

## Challenge 0907-2

July 2009

### *Isospora belli*

The objective of this challenge was to find, identify and report *Isospora belli*.

#### CMPT QA

This sample was verified by two reference laboratories to contain many *Isospora belli* oocysts. Both laboratories also reported *Iodamoeba buschlii* cysts and trophozoites, *Trichuris trichiura* ova and Charcot-Leyden crystals. One laboratory also observed *Blastocystis hominis*.

#### SURVEY RESULTS

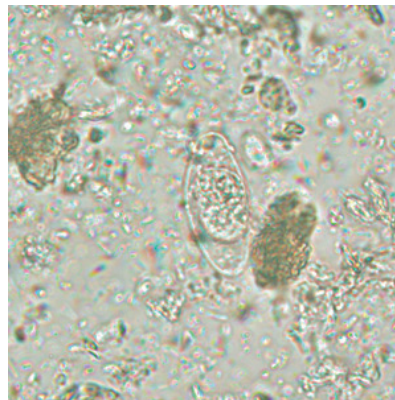
Table 1 shows the results reported by the participant laboratories. Ninety-six percent of the laboratories reported the presence of *Isospora belli* in the sample. *I. butschlii* was observed by both reference laboratories and was reported by 100% of the participants. Other parasites reported included: *B. hominis* (35%), *T. trichiura* (23%), *Enterobius vermicularis*, *Cyclospora cayetanensis* (4% each).

*Isospora belli* was sent twice before as a PT challenge. Laboratories did well on the first challenge (92% received acceptable grade) while the second challenge was ungraded due to lack of consensus between the reference laboratories (Table 2).

#### METHODS

Wet preparation examination of fresh material either as the direct smear or as concentrated material is recommended rather than the permanent stained smear<sup>1</sup>. Under bright-field microscopy oocysts of *I. belli* are oval, elliptical

and very large, measuring 20-30µm long by 10-19µm wide. As the oocysts are very pale, transparent, and have a thin wall (Figure 1) they can be overlooked. They can also be very difficult to see if the concentration sediment is from polyvinyl alcohol-preserved stool.



**Figure 1.** Immature oocyst of *C. belli* in an unstained wet mount, containing a single sporoblast.  
CDC Public Health Image Library.

Mature oocysts contain 2 sporocysts with 4 sporozoites each; however, diarrheic stools usually contain immature unsporulated oocysts that contain a single spherical mass<sup>2,3</sup>

#### MODIFIED ACID-FAST STAIN

A modified acid-fast stain can be useful in identifying the oocysts.

A blue-green background, or contrasting counterstain, of fecal debris allows the oocysts to

#### Grading

Most laboratories reporting *Isospora belli* received an acceptable grade with the exception of laboratories also reporting *E. vermicularis* and *C. cayetanensis*. These parasites were not reported by any other laboratory and the report could lead to mistreatment and thus were graded unacceptable. These laboratories were given the option of sending the slides back for examination. Those laboratories not reporting *I. belli* were graded unacceptable as well.

Historic results: *Isospora belli*  
\*challenge ungraded

Table 2	
Challenge	Results
0907-2	96%
0707-2	52%*
0504-1	92%

#### *Enterobius vermicularis*

Cellulose tape (scotch tape) preparation is recommended as the diagnostic test of choice (a minimum of 4 to 6 consecutive negative tapes is required to rule out infection). Commercial paddles or other collection devices are also acceptable.<sup>1</sup>

Adult worms may be found on or under the surface of the stool specimen, particularly in children. The adult worms can also be found on the tapes.

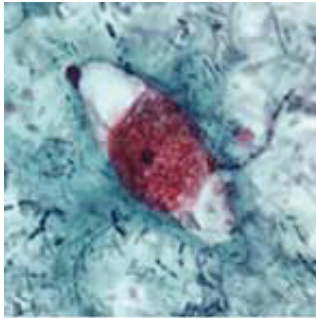
Eggs are occasionally recovered in stool, but this is an incidental finding and not the specimens of choice.<sup>1</sup>

**Table 1:** Combined results received – *Isospora belli* challenge

Reported	No of labs	%	Grade
<i>Isospora belli</i>	25	96	
<i>T. trichiura</i>	6	24	Acceptable
<i>E. vermicularis</i>	1	4	Unacceptable
<i>C. cayetanensis</i>	1	4	Unacceptable
<i>I. butschlii</i>	25	100	Acceptable
<i>B. hominis</i>	8	32	Acceptable
<i>I. butschlii</i> + <i>B. hominis</i>	1	4	Unacceptable

stand out. The cyst wall and sporoblast stain red (Figure 2); however, they are variably stained: some will stain light pink to deep purple, while others may be unstained. Some oocysts may appear collapsed or distorted on one side. This staining method provides a permanent record <sup>1,2</sup>.

Precautions 1: *I. belli* can be distinguished from other modified acid fast parasites, *Cryptosporidium* species (round and 4-6µm) and *C. cayetanensis* (round and 8-10µm), by its size and elliptical shape.



**Figure 2.** Immature oocyst of *C. belli* stained with safranin, showing a single sporoblast.. CDC Public Health Images

Charcot-Leyden crystals derived from eosinophils have also been found in the stools of patients with *I. belli* infections.

Although **PCR is not used as a routine diagnostic tool** in clinical settings the high-throughput (fast, multiple-sample analyzing) potential can be used, together with other collected health and demographic indicators, as a powerful tool for epidemiologic studies or to monitor effectiveness of treatment in *I. belli* infections. A couple of studies have shown great sensitivity and specificity of PCR for the detection of *I. belli* in stool samples <sup>4,5</sup>.

## CLINICAL RELEVANCE

Human isosporiasis seems to be cosmopolitan in distribution, especially in tropical and subtropical regions such as Haiti, Mexico, Brazil, El Salvador, Venezuela, and Southeast Asia. However, the prevalence of this infection is occasionally underestimated because oocysts are usually excreted in small numbers or may not be found in spite of actual infection <sup>3</sup>.

These organisms can infect both adults and children. Intestinal involvement and symptoms are generally transient unless the patient is

immunocompromised.

Transmission is through ingestion of water or food contaminated with mature, sporulated oocysts. The oocysts are very resistant to environmental conditions and may remain viable for months if kept cool and moist; oocysts usually mature within 48 hours following stool evacuation and are then infectious <sup>1</sup>.

Clinical symptoms include diarrhea, which may last for long periods (months to years), weight loss, abdominal colic, and fever; diarrhea is the main symptom. Eosinophilia is found in many patients.

Patients who are immunosuppressed, particularly those with AIDS, often present with profuse diarrhea associated with weakness, anorexia, and weight loss.

## TREATMENT

Currently the drug of choice for treatment for chronic isosporiasis is trimethoprim-sulfamethoxazole (SXT). In patients who are sulfa allergic, pyrimethamine or ciprofloxacin can be used, although ciprofloxacin is less effective than SXT. Up to 50% of immunosuppressed patients relapse following primary therapy <sup>1,2</sup>.

## REFERENCES

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4. ten Hove RJ, van Lieshout L, Brienen EA, Perez MA, Verweij JJ. Real-time polymerase chain reaction for detection of isospora belli in stool samples. *Diagn Microbiol Infect Dis*. 2008;61:280-283.
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6. CDC, DPDx (Division Parasitic Diseases). *Cytoisosporiasis*. Available at: <http://www.dpd.cdc.gov/dpdx/HTML/Cytoisosporiasis.htm>. Accessed August, 2009.

## *Isospora belli* Life Cycle

The entire life cycle of *Isospora* consists of asexual development and sexual reproduction that take place in the same host

At the time of excretion, the immature oocyst contains usually one sporoblast (more rarely two). In further maturation after excretion, the sporoblast divides in two (the oocyst now contains two sporoblasts); the sporoblasts secrete a cyst wall, thus becoming sporocysts; and the sporocysts divide twice to produce four sporozoites each. Infection occurs by ingestion of sporocysts-containing oocysts: the sporocysts excyst in the small intestine and release their sporozoites, which invade the epithelial cells and initiate schizogony. Upon rupture of the schizonts, the merozoites are released, invade new epithelial cells, and continue the cycle of asexual multiplication. Trophozoites develop into schizonts which contain multiple merozoites. After a minimum of one week, the sexual stage begins with the development of male and female gametocytes. Fertilization results in the development of oocysts that are excreted in the stool <sup>6</sup>.

The Committee recommends that all Proficiency Testing samples should be processed as routine samples even when there is a staff shortage or high workload.