cases of PAM. There is no optimal treatment regime for *Naegleria fowleri*. Literature suggests combination therapy works best with amphotericin, rifampicin and azithromycin.

Balamuthia Mandrillaris: Over 200 cases were reported from South America and United States. The true prevalence of disease is unknown in south East Asia. Organism is commonly isolated from soil contact with activities related to soil such as gardening, agriculture pose risk of acquiring the organism. It was first isolated in 1986 from baboon brain that died of meningoencephalitis. The portal of entry of the organism is *via* cutaneous lesions, nasal mucosa and then subsequent spread to brain. CNS lesions mimic acanthamoeba encephalitis and have chronic slowly progressive course over many years. The life cycle of *Balamuthia* involves two stages: trophozoites and the cyst. The morphologically variable trophozoites are 12- 60 microns in size containing single nucleus with large centrally placed nucleolus. Cysts are spherical in shape measuring 12-30µm and contain a single nucleus with double wall having outer ectocyst, middle fibrillar layer and inner amorphous endocyst.The trophozoites, cysts and inflammatory cells are observed in perivascular regions of the infected tissue. In CSF, elevated protiens, reduced glucose are common findings. *Balamuthia* spp. can be grown in tissue cultures such as Monkey Kidney cell lines, Human Lung fibroblast and Human Brain Microvascular Endothelial cell lines. ELISA test is very specific to detect high antibodies titers. The antibodies do not cross react with other free living amoebae. PCR is also highly specific and sensitive test in which primers are developed against mitochondrial rRNA genes. Recently, real time PCR are developed targeting RNAase P gene of *B.mandrillaris*.

Sappinia Species: Two species of Sappinia are well-known cause of CNS infections in humans, *Sappinia diplodea* and *Sappinia pedata. S.diploidea* was first isolated from lizard faeces. As this parasite is found in animal faeces, persons handing livestock are at higher risk. Only one known case of Sappinia encephalitis infection reported in literature. The diagnosis was confirmed on histopathological sample, which showed necrotizing haemorrhagic inflammation of infected tissue, containing trophozoites. The trophozoite of Sappinia is characterized by two opposing nucleus with central flattening. Diagnosis can also be done by amplifying rDNA of both *Sappinia diploidea* and *Sappinia pedata* using SSU primers. The real time PCR can also be used based on 18rRNA gene sequences. Sappinia spp. is cultivated on non- nutrient agar with overlay

of *Enterobacter* or *Escherichia coli*. *Vahlkamphia spp.* and *Paravahlkamfia francinae* are other emerging free living amoebas that were first isolated from CSF of young patient who presented with typical symptoms of primary amoebic meningoencephalitis.

Keywords: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Negaleria fowleri*, *Paravahlkamfia francinae*, *Sappinia* species, *Vahlkamphia* spp.

Free-living amoebae (FLA) are aerobic, mitochondriate, eukaryotic protists and have the capability to exist as free- living organisms in nature and invading living host so they are also called as amphizoic amoebae [1]. The present classification schemes of protists, based largely on their genetic relatedness while the traditional classification scheme which is based on morphological characteristics, is no longer a valid scheme. According to the new classification scheme, the Eukaryotes have been classified into six clusters or 'Super Groups', namely Amoebozoa, Opisthokonta, Rhizaria, Archaeplastida, Chromalveolata and Excavata. The four amoebaehave been classified into two Super Groups, Amoebozoa and Excavata, *Acanthamoeba* and *Balamuthia* are under cluster Amoebozoa: Acanthamoebidae; while *Naegleria fowleri* comes under excavata: Heterolobosia: Vahlkampfiidae; and *Sappinia* under the cluster amoebozoa: Flabellinea: Thecamoebidae [2]. There is an increasing scientific interest in the field of free-living amoebae as determined by published articles over the last five decade. A pubmed search using "Acanthamoeba", "Balamuthia", Naegleria" or "Sappinia" was carried out from 1960 to 2010 which showed constant increase in number of cases from zero to nearly two hundred. Now, the question arises, why they are on the rise? There are many explanations given. Number of immunocompromised patients and contact lens wearers are on the rise [2]. Ability to act as a host or reservoir for other microbial pathogens [3]. These are model organism for motility studies which has led to a significant interest in this organism over the years. Ecologically, infections with free living amoebae have been linked to warm water such as above-ground pipelines, tropical lakes, geothermal water, heated swimming pools or discharges of industrial cooling water and soil [2]. Free living amoeba plays two major ecological roles: Influencing the structure of the microbial community and enhancing nutrient recycling. Both these activities are associated with free living amoebae (FLA). They feed on bacteria thus regulating bacterial populations in the soil. These FLAs cause 60% of the total reduction in bacterial population. Bacteria are primary decomposers of organic material but fail to release minerals from their mass, FLAs act as secondary decomposers thus releasing minerals that are tied up with bacteria. In this way, FLAs make the nutrients available that would

CHAPTER 7

Giardia and Giardiasis

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Abstract: *Giardia intestinalis* (syn. *G. lamblia* or *G. duodenalis*) is a unicellular protozoan parasite that infects the small intestines of humans and animals. The species *G. intestinalis* is composed of eight genotypes (called assemblages) designated from A to H. Only assemblages A and B infect humans, causing diarrhea and other associated symptoms. Giardiasis, the disease caused by the parasite, has a global distribution but is mainly endemic in developing countries where pronounced effects on children manifest in a failure to thrive condition. In adults, giardiasis might predispose for other gastrointestinal disorders such as Irritable Bowel Syndrome (IBS) after parasite clearance. The parasite exists in two forms: the cyst and the trophozoite. The cyst is the infectious form that is transmitted *via* the fecal-oral route. Infections can be asymptomatic, acute or chronic, which might be the result of interplay between parasite and host factors. The parasite is known to induce pathophysiological changes in the small intestines and trigger an immune response that result in parasite clearance. Host immune responses towards *G. intestinalis,* however, are not completely understood. Microscopy is the gold standard in clinical settings to diagnose giardiasis and treatment is usually with metronidazole or tinidazole but other drugs are also available. No human vaccines are available to date but some vaccine trials have shown promising results in animals. The spread of giardiasis is most common *via* water and is usually controlled through monitoring water treatment processes, by implementing a multi-barrier approach, and by monitoring the quality of water in recreational venues. Giardiasis adds to the global health burden and increases the costs of health care in many countries and therefore, a better knowledge of disease transmission and control is required to mitigate the risks of infections.

Keywords: Assemblages, Diarrhea, Diplomonad, Host-parasite interaction, Metronidazole, Protozoa, Van Leeuwenhoek, Variable surface proteins, Ventral disc, Virulence, Water-borne, Zoonosis.

INTRODUCTION

Giardia intestinalis, also known as *Giardia duodenalis* or *Giardia lamblia*, is a

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unicellular protozoan parasite that infects the upper intestinal tract of humans and animals [1]. The disease, giardiasis, manifests in humans as a bout of acute diarrhea that can develop to a chronic stage but the majority of infections remain asymptomatic [2, 3]. Giardiasis has a global distribution with 280 million cases reported annually, with its impact being more pronounced in the developing world, where it is usually associated with poor socioeconomic conditions [4]. Children, elderly people and immunocompromised individuals are the most affected by the disease [5 - 7]. In children specifically, effects on growth, nutrition and cognitive function have been reported [8 - 10]. Currently, it has been suggested that giardiasis could predispose for chronic gastrointestinal disorders such as irritable bowel syndrome (IBS) [11, 12]. In 2004, giardiasis was recognized by the World Health Organization (WHO) as a neglected disease associated with poverty, impairing development and socio-economic improvements [13]. Because giardiasis adds to the global microbial disease burden, an initiative was instigated to implement a comprehensive approach for control and prevention [13]. One area of focus is the potential spread of giardiasis *via* food and food handling [14, 15], daycare settings [16, 17], travel to endemic areas and close human contact [18 - 20]. Potential transmission of giardiasis from animals to humans (*i.e.* zoonosis) has been also the subject of extensive research over the years. Wildlife accessibility to water used for drinking and recreational purposes, as well as living in proximity with animals, have been identified as risk factors associated with zoonosis [21 - 23]. Another body of research addressed the effect of giardiasis on livestock. Not only transmission in livestock but also economic losses associated with poor growth, weight loss, reduced productivity and even death of animals [24 - 28]. Thus, giardiasis is regarded as a disease that has a significant impact on both humans and animals.

Currently, there are eight different genotypes, also called assemblages, of *G. intestinalis*. *Giardia* assemblages are designated from A to H, the majority of which are host-specific [7]. Parasites that belong to assemblages A and B infect humans but are also common in other mammals [29]. The *Giardia* life cycle alternates between an actively dividing trophozoite and an infectious cyst. Cysts are transmitted to humans *via* the fecal-oral route and upon ingestion they break open (*i.e.* excyst) in the duodenum, releasing trophozoites [29]. Trophozoites attach to the intestinal epithelial cells (IECs), to avoid elimination by peristalsis, and start multiplication. The presence of *Giardia* in the host intestines and in close contact with IECs induce functional and structural changes in the intestinal epithelia, which overall results in diarrhea [30]. Nevertheless, given the variability in giardiasis symptoms, infection *versus* symptoms or lack of symptoms is a controversial issue where the interplay between the infecting parasite of a certain

assemblage, isolate virulence and host factors could determine the outcome of infection.

Giardia does not cause overt inflammation in the host small intestines during most infections [31 - 33]. However, inflammatory responses have been seen during acute experimental and human infections [34, 35]. In line with this, IECs produce proinflammatory cytokines in response to the parasite or parasite-excreted products in an isolate-specific mode of induction [36]. At the same time, some parasite factors such as cysteine proteases (CPs) have been shown to attenuate the proinflammatory response by cytokine degradation [37]. Indeed, an accumulating body of evidence shows that cytokine production is rather inhibited during giardiasis [38 - 41]. Effector mechanisms play an early part in defense against *Giardia* and these include nitric oxide (NO) production, lactoferrins, α-defensins, phagocytes, mast cells and dendritic cells [31, 42]. The parasite, however, is able to avert some of these responses, specifically, NO production [43], dendritic cells recruitment [44] and granulocyte infiltration [45]. Patients are able to clear the infection within few weeks and re-infections manifest with reduced symptoms, indicating an acquired immunity against giardiasis [46 - 48]. Antibodies (*i.e.* B cell response) have been suggested to be important players in parasite clearance while the contribution of T cell responses is still unclear [31, 42].

Identification of cysts by microscopy is the gold standard in diagnosing human giardiasis. This is because trophozoites are only seen in stool during periods of acute diarrhea (*i.e.* watery or loose stool). Bright field microscopy coupled with morphometry, using phase contrast or differential interference contrast, is performed on wet mounts or following sample concentration [49]. Usually, multiple stool samples are submitted for analysis, especially when neither trophozoites nor cysts could be detected microscopically at initial examination. Fixation and staining can maintain trophozoite morphology and enhance visualizing trophozoite structure [49]. Antigen detection in stool (coproantigen) can be used for large-scale analyses and polymerase chain reaction (PCR) for increased sensitivity and specificity as well as genotyping [49]. For environmental samples, an initial filtration step is included to concentrate cysts in water samples. Immunomagnetic separation (IMS) followed by fluorescent microscopy is the gold standard for detecting cysts and is used for dual detection of *Giardia* cysts and *Cryptosporidium* oocysts in different water matrices [50]. The technique uses antibodies immobilized to magnetic beads to capture cysts in concentrated samples and fluorescence isothiocyanate (FITC)-linked antibodies to view cysts by fluorescent microscope [50]. The same method is adopted for food, specifically

CHAPTER 8

Microsporidia

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Abstract: Microsporidia the tiny unicellular eukaryotes are intracellular parasites of almost all animals. The diverged and specialized nature of these organisms, show some similarity to fungi. They cause opportunistic infections in animals and humans ranging between asymptomatic and severe life-threatening infections in immunocompromised individuals. Transmission occurs mainly by oral route, but other methods of transmission include inhalation, sexual contact, ocular mucosa, wounds, and insect bites. Food and water are relevant vehicles of infection. Animals act as reservoirs as they harbor most of the species that can also infect man and might contaminate water and environment with spores expelled in feces and/or urine. Clinical presentation is mainly intestinal with chronic diarrhea, mal-absorption, and loss of weight in immunocompromised persons, and self-limiting diarrhea in the immunocompetent individuals. Dissemination to other organs, may threaten the life of patients. Clinical picture of disseminated infection includes fever, cerebral manifestations or some other unexplained symptoms. Diagnosis of spores in feces, urine, CSF, sputum and in tissue is difficult and necessitates the use of special stains. Other methods of laboratory diagnosis include immunofluorescence, Electron Microscopy, and DNA detection. Treatment with Albendazole is effective for intestinal and other deep infections of various species of microsporidia except *E. bieneusi*, where fumagillin, can be considered. This drug is also used as topical treatment for eye infections by *E. hellem* and other species. Trials to produce vaccine against microsporidia are still under study. The increasing awareness will lead to a better understanding of the epidemiology, clinical relevance and control of microsporidiosis in humans and animals.

Keywords: AIDS, Dissemination, *Encephalitozoon*, *Enterocytozoon*, HIV, Microsporidia, Microsporidiosis, Parasitophorous vacuole, Protista, Polar tubule, *Septata*, Spores.

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INTRODUCTION

Microsporidia are unicellular, obligate intracellular spore formers of eukaryotic origin. They parasitize almost all animals. Understanding the basic biology of Microsporidia, have taken almost about 150 years of scientific research. The identification of DNA of organisms, created a new era of molecular phylogeny. Microsporidia, which were considered as protozoa (Kingdom Protista), are now considered as highly specialized fungi [1, 2]. There are more than 170 genera and approximately 1300 species of microsporidian organisms that parasitize a wide variety of vertebrates and invertebrates with at least 14 species and 8 genera known to infect humans [3 - 5]. The genera of microsporidia that cause human diseases are: *Nosema* [6, 7], *Brachiola* [8, 9], *Vittaforma* [10], *Pleistophora* [11, 12], *Trachipleistophora* [13], *Enterocytozoon* [14, 15], *Encephalitozoon* [16 - 18], *Septata* [19], and *Anncaliia* [20]. Microsporidia have been known to cause seriously damaging diseases in honeybees and silk worms, that consequently led to a serious economic loss [21]. Infection also was detected in different animals as rabbits, laboratory rodents and furred animals [22]. However microsporidia were considered as opportunistic pathogens in humans after the emergence of AIDS pandemic [23], and also have been detected in immunocompetent persons [24]. Infected cases may be asymptomatic or they may suffer severe life threatening disease according to the tissue or organs affected, as microsporidia may infect almost any part of the body. The most common site of infection is the intestine, which may account for up to 50% of all infections, with chronic diarrhea and wasting as the predominant manifestations [23 - 26].

HISTORY

There was an important economic problem in the year 1850, due a decline in the European silk industry as a result of a disease that affected the silk worms. This disease was called the pepper-disease (pébrine). Investigations were carried out in scientific centers in order to identify the microbial causative agents of the disease. There was some association between the disease and characteristic globular organisms, which were described, later by the Swiss microbiologist Karl Wilhelm von Nägeli in 1857 as the first microsporidium and he gave them the name *Nosema bombycis* [27]. Nägeli described *N. bombycis*, as a yeast-like fungus and included it in the Schizomycetes, which fits into the tree of eukaryotes according to the recent classification [27]. In 1870, Louis Pasteur incriminated microsporidia as a cause of infection of silkworm and cause of decimation of the silk industry. Aided by his colleagues, they could identify the nature of this parasite [28], with subsequent improvement of the European silk industry [29].

Further studies by Edouard-Gérard Balbiani by the year 1882, has created a new group for *Nosema* organisms, gave them the name 'microsporidies' and included them in the group of sporozoa within the Kingdom Protozoa [30]. Sporozoa is an old group of pathogens united together in an assemblage based on their similarity as spore formers. Recently, studies showed that they have distant relations and were subgrouped as members of Apicomplexes, haplosporidians and the Cnidosporidia. The last subgroup included Myxsosporidia (affecting animals), *Actinomyxidae* (of unknown origin), *Helicosporidia* (green algae) and the Microsporidia [31].

In the year 1976 Sprague created the Phylum *Microspora*, which was later included in the subkingdom Protozoa, a subdivision of the Kingdom Protista created in 1980 by Levine [30 - 33]. Shortly after, Sprague and Bencil changed the name of the phylum to Microsporidia, Balbiani 1882 [34]. This was in honor of Balbiani, who has created the order Microsporidia in 1882 [30].

Phylogeny and Taxonomy Considerations

Species of microsporidia have been classified according to studies based on their habitat, morphological and ultrastructural details. However the most important was the recent molecular phylogenetic classification [35, 36]. The most spectacular features of the spores of microsporidia have been explored by electron microscopy [37, 38],

Electron microscopy studies showed that the microsporidial spores lack some important structures of the Eukaryotic cells as mitochondria, peroxomes, Golgi apparatus, flagella and microtubules [39].

Intimate resemblance to fungi was proved by ultrastructural morphological studies based on the spore size, number of coils of the polar tube inside the spore as well as the life cycle and the host parasite relationship [40 - 43]. On the other hand, the resemblance to prokaryotes was revealed by biochemical analysis, after detecting that microsporidia include 70S ribosomes as in case of prokaryotes [44, 45]. Study of the rDNA sequences for the phylogenetic classification of microsporidia, suggested that they were among the deep-branching early eukaryotes [46]. This is based on lacking mitochondria, Golgi bodies and peroxisomes and having the small ribosomes of the prokaryotes as mentioned before [46].

Phylogenetic study of the sequence of the small subunit rRNA gene of *Variamphora necatrix*, one of the species of microsporidia, showed that there is closer resemblance to prokaryotes than to eukaryotes, suggesting that they have

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