Infections by Intestinal Coccidia and *Giardia duodenalis*

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KEYWORDS

• *Cryptosporidium* • *Cyclospora cayetanensis* • *Giardia duodenalis*
• *Cystoisospora belli* • Human enteric coccidian

KEY POINTS

- Sample collection and preservation are important steps, and examination of 3 specimens from different days increases the accuracy of diagnosis.
- *Giardia* can be detected by light microscopy during ova-and-parasite (O&P) examination, antigen detection methods (laboratory and rapid diagnostic devices), or fluorescent microscopy.
- *Cryptosporidium* and *Cyclospora* are not easily detectable by O&P examination, and parasite-specific tests must be requested, such as modified acid-fast (MAF) microscopy. For *Cryptosporidium*, there are antigen detection assays (laboratory or rapid diagnostic devices) or antibody-based fluorescent microscopy. Properly equipped fluorescent microscopes can be used in research laboratories for confirmation of *Cyclospora*, as this parasite autofluoresces with the appropriate excitation wavelength.
- *Cystoisospora* can be detected by light microscopy, and confirmation is accomplished through morphometric characteristics of samples that have been stained with safranin or acid-fast stain and also by autofluorescence.

INTRODUCTION

The protozoa, as typically delineated in public health, are a nonmonophyletic conglomerate of unicellular eukaryotic organisms that are characterized by having animallike affinities. Most protozoa that infect the human enteric tract are characterized by having an environmentally stable stage such as a cyst or oocyst. Cysts and

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oocysts confer protection from environmental factors, allowing these parasites to infect other susceptible hosts through either the water or food-borne routes.

There are several parasitic protozoa that can cause enteric infections in humans, the focus here being *Cryptosporidium* spp, *Cyclospora cayetanensis, Giardia duodenalis* (syn. *Giardia lamblia, Giardia intestinalis*), and *Cystoisospora belli* (previously *Isospora belli*). Infections are usually characterized by gastrointestinal clinical manifestations that may include diarrhea, vomiting, abdominal cramps, and general malaise.[1,2] Three of these protozoa, *Cryptosporidium, Cyclospora*, and *Cystoisospora*, were previously classified as coccidian parasites because of their intracellular location (these parasite infect enterocytes) and a complex life cycle that includes asexual (meronts) and sexual (microgametocytes and macrogametocytes) reproductive stages.[3] *Giardia* is a flagellate, does not invade epithelial cells, and reproduces only asexually by binary fission.

The classification of eukaryotic parasites is in frequent revision because of modern systematics that incorporate bioinformatic data and cladistics classification into the traditional morphometric-based taxonomy. As of 2012, the formerly known coccidians are classified in the subgroup Apicomplexa, with *Cyclospora* and *Cystoisospora* being classified as Eimeriorinas (the sporozoites, which are the infective stages, are always enclosed in sporocysts within an oocyst) and *Cryptosporidium* grouped alone as a single clade (oocysts without sporocysts, containing 4 naked sporozoites).[4]

These parasites produce resistant stages (cysts in *Giardia*,[5] oocysts in the coccidia), which are released into the environment. The excretion intensity of these parasites can vary significantly, from very high to low, and can be sporadic.[6] Therefore, the diagnostic success of a single stool sample can be suboptimal.[7] It is currently recommended to test 3 samples,[8] ideally collected every other day, over a period of at least 1 week.[9]

Samples have to be properly preserved to assure success of the assays to be conducted. The most widely used method relies on a 2-vial collection system with sodium acetate-acetic acid-formalin (SAF); 10% buffered formalin; polyvinyl alcohol (PVA) containing fixatives such as mercury, zinc, or copper (Zn PVA, Cu PVA); or Schaudinn fluid. However, there is a trend to minimize the use of formalin (because of toxicity) and mercury (environmental impact)[10]; however, those alternatives may not always have high parasite recovery rates and not are always compatible with immunoassays.[7]

See Appendix 1 for laboratory procedures for the microscopic detection of coccidian parasites (*Cryptosporidium* spp, *C cayetanensis*, and *C belli*).

CRYPTOSPORIDIUM SPP

The genus *Cryptosporidium* was first described in 1910 by Tyzzer,[11] who in 1912 also described *Cryptosporidium parvum* in the small intestine of mice.[12] For several decades, human cryptosporidiosis was considered a benign self-resolving infection that was caused by *C parvum*.[13] This parasite was considered to have the potential to infect a broad range of mammalian species. With the advent of the human immunodeficiency virus (HIV)/AIDS epidemic, cryptosporidiosis became an important infection, where immunocompromised patients developed nonretractable life-threatening diarrhea.[14,15] Cryptosporidiosis was classified as opportunistic infection and also as an AIDS-defining illness.[16] Given its public health importance, numerous studies were conducted to better understand its pathogenesis, transmission routes, therapeutic approaches, and disease-prevention strategies. The renewed interest led to important discoveries that highlighted the importance of the immune system, more specifically CD4 cells, in the clearance of infections.[17,18] It was also confirmed that