Challenge PA2207-3

July 2022

Stool: *Giardia lamblia, Entamoeba histolytica/dispar, Blastocystis* species, *Endolimax nana, Entamoeba hartmanni*

CMPT QA/QC/ Statistics

This sample was verified by two reference laboratories. Laboratories were expected to report the presence of *Giardia lamblia, Entamoeba histolytica/dispar, Blastocystis species, Endolimax nana, Entamoeba hartmanni*

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 reference laboratories reported *Giardia lamblia, Entamoeba histolytica/dispar, Blastocystis* species, *Endolimax nana, Entamoeba hartmanni*, CLC. One of the laboratories also reported *Entamoeba coli* and *Retortamonas intestinalis*

Participants: 13/14 (93%) participants reported *Giardia lamblia* and *Entamoeba histolytica/dispar*; One participant reported *Giardia lamblia* but did not report E. histolytica/dispar. In addition to *G. lamblia* and *E. histolytica/dispar*, laboratories reported *Blastocystis* species, *Endolimax nana*, *Entamoeba coli*, *Entamoeba harmanni*; and *Retortamonas intestinalis* (Table1).

Three participants performed PCR testing: the three reported it negative for *Cryptosporidium* and *Entamoeba hist*olytica, and positive for *Giardia lamblia*. The three labs used BD max which accepts SAF and thus, the organism reported as E. histolytica/dispar is probably *E. dispar.*

This critique will focus on *Entamoeba histolytica/dispar*. *Giardia lamblia* has been recently reviewed in PA2110-3

IDENTIFICATION

Microscopic examination

The identification of *E. histolytica/dispar* trophozoites and cysts in stools requires concentration procedures and permanent stained smears.

E. histolytica and *E. dispar* are two separate and distinct species however they are morphologically indistinguishable using routine diagnostic methods unless erythrophagocytosis is observed. [1-3]

Erythrophagocytosis (ingestion of red blood cells by the parasite) is the only morphologic characteristic that can be used to differentiate pathogenic *E. histolytica* from the non-pathogenic *E. dispar*. It can be observed on wet mounts or stained smears but this is rare.

Grading

Participants reporting both pathogens - Giardia lamblia and Entamoeba histolytica/dispar were graded accelptable.

Not reporting a pathogen was graded unacceptable.

Table 1. Results reported

Reported	Total	Grade
Giardia lamblia, Entamoeba histolytica/dispar +/-:	13	Acceptable
Blastocystis species		
Endolimax nana		
Entamoeba coli		
Entamoeba hartmanni,		
Retortamonas intestinalis		
CLC +/- erythrocytes		
Giardia lamblia, *Blastocystis* species, *Endolimax nana, Entamoeba hartmanni, Entamoeba coli*	1	Unacceptable
Total	**14**	

Pathogenic *E. histolytica* trophozoites have active, progressive and directional motility when observed in unpreserved specimens.

E. histolytica/dispar trophozoites. [1,2]

• Usual size 15-20 µm (range 12 to 60 µm), invasive forms are usually >20 µm tending to be more elongated in diarrheal stool.

• Single nuclei, on a permanently stained smear, consist of uniformly arranged chroma-tin on the nuclear membrane and a small, compact, centrally located karyosome.

•The cytoplasm is characterized as finely granular ("ground glass") with few bacteria or no debris in vacuoles.

• *E. hartmanni* trophozoites (4-12 µm) and cysts (5-10 µm) are morphologically similar to *E. histolytica/E. dispar* trophozoites, but are smaller in size.

E. histolytica/E. dispar cysts. [1,2]

• Usual size 12-15 µm (range 10 to 20 µm)

• 1 to 4 spherical nuclei with central karyosome and evenly distributed peripheral chromatin (mature cysts can have up to 4 nuclei; immature cysts have 1 or 2 nuclei).

• Distinctive chromatoidal bars with smoothly rounded ends may be present.

• Glycogen mass may be evident (should not be confused with *Iodamoeba butschlii* cysts).

Cyst formation only occurs in the intestinal tract. The mode of transmission from one host to another is through the cyst form.

Cysts are highly resistant to desiccation and to certain chemicals such as chlorinated compounds and fluorides. Cysts can survive up to a month in water, up to 12 days on dry land and can tolerate temperatures up to 50°C.

Serological tests

Serum antibody detection is considered to be of crucial importance in detecting extra-intestinal infections such as amoebic liver abscesses. The sensitivity of serology is about 95% for amoebic liver abscess and 70-84% for invasive intestinal disease and 10% for asymptomatic patients passing cysts of *E. histolytica*. [4]

A positive antibody test confirms the suspicion of invasive amoebic disease provided the patient has not had a disease episode in the recent past as antibody titers can remain high for years after successful therapy. [4]

Serological tests are not useful for diagnosis in amebiasis-endemic countries, because of a high percentage of asymptomatic individuals already carrying antibodies specific to *E. histolytica*. [5]

Antigen detection methods

These methods use monoclonal antibodies directed against various proteins of *E. histolytica*.

The main advantage of these tests is that they can distinguish between pathogenic *E. histolytica* and non-pathogenic *E. dispar*. They are relatively quick to perform with a quick turnaround time, compared to the gold standard, Ova & Parasite examination. They may be useful after the microscopic diagnosis *E. histolytica/dispar* to determine if the patient has the pathogenic or non-pathogenic strain and whether treatment is warranted. However, these tests require an unpreserved sample that needs to reach the testing laboratory in less than 24 hours. If the transport is going to be delayed, the sample should be frozen.

Molecular methods

Nucleic acid testing (NAT) can be used to identify *E. histolytica* by amplifying *E. histolytica* genes from extracted fecal DNA. Sensitivity and specificity are high (80–100% and 100%, respectively).[6,7]

The advantage of molecular detection is that it is extremely sensitive and reliably able to differentiate non-pathogenic *Entamoeba* species from *E. histolytica*.

Drawbacks of this method are the difficulty of maintaining quality control for quality assurance purposes, [5] and if performed alone, the presence of other parasites will not be identified.

CLINICAL RELEVANCE

Infection in humans occurs after the ingestion of mature cysts via water or food (raw vegetables) contaminated with feces, sexual contact, or by contaminated hands of food handlers. After excystation in the small intestine, trophozoites inhabit the large intestine and can either invade the tissue (if pathogenic *E. histolytica* present) or are eliminated in the stool. Humans, chronically ill or asymptomatic, can excrete 15 million cysts per day.

The incubation period varies from a few days to several months, but is usually 2 - 4 weeks. [1,2] Trophozoites do not survive out-side the body.

E. histolytica has the unique ability among the intestinal amoebae parasitizing humans of being able to invade tissue.

Clinical syndromes associated with amebiasis vary with the host and the organism and may range from an asymptomatic infection to a disseminated fatal disease. When the infection disseminates to extra-intestinal sites, it is found most frequently in the right lobe of the liver

Non-invasive infection may present nonspecific gastrointestinal symptoms, including abdominal pain and increased frequency of bowel movements.

Symptoms associated with invasive intestinal amebiasis usually start with abdominal pain, diarrhea, dysentery, or weight loss, depending on the severity of the disease. Almost all patients have occult blood in the stools, and nearly one-third exhibit fever.

Dissemination of amoebic infection to extra-intestinal sites most frequently involves the liver, lungs, pericardium, brain, and skin.[8]

Amoebic liver abscess is observed more frequently in men than in women (with male-to-female ratios of up to 10:1). Although the reason for this is unknown, speculations regarding the protective role of estrogen versus invasiveness have been postulated.

Four of the *Entamoeba* species have been described that infect humans are morphologically indistinguishable: *E. histolytica, E. dispar, E. moshkovskii,* and *E. bangladeshi.*

E. dispar, a non-pathogenic commensal species, is perhaps 10 times more common than *E. histolytica* worldwide. Evidence is now building that *E. moshkovskii*, could also be associated with human disease. [9]

In 2010 to 2011, a new species: *E. bangladeshi* was identified by NAT.[10] Under light microscopy, it has a similar appearance to *E. histolytica.* The epidemiology and potential pathogenicity of this novel species requires more investigation. [5]

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