CMPT Enteric Parasitology Program

Innovation, Education, Quality Assessment, Continual Improvement

Challenge PA1604-1

CMPT Enteric

Stool: *Dientamoeba fragilis*, *Blastocystis hominis*

CMPT QA/QC/ Statistics

This sample was verified by two reference laboratories. Laboratories were expected to report the presence of *Dientamoeba fragilis* and *Blastocystis hominis*

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 *Dientamoeba fragilis* and *Blastocystis hominis.*

Participants (Table 1): All participants reported the presence of *D. fragilis* in the sample, 19/20 (95%) also reported *B. hominis*, 9 laboratories reported *Endolimax nana*, and 6 also detected *Entamoeba coli*. One laboratory reported *Entamoeba histolytica/dispar* on both the direct stain and on the concentrate.

19/20 participants received an acceptable grade; the laboratory that reported *Entamoeba histolytica/dispar* in addition to *D. fragilis* was given an unacceptable grade.

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.

IDENTIFICATION

Diagnosis of *D. fragilis* infection depends on proper feces sample collection and processing techniques. Immediate fixation of feces is necessary to preserve *the* morphology of *D. fragilis* as the trophozoites degenerate rapidly in unpreserved stools. [1]

As daily shedding of *D. fragilis* trophozoites is highly variable, multiple samples may be required to maximize the chances of detection. Additional stool examinations have shown to increase the percentage of positive results by 31.1% for *D. fragilis*. [2]

Identification of trophozoites in wet mounts is difficult as they may be encountered as refractile, rounded forms, varying in size. [1] As the nuclear structure can be challenging to see in saline or iodine preparations, the trophozoites may be dismissed as artifacts. [3] *D. fragilis* trophozoite identification requires examination of a permanent stained smear using an oil immersion lens (total magnification of 1,000X) as the stained smear is the only method that ensures maintaining the morphology of the organism.

Grading

Reporting *Dientamoeba fragilis* was considered acceptable.

Reporting *Entamoeba histolytica / dispar* in addition to *D. fragilis* was considered unacceptable.

Table 1. Results reported

Reported	Total	Grade
Dientamoeba fragilis	**19**	Acceptable
+ *Blastocystis hominis*	18	
+ *Endolimax nana* (one lab would not report it)	9	
+ *Entamoeba coli*	6	
Dientamoeba fragilis, Blastocystis hominis, Entamoeba histolytica / dispar	**1**	Unacceptable
Total	**20**	

Trophozoites measure 5-15 µm (range, 4-30 µm) in diameter and contain 1-2 nuclei. The most common form is binucleated, but approximately 20-30% are uninucleated. [4] The diameter of the nuclei varies from 1 to 3 µm, but depends largely on the size of the trophozoite. Internally, the nuclei appear fragmented, usually containing four to eight granules, without peripheral chromatin. [3]

Permanent stain smears need to be examined carefully because the trophozoites may be pale-staining and can be easily missed. [1]

Other diagnostic methods

Interpretation of stained slides requires experienced personnel to distinguish *D. fragilis* from other protozoa.

Other possible diagnostic/research methods for *D. fragilis* include parasite culture techniques, PCR, and RT-PCR however these techniques are not routinely available to the clinical laboratory.

While not routinely performed, *D. fragilis* can be detected by culture techniques. Apart from being complex and needing fresh stool samples, culture techniques are highly influenced by the time elapsed from collection and refrigeration, therefore, only fresh unrefrigerated samples should be cultured which may be impractical for most patients and laboratories. [5]

Stark et. al [5] compared microscopy, parasite culture using different media, and the molecular techniques, PCR and RT-PCR, for the detection of *D. fragilis* in 650 samples.

RT-PCR showed the highest sensitivity (assigned 100%), detecting 35 positive samples, while conventional PCR detected 15 cases (43%), culture detected 14 positive samples (40%), and microscopy detected *D. fragilis* in 12 stools (34%). Although these techniques are more sensitive than microscopy, they are not routinely performed in the clinical setting.

Differential diagnosis

Organisms with one nucleus can easily be confused with *Endolimax nana* or *Entamoeba hartmanii* [2, 3] *E. nana* trophozoites may appear delicate and similar to *D. fragilis*.

The nucleus of *E. nana* has a large flat karyosome, however, in some trophozoites, the karyosome may be divided into several parts. [6]

In some trophozoites of *D. fragilis*, the nuclear chromatin tends to mimic that of *E. nana* or *E. hartmanni*, particularly if the organisms are over-stained. [6]

LIFE CYCLE

The life cycle of *D. fragilis* has not been definitively described. Until recently, there was no recognized cyst stage and thus infection between humans was thought to occur through direct fecal-oral spread of the trophozoite stage.

Since the trophozoite cannot survive the digestive juices in the upper regions of the digestive tract, its mode of transmission was believed to be through the co-infection of eggs of *Enterobius vermicularis* or *Ascaris lumbricoides*. [6, 7]

In an article published in the International Journal of Parasitology, Munasinghe et. al. [8] described, for the first time, a cyst stage in the life cycle of *D. fragilis*.

D. fragilis cysts are described as having a distinct thick wall with a peritrophic space between the outer cyst wall and the encysted parasite. The authors describe numerous spherical, double membrane-bound vesicles inside the peritrophic space and an amoebic parasite enclosed within the cyst wall. Cysts also contain one or two nuclei with a centrally located nucleolus and a basal body.

Pre-cysts have been confirmed recently by Stark et al. [9] who described them having a compact spherical shape, approximately 50% smaller in size than the trophozoite form. The cytoplasm is described as dense and homogeneous rarely containing any inclusions. [10]

Retrospective studies in Australia and US examined permanently stained smears positive for *D. fragilis* with special care to identify forms bearing a strong resemblance to the cysts described by Munasinghe et al. [8] the cysts were detected independently in both studies. [9]

In conclusion, transmission via helminth eggs such as those of *Ascaris* and *Enterobius* spp. has been postulated. [3] With the confirmation of cyst and pre-cyst stages, [8 9] the fecal-oral transmission of *D. fragilis* has therefore been confirmed. [10]

CLINICAL RELEVANCE

Intestinal infection with *D. fragilis* can be asymptomatic or cause a wide range of symptoms [3]. Intermittent diarrhea, abdominal pain, nausea, anorexia, malaise, fatigue, and poor weight gain, have been associated with *D. fragilis* infection.

The presence of eosinophilia[11,12] in approximately 50% of patients has prompted experts to recommend that *D. fragilis* be included in the differential diagnosis of chronic diarrhea and eosinophilic colitis. [10]

RECENTLY PUBISHED

Dientamoeba fragilis, one of the neglected intestinal protozoa. Lynne S. Garcia J Clin Microbiol. 2016 Apr 6

REFERENCES

1. Yang J, Scholten T. *Dientamoeba fragilis*: A Review with Notes on its Epidemiology, Pathogenicity, Mode of Transmission, and Diagnosis. *Am J Trop Med Hyg*. 1977;26:16-22.

2. Hiatt RA, Markell EK, Ng E. How many stool examinations are necessary to detect pathogenic intestinal protozoa? *Am J Trop Med Hyg*. 1995;53:36-39.

3. Johnson EH, Windsor JJ, Clark CG. Emerging from Obscurity: Biological, Clinical, and Diagnostic Aspects of *Dientamoeba fragilis*. *Clin Microbiol Rev*. 2004;17:553-570.

4. Bogitsh BJ, Carter CE, Oeltmann T.N. Visceral protozoa II: Flagellates. In: *Human Parasitology.* third ed. Burlington, MA: Elsevier Academic Press; 2005:90.

5. Stark D, Barratt J, Roberts T, Marriott D, Harkness J, Ellis J. Comparison of microscopy, two xenic culture techniques, conventional and real-time PCR for the detection of *Dientamoeba fragilis* in clinical stool samples. *Eur J Clin Microbiol Infect Dis*. 2010;29:411-416.

6. Garcia LS. Intestinal protozoa: Flagellates and ciliates. In: *Diagnostic Medical Parasitology*. 5th ed. Washington, DC: ASM Press; 2007:6 - 56.

7. Girginkardeşler N, Kurt Ö, Kilimcioğlu AA, Ok ÜZ. Transmission of *Dientamoeba fragilis*: Evaluation of the role of *Enterobius vermicularis*. *Parasitol Int*. 2008;57:72-75.

8. Munasinghe VS, Vella NG, Ellis JT, Windsor PA, Stark D. Cyst formation and faecal-oral transmission of *Dientamoeba fragilis* - the missing link in the life cycle of an emerging pathogen. *Int J Parasitol*. 2013.

9. Stark D, Garcia LS, Barratt JL, et al. Description of *Dientamoeba fragilis* Cyst and Precystic Forms from Human Samples. *J Clin Microbiol*. 2014;52:2680-2683.

10. Garcia LS. *Dientamoeba fragilis*, one of the neglected intestinal protozoa. *Journal of Clinical Microbiology*. 2016.

11. Gray TJ, Kwan YL, Phan T, Robertson G, Cheong EYL, Gottlieb T. *Dientamoeba fragilis*: A Family Cluster of Disease Associated With Marked Peripheral Eosinophilia. *Clinical Infectious Diseases*. 2013;57:845-848.

12. Cuffari C, Oligny L, Seidman EG. *Dientamoeba fragilis* masquerading as allergic colitis. *J Pediatr Gastroenterol Nutr*. 1998;26:16-20.