Incidence of *Blastocystis hominis* in faecal samples submitted for routine microbiological analysis

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Accepted: 9 June 2002

ABSTRACT

Over a one-year period, 1390 faecal samples were submitted to Aberystwyth Public Health Laboratory for routine microbiological examination. All were stained using a commercial trichrome method. *Blastocystis hominis* was detected in 96 (6.9%), making it the most common parasite found in the study. Of the *B. hominis*-positive specimens, 73% were missed on direct microscopy. Molecular typing of *B. hominis* has revealed extensive genetic diversity in morphologically identical strains and thus detection by microscopy alone may not be sufficient to confirm the role of this organism in human disease.

KEY WORDS: Blastocystis hominis.
Feces.
Microbiological techniques.

Introduction

Blastocystis hominis is a controversial human parasite, both in terms of its taxonomic history and its pathogenicity. Alexeieff[1] defined the genus in 1911, although it was not until the following year that Brumpt[2] proposed the name for the organism when found in humans. These early workers regarded *B. hominis* as a yeast.[1,2] Over the ensuing years *B. hominis* continued to confound, and it was thought to be a degenerate or cyst form of a flagellate by some,[3] while others classified it as an amoeba or a sporozoan.[3,4]

There is little doubt that the presence of multiple morphological forms in some clinical specimens hindered early workers in the field, adding to this taxonomic enigma. Silberman *et al*.,[5] using phylogenetic analysis of ribosomal RNA, finally solved the mystery and classified *B. hominis* as the only member of the Stramenopiles found to infect humans. Other members of this diverse group of eukaryotes include kelp, diatoms, slime nets and water moulds.

B. hominis varies greatly in size (<5 to >15 μm), shape and number of nuclei.[6] In addition, four different morphological forms have been described: amoeboid, central body, granular and cystic.[4,7] *B. hominis* is a strict anaerobe and exposure to oxygen may also adversely affect its morphology. This morphological diversity can pose difficulties for laboratory staff, and result in it not being recognised in clinical samples.

The central body (CB) form is perhaps the most commonly seen in the diagnostic laboratory.[8] Vdovenko[9] considered CB and granular forms of *B. hominis* to be degenerative and due to environmental exposure. The CB form measures 10-15 μm in diameter and can often be recognised in unstained faecal preparations.

The cystic form, however, is much smaller (4-5 μm) and can be easily overlooked in unstained preparations.[7] Permanent smears are considered to be the method of choice for light microscopic diagnosis.[4] Even when using suitable stains, such as a trichrome method or iron haematoxylin, much variability can occur, especially with the central vacuole (Figure 1).

Evaluation of data on the pathogenicity of *B. hominis* is problematical as many of the reported cases associated with symptomatology are anecdotal. Conflicting reports and uncontrolled studies add to the confusion, and some studies have failed to eliminate all other infectious causes of intestinal symptoms.

A surge of interest in the 1980s saw reports of *B. hominis* infection in immunocompetent and immunocompromised patients.[8] Garavelli *et al*.[10] described five cases of blastocystosis in symptomatic human immunodeficiency virus (HIV)-positive patients that responded to treatment with metronidazole. They concluded that *B. hominis* might be responsible for HIV-related diarrhoea.

Cirioni *et al*.[11] examined 1216 adults from different patient cohorts and found *B. hominis* to be the most frequently detected parasite in each group. Interestingly, symptoms were only found in *B. hominis*-positive patients when severe immunosuppression was present. *B. hominis* has also been associated with irritable bowel syndrome,[12,13] patients with intestinal carcinoma,[14] and reported in asymptomatic individuals.[15-17]

B. hominis has been described as probably the most common human intestinal protozoan, worldwide.[18] Originally, it was associated with diarrhoea in the tropics and subtropics, although since found in patient populations throughout the world.[4] Reported prevalence rates vary from 1.5% to 10% in developed countries, with much higher rates of 30% to 50% in developing countries.[4]

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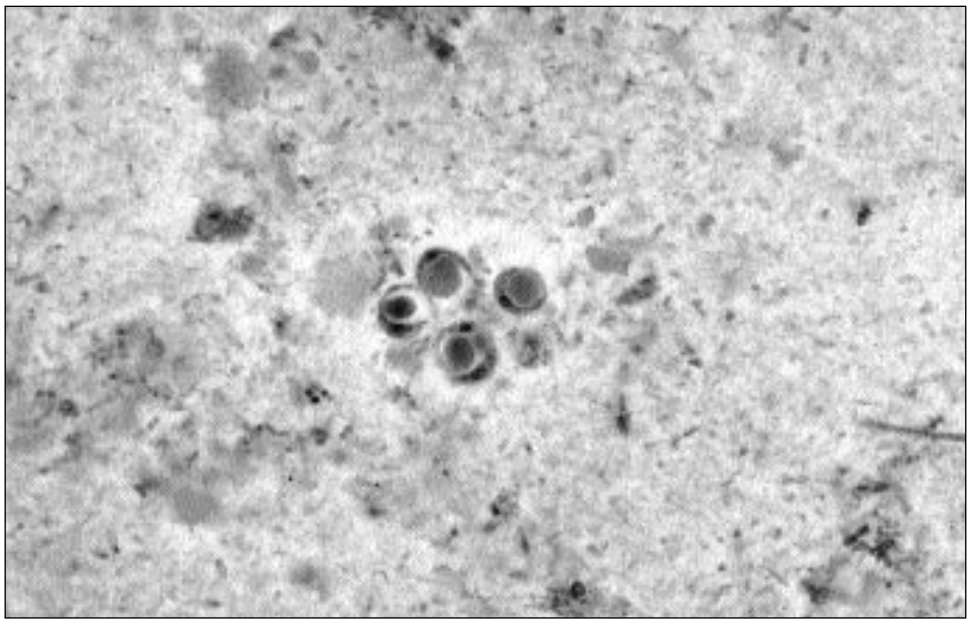

Fig. 1. Trichrome-stained smear of *Blastocystis hominis* (original magnification x600).

Pakianathan and McMillan[19] examined 175 homosexual men in Edinburgh and reported *B. hominis* in 26%, although it was not associated with diarrhoea. Other than this study, in a population subgroup that usually has a high carriage rate of intestinal protozoa, little has been published on the incidence of *B. hominis* in the UK.

The purpose of the present study is to determine the incidence of *B. hominis* in faecal samples submitted for routine microbiological examination to Aberystwyth Public Health Laboratory (PHL).

Materials and methods

During a one-year period from July 1999, all faecal specimens submitted to Aberystwyth PHL were examined for the presence of *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. and *Escherichia coli* 0157, using standard bacteriological techniques. Specimens were not examined for the presence of viruses other than rotavirus investigations on selected samples. Rotavirus and *Clostridium difficile* toxin results were not included in the analysis. A phenol/auramine stain was used to detect cryptosporidium oocysts.[20]

In order to detect *B. hominis*, all samples were fixed overnight in sodium acetate/acetic acid/formalin (SAF; Intersep Inc., Florida, USA). The following day they were washed (x3) in saline, centrifuged at 1000 x*g* for 5 min each time, mixed with a drop of Mayer's albumin, spread on a slide and allowed to dry. Resulting smears were stained with a commercial trichrome stain (Intersep Inc.) and mounted in DPX (BDH Laboratory Supplies, Poole, UK). Smears were examined under oil immersion (x50 and x100).

Table 1. Parasites detected in 1390 unselected faecal specimens (July 1999 to June 2000)

Organism	Number	Percentage
Blastocystis hominis	96	6.9%
Cryptosporidium parvum	35	2.5%
Dientamoeba fragilis	17	1.2%
*Entamoeba coli**	13	0.9%
Giardia lamblia	10	0.7%
Endolimax nana	4	0.29%
*Iodamoeba butschlii**	3	0.22%
*Entamoeba hartmani**	2	0.14%
*Chilomastix mesnili**	2	0.14%
Entamoeba histolytica/dispar	1	0.07%
Cyclospora cayetenensis	1	0.07%

* *non-pathogenic species*

Results

B. hominis was detected in 96 (6.9%) of the 1390 specimens examined. Repeat positive specimens were excluded from the study. Of the 96 positives, 45 were from men (age range: 1-80 years [mean: 48.6 years]), 47 from women (age range: 5-90 years [mean: 46.7 years]) and sex was not stated in four. There was no apparent seasonality, although a longer study would be needed to confirm this observation.

Cryptosporidium sp. was the second most common parasite observed after *B. hominis* (Table 1). Other bacterial enteropathogens are presented in Table 2. *B. hominis* was found with other parasites/bacterial pathogens in 15 specimens: five with *Entamoeba coli*; three with *Dientamoeba fragilis* (one also had *Cryptosporidium* sp. present); two with *Campylobacter* spp.; and one each with *Salmonella* sp., *Giardia lamblia*, *Iodamoeba butschlii* and *Cryptosporidium* sp. One specimen contained *B. hominis*, *G. lamblia* and *Endolimax nana*.

Of the *B. hominis*-positive specimens, 73% (70/96) were detected by the trichrome stain alone. Although *B. hominis* was seen by direct (unstained) microscopy on 26 occasions, the false-positive rate was high (i.e. 22 possible *B. hominis*-positives on direct microscopy were negative by the trichrome method).

B. hominis was detected alone (without other parasites or pathogenic bacteria) in 81 patients but clinical data (obtained

directly from the specimen request form) was only available in 57 cases. Eight patients had a history of recent foreign travel (Cyprus, Turkey, Tanzania, El-Salvador, India, Thailand, Nepal, and South America). The most common symptom was diarrhoea, and was present in 40 (70%) patients. Other symptoms included abdominal pain, diarrhoea and vomiting, flatulence and pyrexia.

Discussion

B. hominis was by far the most common faecal parasite detected in the laboratory over the period of the study, and results indicate that it is a common parasite in Wales. Despite this, only two laboratories regularly report its presence in Wales.[21] Indeed, *B. hominis* numbers reported to the UK Public Health Laboratory Service (PHLS) Communicable Diseases Surveillance Centre (CDSC) fell from 551 in 1996 to 293 in 1999.[22] However, these numbers may reflect unsuitable laboratory techniques, inexperience or reporting practices, and thus may not be representative. Without the use of the trichrome-stained smear, a large number of *B. hominis* cases would have been missed in our study. In view of this, we recommend the use of suitable permanent staining methods when assessing the incidence of *B. hominis*.

The main controversy surrounding *B. hominis* is the vexing question of its role in human disease. Zierdt[23] proposed that it is pathogenic when present in large numbers (≥ 5 organisms per x1000 field). Similarly, other workers used a cut-off of ≥ 5 organisms per x400 field.[24,25] However, Shlim *et al*.[15] found that increased *B. hominis* numbers are not associated with increased severity of symptoms, and they concluded that a quantitative cell count is not a reliable indicator of infection. Keystone[26] commented that this study, although failing to find an association between *B. hominis* and human disease, might have been weakened by design flaws. The selection criterion employed for controls was faulty and, once other known pathogens were excluded, only 19 patients with diarrhoea and 26 controls were infected with *B. hominis*.

Pathogenicity studies have shown that *B. hominis* modulates immune responses and cytokine release in colonic epithelial cells.[27] In addition, Dagci *et al*.[28] monitored intestinal permeability (IP) in patients with pathogenic and non-pathogenic protozoan infections. They found that IP increases in patients with *G. lamblia* and *B. hominis* but not in patients harbouring *Entamoeba coli*. Intestinal permeability is said to demonstrate the intactness of the intestinal mucosa and is increased during damage to the intestinal wall. Accordingly, they concluded that *B. hominis* could be pathogenic.

Perhaps the most promising breakthrough has been the use of molecular methods for typing *B. hominis*. Clark[29] investigated *B. hominis* isolates using ribotyping to study variation in the small subunit ribosomal RNA genes. He found extensive genetic diversity, with seven morphologically identical strains that differed genetically. It is possible that a particular subtype of *B. hominis* may be found in the future with the potential to cause human disease. Lanuza *et al*.[30] compared soluble-protein and antigenic heterogeneity in axenic *B. hominis* isolates and found differences between those isolated from patients with chronic and acute diarrhoea.

Table 2. Pathogenic bacteria isolated from 1390 unselected faecal specimens (July 1999 to June 2000)

Organism	Number	Percentage
Campylobacter spp.	69	5.0%
Salmonella spp.	22	1.6%
Shigella sonnei	3	0.22%
Aeromonas hydrophila	2	0.14%

As faecal specimens were not examined for viruses in the present study, it would be unwise to directly correlate carriage of *B. hominis* with gastrointestinal symptoms; however, we have demonstrated that *B. hominis* is perhaps more common in the UK than was previously thought.

Certainly, much remains to be elucidated about the pathogenicity of *B. hominis*, and discovery of its genetic diversity means that detection by microscopy alone may not be sufficient to link this controversial parasite with infections in humans.

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