Blastocystis or Dientamoeba? Morphological similarities when fixed and stained

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Many laboratories will use a wet preparation of faeces to check for enteric protozoa. The appearance of *Blastocystis hominis* in these films is straightforward. Dientamoeba can be present as well but it requires an enormous amount of skill to identify if you have not prepared a fixed and stained smear. The appearance of these two organisms in the iron haematoxylin stain can be very similar.

This article aims to demonstrate these similarities and, hopefully, laboratories that are considering the use of fixed and stained slides for the identification of enteric protozoa may find the information useful.

In the author's laboratory, enteric protozoa are identified in slides prepared from stools using Sodium Acetate/Acetic Acid/Formalin as fixative. They are stained using the *Para-Stain* [1] protozoa staining kit which combines a modified Ziehl-Neelsen step as well as an iron haematoxylin step in the same stain. Identifications were confirmed using the modified Boeck and Drbohlav's Locke-egg-serum medium [2].

Over the past 3 years we have noticed that if Blastocystis is present, not all of the cells have the classic textbook central body (CB) that characterises this organism when viewed in the wet preparation. The CB form is what we see in the wet film but there is also the granular form and the amoeba form, which are identified using cultures and electron microscopy [3].

Blastocystis hominis: Commonly occurs in both symptomatic and asymptomatic patients and its pathogenicity is controversial.

Dientamoeba fragilis: Infection may be symptomatic or asymptomatic. It has, however, been strongly implicated in gastrointestinal infections and infections in children [4, 5]. At this facility we see many symptomatic patients who are carrying Dientamoeba by itself; some of these patients having repeat episodes of diarrhoea after appropriate treatment. Trophozoites colonise the caecum and ascending colon where they cause surface irritation but they do not invade the mucosa [5].

Work done in Canada and Great Britain indicates that this organism is the chief cause of parasitic gastrointestinal disease [6, 7]. There is no cyst stage, hence it is rarely implicated in outbreaks.

A report using a population of 27,058 specimens in Tunisia makes reference to an association with Blastocystis [8]. The incidence of Dientamoeba is worldwide and indications from the literature are that more laboratories should be testing for it.

Slides were prepared, fixed and stained on specimens from outpatients with unformed or fluid faeces and those on inpatients with unformed or fluid specimens, provided those patients had only been admitted in the last 2 days.

The following *Para-stain* slides demonstrate what can happen to Blastocystis and Dientamoeba morphology once they have been fixed and stained (Figures 1-4).

Figure 1. Blastocystis with yellow CB forms. The Blastocystis without the CB has a clear cytoplasm and two nuclei resembling Dientamoeba, but lacks packets of chromatin typically located at the centre of the nucleus.

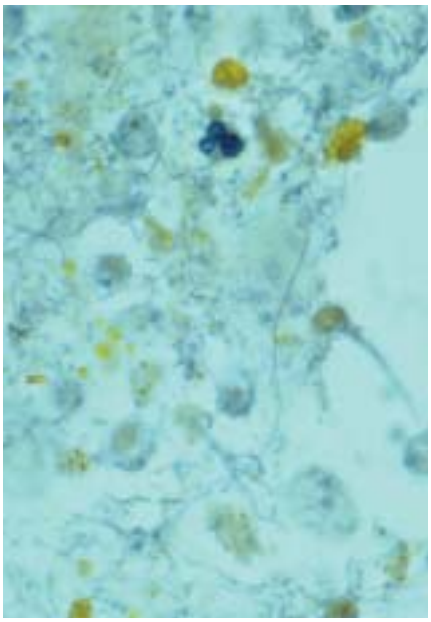


Figure 2. Blastocystis showing many CB forms. Note the pale organism that has two nuclei.

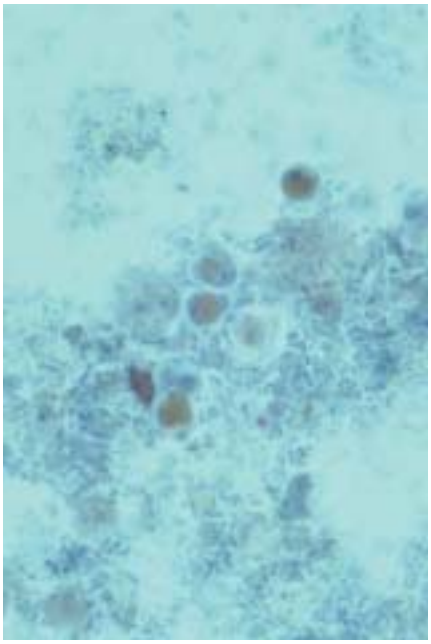


Figure 3. Typical Blastocystis grouped in one corner of the slide but the cell at the other end may cause some confusion. It is *Blastocystis hominis*.

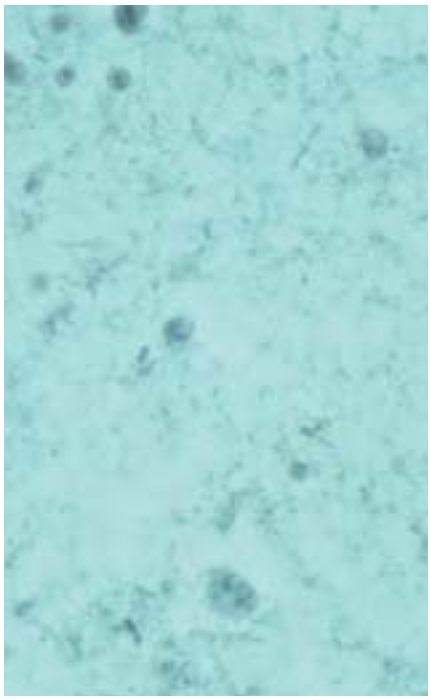
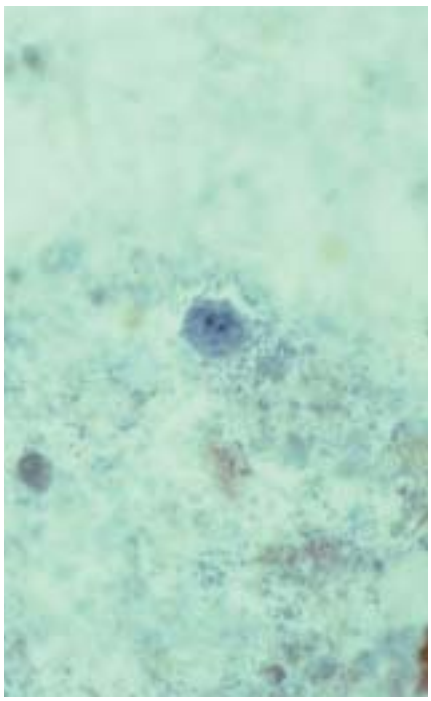


Figure 4. A good example of Dientamoeba fragilis. The nucleus contains central packets of chromatin (beads not fragments) which avoid the edge of the nucleus. The cytoplasm is more coarse and contains ingested bacteria and vacuoles.

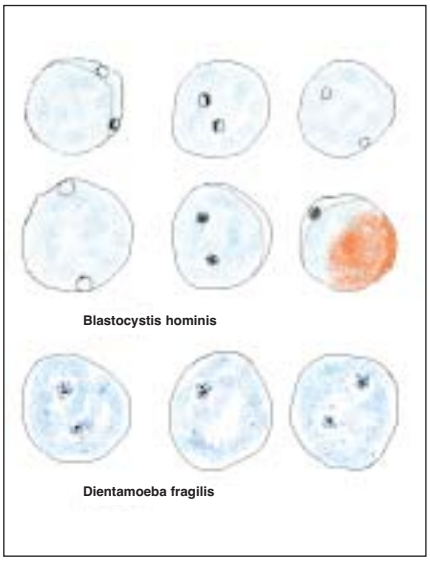


The fragmentation of the nucleus is, at this point in time, the real marker for accurately identifying these two protozoa, since it has been reported that Dientamoeba may look paler in stained smears[3]. Figure 5 may help microscopists.

The following suggestions will help to improve the reporting of these two organisms:

- Encourage as many GPs and microbiologists as you can to fix their COP specimens as soon after passage as possible.
- Always stain for enteric protozoa – you simply cannot rely on wet mounts to distinguish these two organisms. The stained slide can be challenging enough.
- Follow up trained staff with frequent slide review meetings.
- Conduct a more thorough search when *Blastocyctis hominis* and/or Dientamoeba fragilis are present.
- Have complicated slides and those containing numerous species checked by another staff member.
- Participate in an approved external Quality Assurance Program for the enteric protozoa. Monitor the figures from other laboratories and network with people who are interested in parasitology.

Figure 5.



Many laboratories are still not staining specimens that carry a request for cysts, ova and parasites. It is incumbent on these laboratories to accurately identify all organisms that are present, otherwise they will be doing patients a disservice. Pus cells stain well so their presence is also a valuable marker for infection; such information is useful to clinicians and can be reported in conjunction with the bacterial culture report.

This technique is accurate, especially if used in conjunction with an automated glass coverslipping machine which provides better resolution than the manually applied or automated plastic cover slips. It is also affordable.

To improve the reporting of these two parasites, staining is the best available method at present and is a valuable diagnostic tool, but it can have its pitfalls. Hopefully, emerging molecular methods eventually will further clarify the exact role that these two protozoa play in a clinical setting.

Acknowledgements

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