

Challenge 1604-3

April 2016

Stool: *Cyclospora cayetanensis***CMPT QA/QC/ Statistics**

This sample was verified by two reference laboratories. Laboratories were expected to report the presence of *Cyclospora cayetanensis*.

All challenge components are confirmed before shipping by the reference laboratories. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

SURVEY RESULTS

Reference laboratories: Both laboratories reported the presence of *Cyclospora cayetanensis*.

Participants (Table 1): 17/20 (85%) laboratories correctly reported the presence of *Cyclospora* species / *C. cayetanensis* and received an acceptable grade. Two participants did not report the organism, this was considered unacceptable.

Two laboratories reported finding *Entamoeba coli* and one of them also *Blastocystis hominis* but not *C. cayetanensis*. The unacceptable grade was not for the reference to *Entamoeba coli* and *Blastocystis hominis* but rather for not reporting a *C. cayetanensis*.

Suitability for Grading

A challenge component is considered suitable for grading if agreement is reached by both (100%) reference laboratories and at least 70 percent of the participants.

Parasite identification was correctly performed by both reference laboratories and greater than 70 percent of all laboratories and was thus, determined to be suitable for grading.

COMMENTS ON RESULTS

This is the first time a *Cyclospora* challenge has reached consensus among participants and therefore been suitable for grading.

One participant reported *Cyclospora* species; although this report is not technically wrong, there is only one species that affects humans and thus, the laboratory should have identified the organism as *Cyclospora cayetanensis*.

Grading

Reporting *Cyclospora cayetanensis* was considered acceptable.

Not reporting *C. cayetanensis* was graded unacceptable.

IDENTIFICATION

Standard laboratory procedures, using a sequential iron haematoxylin and acid-fast stain or modified iron-haematoxylin stain for ova and parasites should be able to identify *C. cayetanensis* in stool samples.

Fecal specimens should be concentrated prior to staining because the chances of detecting *Cyclospora* oocysts in specimens that have not undergone a concentration procedure are reduced by up to 40%.¹

Stool examination for oocysts is the standard procedure for diagnosing *C. cayetanensis*. Other specimens that may contain oocysts include intestinal aspirates, duodenal or jejunal biopsy samples.¹⁻⁴

Table 1. Results reported

Reported	Total	Grade
<i>Cyclospora cayetanensis</i>	17	Acceptable
+ <i>Entamoeba coli</i>	7	
+ <i>Blastocystis hominis</i>	2	
<i>Cyclospora</i> species	1	Acceptable
<i>Entamoeba coli</i> ± <i>Blastocystis hominis</i>	2	Unacceptable
Total	20	

When viewing coccidia microscopically, calibration of the microscope's ocular micrometer is important to measure the oocysts in order to differentiate *C. cayetanensis* from *Cryptosporidium* species. *Cyclospora* oocysts measure 8 to 10 µm and *Cryptosporidium* oocysts measure 4 to 6 µm.^{2,4}

Although *C. cayetanensis* is an acid-fast organism, the oocysts stain variably with acid-fast stains (no staining, to mixed or variable staining, to complete staining - See Figure 1), which can complicate identification. A strong decolorizer should not be used. One per cent sulfuric acid solution is recommended and also works well for modified acid-fast stains for *Cryptosporidium* species and/or *Isospora belli*. Even with the 1% sulfuric acid solution, some oocysts may appear unstained or very pale.⁴

Another method of identification on wet mounts is to use fluorescent microscopy. Wet preparations of *C. cayetanensis*, independent of specimen age or type, exhibit an intense color when viewed with a fluorescence microscope.

With a UV excitation filter set at 330-365 nanometers, the color is an intense blue. With a filter set at 450-490 nanometers, the color is a less intense green.^{1-3,5}

CLINICAL RELEVANCE

C. cayetanensis infection occurs only in humans. No animal reservoir for *C. cayetanensis* is known or suspected.²

C. cayetanensis transmission is considered an emerging disease of public health concern primarily in the developed countries where it has

been identified as the cause of several outbreaks in North America⁹ and Europe. In these areas, *C. cayetanensis* transmission has primarily been linked to foods imported from developing countries and has been demonstrated in source waters in several countries.⁵ *C. cayetanensis* has also been associated with traveler's diarrhea.

Individuals infected with *C. cayetanensis* generally describe a "flu-like" illness that involves nausea, vomiting, anorexia, fatigue, weight loss, and explosive watery diarrhea.⁶

The diarrhea usually occurs at least six times daily. Fever occurs only in about 25% of patients.⁷ The illness usually lasts from two to seven weeks, but can be prolonged in immunosuppressed hosts. *C. cayetanensis* infection is common in HIV patients and the illness tends to be more severe and of a longer duration.⁸

LIFE CYCLE

The life cycle begins with the ingestion of the sporulated oocyst in contaminated water or food. Upon ingestion, the oocysts excyst in the gut, releasing the sporozoites, which proceed to invade the epithelial cells of the small intestine.

The next process is schizogony, which begins with the formation of a trophozoite that grows into a mature schizont that contains 8-12 merozoites, which are then released, presumably by cell rupture, to invade other epithelial cells and repeat the process. These merozoites are called type I meronts, which are asexual forms.

After several cycles of type I schizogony, type II meronts (sexual forms) develop, with each cell

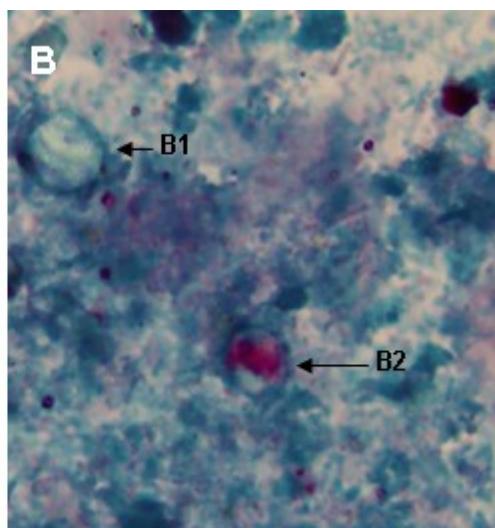
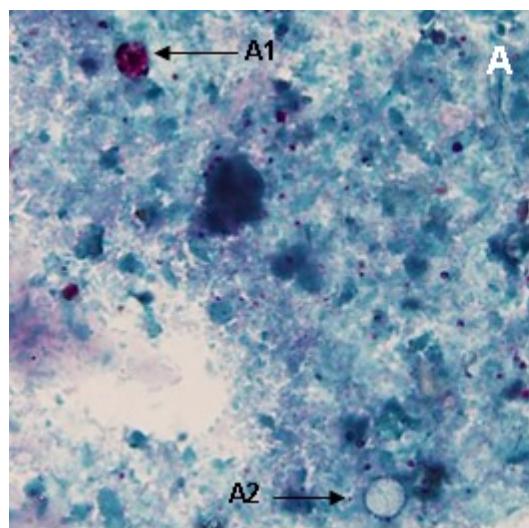


Figure 1. Stool stained with modified acid-fast stain. A: two *Cyclospora cayetanensis* oocysts in the field of view, one properly stained (A1) the other unstained (A2); B: two oocysts one partially stained (B1) and the other unstained (B2). Images courtesy of Quantine Wong, BCCDC PHL.

containing 4 merozoites. After invading epithelial cells, some of these form single macrogametes and others divide multiple times to form microgametes.

When released, a microgamete fertilizes a macrogamete, which develops into a zygote. The zygote, in turn, develops into an oocyst with an environmentally resistant wall. The oocyst passes into the environment in the feces, as a non-sporulated noninfectious oocyst.^{2,5}

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