



CMPT Enteric Parasitology Program

Innovation, Education, Quality Assessment, Continual Improvement

Challenge 0904-2

April, 2009

Entamoeba histolytica / dispar, Blastocystis hominis

CMPT QA

This sample was verified by two reference laboratories. Both laboratories reported *Entamoeba histolytica/dispar* cysts and trophozoites. *Blastocystis hominis* was also reported by both laboratories and in addition one of them reported *Entamoeba coli*.

Although *Giardia lamblia* was not observed in the QA samples, one of the reference laboratories reported it when processing the survey.

SURVEY RESULTS

E.histolytica was reported by 23/26 laboratories (85%), most reporting *E.histolytica/dispar* while two laboratories reported *E.histolytica* only.

Two out of 26 (8%) laboratories did not report *E. histolytica*, and 4% (n=1) did not send a report due to high workload.

Seven laboratories (27%) reported *Giardia lamblia*.

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

METHODS

Microscopic examination

In tissue specimens (e.g., liver), only the trophozoite is found, and it is also diagnostic for invasive *E. histolytica*¹. The identification of *E. histolytica/E. dispar* trophozoites and cysts in stools requires concentration procedures and permanent stained smears.

Entamoeba histolytica and *Entamoeba dispar* are two separate and distinct species, however, their trophozoites and cysts cannot be identified to the species level on the basis of morphology; they are indistinguishable using routine diagnostic methods.^{1,2}

Grading

It was considered Unacceptable not to report *E. histolytica/dispar* in this sample.

The amoebiasis section of Diagnostic Medical Parasitology¹ warns about too dark or too thin permanent stained smears for the differentiation between *E. coli* and *E. hystolitica/dispar*.

The authors note that if the smear is too dark, a delicate *E. hystolitica/dispar* organism may appear more like *E. coli*; on a very thin, pale smear, *E. coli* can appear more like *E. hystolitica/dispar*.

The number of laboratories reporting *Giardia* suggests the parasite was present in the sample and thus it was not considered for grading

It is always unacceptable not to process a PT sample.

Table 904-2-1: Combined results received – E. histolytica/dispar challenge

Reports	No. Reported	%	Grade
<i>E.histolytica/dispar</i>	23	88	Acceptable
and <i>B. hominis</i> (n=20)			Acceptable
and <i>E. coli</i> (n=1)			Acceptable
and <i>E. hartmanni</i> (n=1)			Acceptable
and <i>E. nana</i> (n=1)			Acceptable
and <i>Giardia lamblia</i> (n=7)			Acceptable
<i>Entamoeba coli, B. hominis</i>	1	4	Unacceptable
<i>Giardia lamblia, Entamoeba coli, B. hominis</i>	1	4	Unacceptable
No report due to high workload	1	4	Unacceptable
Total	26	100	

Major parasites in bold

Table 904-2-2: Historic results – *E. histolytica*/dispar challenge

Challenge	904-2	804-2	704-3	510-3	504-3	310-2	304-2	210-3
Results	88%	83%	71%	100%	100%	96%	89%	93%

***E. histolytica*/dispar trophozoites**

- Usual size 15-20µm (range 10 to 60 µm), invasive forms are usually >20µm; tending to be more elongated in diarrheal stool.
- Single nuclei, on a permanently stained smear, consist of uniformly arranged chromatin on the nuclear membrane and a small, compact, centrally located karyosome¹.
- The cytoplasm is characterized as finely granular (“ground glass”) with few bacteria or no debris in vacuoles.
- *Entamoeba hartmanni* trophozoites and cysts are morphologically similar to *E. histolytica*/*E. dispar*, but are smaller in size.
- **Erythrophagocytosis (ingestion of red blood cells by the parasite) is the only morphologic characteristic that can be used to differentiate *E. histolytica* from the nonpathogenic *E. dispar*.** However, erythrophagocytosis is not typically observed on stained smears of *E. histolytica*.

***E. histolytica*/*E. dispar* cysts –**

- Usual size 12-15µm (range 10 to 20 µm)
- 1-4 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 *I.bütschlii* cysts).

Cyst formation only occurs in the intestinal tract; cysts represent the mode of transmission from one host to another.

Unfortunately, *E. histolytica* and *E. dispar* cannot be differentiated by observing cyst

morphology.

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.

Serology tests

Serum antibody detection is considered to be of crucial importance in detecting extra intestinal infections such as amoebic liver abscess.

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*. However, antibody testing to diagnose carriage of *E. histolytica* is unhelpful⁶.

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 titres can remain high for years after successful therapy⁶.

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 *E. histolytica* and *E. dispar*, in order to determine whether treatment is necessary.

The disadvantages of these tests is that they are expensive, limited to one organism only and they require an unpreserved sample.

Molecular methods

PCR can be used to identify *E. histolytica* by amplifying *E. histolytica* genes from extracted fecal DNA. Sensitivity and speci-

**ANTIGEN DETECTION
Sample submission
procedure**

One of the reference laboratories performing *E. histolytica* specific antigen detection ELISA notifies the client about the availability of the test if *E. histolytica*/*dispar* has been confirmed on the initial SAF preserved stool sample.

The patient is asked to re-submit a paired stool sample (collected at the same time): 1 SAF sample to reconfirm the presence of *E. histolytica*/*dispar* and 1 unpreserved sample for the stool antigen test.

The unpreserved stool sample should be refrigerated immediately after collection and sent to the laboratory within 48 hrs on ice or frozen until sent.

ficity are high (80–100% and 100%, respectively) ^{4, 6}.

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

Draw-backs of this method are the high level of expertise required and the difficulty of maintaining quality control for quality assurance purposes. The availability of the test is limited and the specimen must be collected in absence of preservatives.

CLINICAL RELEVANCE

Entamoeba dispar is the non-pathogenic organism and *Entamoeba histolytica* is the intestinal protozoa attributed to amoebic dysentery or amoebiasis. Estimates suggest that almost 500 million people in the world are carrying *E. histolytica* ^{1,2}.

Roughly 50 million people develop invasive disease each year, and 50,000-100,000 people die of amoebiasis each year, resulting in a mortality rate of 1 in 500-1000 diagnosed cases.

A study in Mexico using ELISA for detection of galactose/N-acetyl D-galactosamine lectin for *E. histolytica* in stools to differentiate between *E. histolytica* and *E. dispar* in stools obtained from 120 children revealed that the majority (88%) were *E. dispar*; 93% infected with *E. histolytica* were found to have symptoms, while in the group infected with *E. dispar* only 1% was found symptomatic, showing a 98% correlation between *E. histolytica* and the presence of symptoms¹⁰.

The same study cited a few more studies where the prevalence of *E. dispar* was close to 90% compared to 10% of *E. histolytica*.

There are no known animal vectors or reservoirs. Mature cysts are ingested via fecally contaminated water or food (raw vegetables), sexual contact, or by fecally contaminated hands of food handlers. Af-

ter 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 stools.

The incubation period varies from a few days to several months, but is usually 2-4 weeks ⁵. Trophozoites do not survive outside the body.

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 has been postulated.

Humans, chronically ill or asymptomatic, can excrete 15 million [15 x 10⁶] cysts per day.

ENTERIC PARASITIC INFECTIONS IN CANADA

The National Enteric Surveillance Program (NESP) reported 16.8 cases of parasitic infections in Canada per 100,000 people in 2006. The report also states the national isolation rate has remained relatively constant during the last 5 years.

The most prevalent parasite was *Giardia*, representing 73% of the cases, followed by *Cryptosporidium* with 13%, *Entamoeba histolytica/dispar* with 12% and then *Cyclospora* with 3% of the parasitic infections (2006 data)¹².

TREATMENT

Treatment of *E. dispar* is not recommended. It is up to the physician to evaluate the patient's clinical presentation to decide if treatment is warranted with a report of *E. histolytica/E. dispar*.

In a low endemic area all asymptomatic *E. histolytica* infections should be treated

AMOEBIASIS IN CANADA

According to the reports of the C-EnterNet¹¹, during 2005-2006 there were only 20 reported cases of amoebiasis in Canada. Of these 20 cases, 7 were travel related and 13 were classified as endemic.

However, in 2000, amoebiasis was removed from the list of national surveillance targets; therefore, comparative incidence data cannot be provided for Canada, although in 2004, 655 cases were reported in Ontario.

C-EnterNet is a multi-partner initiative facilitated by the Public Health Agency of Canada and funded by Agriculture and Agri-Food Canada through the Agricultural Policy Framework initiative.

It is designed to support activities that will reduce the burden of enteric (gastrointestinal) disease, by comprehensive sentinel site surveillance implemented through local public health units.

because of the potential for invasive disease and the risk of transmission to others. *E. histolytica* is susceptible to metronidazole, although the efficacy of the therapeutic regimens varies for treatment of patients with non-invasive intestinal disease, invasive recto colitis, and liver abscess.

If indicated, treatment for asymptomatic presentation of *E. histolytica* intraluminal infection is paromomycin or iodoquinol. Amoebic colitis and any invasive process should be treated with metronidazole for 10 days, followed by intraluminal treatment with paromomycin or iodoquinol for 21 days ^{2,8}.

CONCLUSIONS

There is a need for simpler, cost-effective tools suitable for identification of *E. histolytica* and *E. dispar* in clinical specimens, not only for diagnostic purposes and patient care management, where *E. dispar* infected patients could be treated unnecessarily with antiamebic chemotherapy, but also for a better understanding of the epidemiology of these parasites in the human population ⁹.

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