

Epidemiology and Pathogenicity of *Blastocystis hominis*

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Received 15 May 1989/Accepted 2 October 1989

A prospective study was performed on a large outpatient population to evaluate the epidemiology and pathogenicity of *Blastocystis hominis*. Patients with stool specimens positive for *B. hominis* and negative for other bacterial and parasitic pathogens were sent a questionnaire and were requested to submit a follow-up specimen for ova-and-parasite examination. *B. hominis* was identified in 530 of 16,545 specimens (3.2%). There was a spectrum of clinical-pathological presentations in the 143 patients evaluated. An asymptomatic carrier state was seen in 19 patients. Fifteen patients had an illness consistent with acute self-limited *B. hominis* gastroenteritis, and 21 patients had chronic gastroenteritis associated with *B. hominis*. In the epidemiological evaluation of 130 patients, the most common symptoms were watery diarrhea, abdominal pain, and gas. We did not find a statistically significant association between the number of organisms present and the disease state. In summary, our results are consistent with a role for *B. hominis* in acute and chronic gastroenteritis; however, further detailed studies are necessary to determine whether that role is one of association or causation.

In 1976, Zierdt and Tan recommended reclassification of *Blastocystis hominis* as a protozoan in the subphylum Sporozoa (20). *B. hominis* was named by Brumpt in 1912 (1). Reports in the early 1900s of a possible pathogenic role for *B. hominis* were largely ignored, and it was generally considered a nonpathogenic intestinal yeast until Zierdt renewed interest in the organism.

In 1967, Zierdt et al. (19) gave morphological and physiological evidence that *B. hominis* was in fact a protozoan. Since that time there have been many reports, mostly anecdotal and retrospective, supporting a role for *B. hominis* as a potential pathogen in humans (2-4, 6, 7, 12, 15-17). There is some experimental evidence to support a pathogenic role from studies done on gnotobiotics (11). Many parasitologists now believe that when *B. hominis* is present in large numbers (over five organisms per oil immersion field) in the absence of other known bacterial, viral, or parasitic agents it should be recognized as a pathogen and treated.

This study was undertaken to prospectively evaluate the epidemiology and pathogenicity of *B. hominis* in an outpatient population that is representative of that seen in general medical practice. When *B. hominis* is found in the absence of other potential pathogens, it is for these patients that most clinical decisions regarding its pathogenicity and treatment must be made. This study is predominantly descriptive in nature and includes frequency tabulations of epidemiological findings. We have also endeavored to group patients (based on generally recognized criteria) into clinical-pathological groups that would be of assistance to the practicing clinician. This study was not designed to determine the effectiveness of therapy.

(These data were presented in part previously [P. W. Doyle, M. M. Helgason, and R. G. Mathias, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 34, 1988].)

MATERIALS AND METHODS

Patient population. Metro-McNair Clinical Laboratories is a large outpatient laboratory in Vancouver, British Columbia, Canada, that processes more than 15,000 stool specimens per year. Patients were included in the study when stool specimens submitted to our laboratory contained *B. hominis* as the sole enteric pathogen after examination for ova and parasites (O+P) and bacterial culture and susceptibilities (C+S). A questionnaire was sent to the referring doctor of these patients. The questionnaire requested clinical information and a follow-up stool for O+P examination.

Follow-up clinical information and follow-up specimens for O+P were required for inclusion in the evaluation, except if the patient was found to be asymptomatic when the first specimen was taken. The follow-up stool examination for O+P (and C+S if performed) also had to be negative for all pathogens other than *B. hominis*, or the case was excluded.

Stool collection. Initial specimens were ordered by the referring physicians, collected by the patients, processed by our branch laboratories, and sent to our central laboratory for analysis. Transport containers contained sodium acetate-acetic acid-Formalin fixative for O+P and a sterile container with no fixative for bacterial culture. Most of the specimens were set up for culture on the day that they were received. Delayed specimens were held at room temperature and processed within 24 h.

Stool analysis. All specimens were examined for O+P by the Formalin-ether concentration method, the trichrome stain, and the modified acid-fast stain for cryptosporidia. The size and number of *B. hominis* cells per high-power field (400×) were recorded from both the iodine preparation and the trichrome stain. The presence of other parasitic pathogens and nonpathogens was recorded. For the study, the following protozoa were considered nonpathogens: *Endolimax nana*, *Entamoeba coli*, *Entamoeba hartmanni*, *Iodamoeba buetschlii*, and *Chilomastix mesnili*. *Dientamoeba fragilis* was considered a pathogen in this study. The presence of polymorphonuclear leukocytes was also recorded.

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Stool cultures were performed on the following media and incubated for the indicated times and temperatures: Columbia sheep blood agar, MacConkey agar, sorbitol MacConkey agar, and Hektoen agar for 24 h at 35°C and then 24 h at 20°C; yersinia selective agar for 48 h at 20°C; campylobacter agar for 2 days at 42°C; selenite broth subcultured after 24 h of incubation to Hektoen agar; and phosphate-buffered saline for cold enrichment, which was incubated at 4°C for 10 days and then subcultured to yersinia agar at 20°C for 48 h. All media were supplied by Prepared Media Laboratory Ltd., Richmond, British Columbia, Canada. We did not examine stools routinely for *Clostridium difficile* or for enteric viruses. Other studies in our laboratory have found a very low incidence of *C. difficile* in our patient population (unpublished data, available on request).

Data analysis. Data were coded onto spreadsheets by one of us using Symphony Software Release 1.2 (Lotus Development Corp., Cambridge, Mass.) and analyzed using the computer program SPSX on a mainframe computer. Statistical differences were analyzed using the Fischer exact probability two-tailed test (14). Patients were classified as per the definitions given below. Patients' identities were kept confidential.

Clinical-pathological evaluation. Clinical data were taken from information recorded on the returned questionnaires and also from phone conversations with referring physicians.

Patients' clinical and laboratory data were analyzed, and each case was grouped as per the following predetermined definitions. (i) Asymptomatic carrier: stool specimen positive for *B. hominis* in the absence of symptoms; (ii) acute gastroenteritis: spontaneous clearance of symptoms in less than 2 weeks, with simultaneous clearance of *B. hominis*; (iii) chronic gastroenteritis: symptoms present for 2 or more weeks resolved with clearance of *B. hominis* (these patients were subclassified into two groups, spontaneous resolution [self-limited] and resolution after a course of therapy [treated]); and (iv) symptomatic carriers of *B. hominis*: clinical and laboratory evidence that *B. hominis* was not the cause of symptoms (these patients were subclassified into two groups, spontaneous resolution [self-limited] and resolution after a course of therapy [treated]).

Upon analyzing the data, it became apparent that two large groups of patients existed that did not fit into the above-predetermined groups, and these groups are defined as follows. (v) Postdiarrheal carriers: persistence of *B. hominis* after spontaneous resolution of symptoms; and (vi) persistent blastocystosis: chronic or intermittent symptoms and persistence of *B. hominis* at follow-up (these patients were subclassified into two groups, no treatment and treated).

For the purpose of this study, metronidazole (Flagyl) and iodoquinol (Diodoquin) were assumed to have activity versus *B. hominis* and were recommended only for patients with chronic symptoms as a "trial of therapy." This study was not intended to determine the efficacy of either form of therapy. For the purposes of this study, we used the results of therapy with these agents as a tool to help in the classification of patients with *B. hominis*.

Epidemiological evaluation. Data for the epidemiological evaluation were taken only from returned questionnaires. Data collected directly from physicians or patients by the investigators were not used for this part of the evaluation. These data thus represent a subset of the above-described data.

TABLE 1. Asymptomatic carriers^a

Reason tested	No. of carriers
Posttravel screen	8
Immigrant screen	2
"Screen"	2
Gay bowel screen	2
Mistake in ordering	1
Eosinophils only	1
Perianal itch, no pinworm	1
B ₁₂ deficiency only	1
No reason	1

^a Positive for *B. hominis*, no symptoms.

RESULTS

All stool specimens received for O+P examination over a 12.5-month period in 1986 to 1987 were tested for *B. hominis*. *B. hominis* was identified in 530 of 16,545 stool specimens (3.2%) from 425 patients examined over this period. The average number of stool specimens per patient was 1.42 in a subsequent study at Metro-McNair Laboratories and is a good estimate for this study (unpublished data from S. Champagne). Excluded from the study were 101 patients in whom other pathogens were detected on O+P and/or C+S or for whom a C+S was not performed. Of 324 questionnaires sent to referring physicians, 130 were returned; and the data were evaluated epidemiologically. Information on a further 13 patients was obtained directly from the physicians and used for the clinical-pathological evaluation. The response rate for eligible individuals was 44%.

Clinical-pathological evaluation. The overall results of the clinical-pathological evaluation on 143 patients are given below. The patients have been grouped as per the definitions given above.

Of the 143 patients evaluated, 19 were classified as asymptomatic carriers. Table 1 shows the reasons for testing these patients. Eleven patients were classified as symptomatic carriers of *B. hominis*, since there was evidence that *B. hominis* was not the cause of their symptoms. Metronidazole was given to 10 of these 11 patients with clearance of symptoms or *B. hominis* but not both. In one patient, there was spontaneous clearance of *B. hominis* with persistence of symptoms.

We classified 23 of the 143 patients as postdiarrheal carriers. Many of these patients had spontaneous resolution of symptoms of acute gastroenteritis, with virtual elimination of *B. hominis* from their stool specimens.

We classified 15 of the 143 patients as having acute gastroenteritis. These cases are consistent with a role for *B. hominis* in acute gastroenteritis.

We classified 21 of the 143 cases as consistent with chronic *B. hominis* gastroenteritis. In 8 of the 21 patients, there was spontaneous (self-limited) resolution of symptoms and clearance of the organism. In 13 of the 21 patients, there was resolution of symptoms with clearance of the organism after a course of therapy (treated). Metronidazole was the drug of choice. Iodoquinol was given to only one patient, after a failure to respond to metronidazole.

Of 143 patients, 13 were classified as having persistent blastocystosis, with chronic or intermittent symptoms and persistence of *B. hominis* at follow-up. Chronic symptoms were present in 9 of the 13. Some of these patients received therapy. Intermittent symptoms were present in 4 of the 13.

Forty-one of the 143 patients were initially included and

TABLE 2. Patients initially included and subsequently excluded

Reason for exclusion	No. of patients
No follow-up O+P	10
No follow-up symptoms	2
No follow-up symptoms or O+P	
No therapy.....	10
Postmetronidazole	2
Other therapy during study	
Antibiotics	6
Milk products restricted	1
Original specimen	
Postsymptoms	3
Posttherapy	3
Gastrointestinal illness	4

subsequently excluded from further evaluation for the following reasons: (i) original stool specimen was collected after symptoms had cleared, (ii) original specimen was collected after therapy for a gastrointestinal illness, (iii) patients had another diagnosed gastrointestinal disorder, and (iv) patients received antibiotics or other therapy during the period of evaluation (Table 2).

Four patients included in the study had symptoms of acute gastroenteritis and received nonspecific antidiarrheal medication because of the severity of their acute disease. Two of these patients were classified as postdiarrheal carriers, and the other two were classified as having acute gastroenteritis.

Epidemiological evaluation. The epidemiological evaluation was performed on 130 patients. The sex distribution (male:female) was 1:1.2. The urban-rural ratio was 3.4:1.0. Figure 1 shows that the peak incidence of *B. hominis* was in the 30- to 39-year age group. The mean age was 37 years.

A travel history was found in 68 of the 130 patients (52%). Of the 68 travelers, 22 traveled to Asia, 21 traveled to South America, 19 traveled within North America, 10 traveled to Europe, 7 traveled to Africa, and 1 traveled to Australia. Eleven people traveled to multiple destinations.

Twelve patients were recent immigrants. Six of the 12 immigrants were from Asia, which is approximately the norm for our immigrant population.

In 53 of 121 responders (44%), there was a history of animal exposure. The pet animal-to-farm animal ratio was 14:1.

Only 3 patients of 108 responders were noted to be immunocompromised. Two of these patients had cancer.

Symptoms were present in 109 of the 130 patients (84%), and 21 (16%) were asymptomatic. The duration of symptoms was noted in 104 of the 109 patients. In 28 patients, symptoms were of less than 14 days' duration; in 15, duration was less than 1 month; in 21, duration was less than 3 months; in 21, duration was less than 6 months; in 8, duration was less than 1 year; and in 11, duration was greater than 1 year.

The severity of symptoms was documented in 91 of the 109 symptomatic patients. Thirty-one patients described their symptoms as mild, 2 described them as mild to moderate, 47 described them as moderate, 2 described them as moderate to severe, and 9 described them as severe.

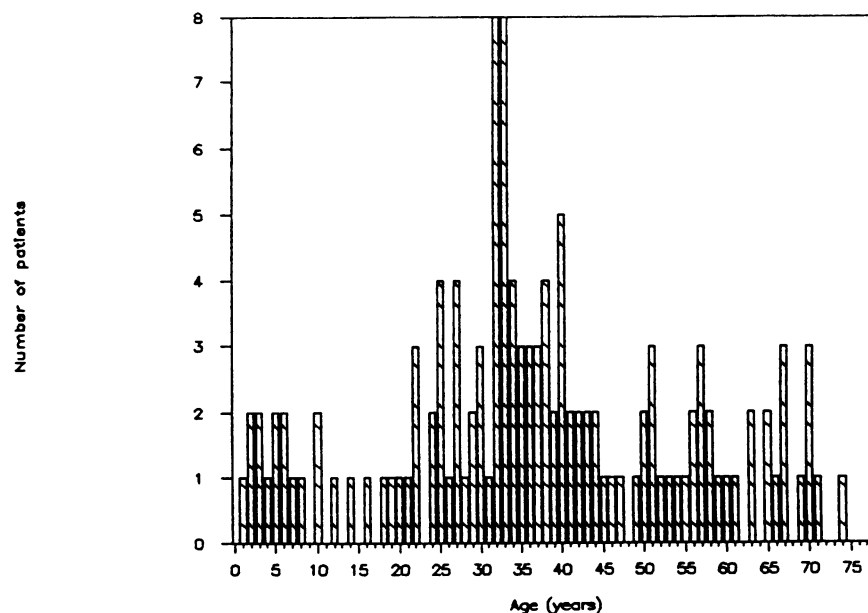
From 125 of the 130 patients, data were available on type of symptoms (if present), as shown in Fig. 2. Diarrhea was the most common symptom and was present in 93 of the 125 patients (74%), followed by abdominal pain in 72 patients (58%) and gas in 52 patients (42%).

The number of stools per day varied from 1 to 25, with a mean of 4.8 for the 72 patients responding.

Of 69 responders, 56 had watery stools, 19 had stools with mucus, and 8 had bloody stools. In some patients, diarrhea was a mixture of these types.

Endoscopy was performed on five patients. In four of them, no pathology was seen. One patient had a colonic adenocarcinoma.

Although some patients classified as having acute *B. hominis* infection had high numbers of organisms in their stool specimens, there was no statistically significant difference between the number of organisms seen in patients classified as having acute gastroenteritis and those classified as asymptomatic carriers.

FIG. 1. Age distribution of patients with *B. hominis*.

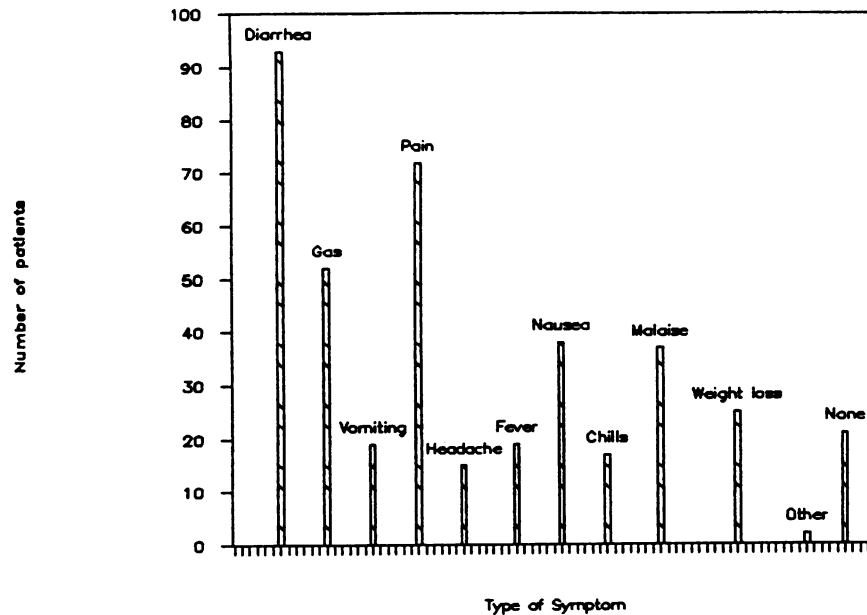


FIG. 2. Frequency of specific symptoms in patients with *B. hominis*.

We analyzed the size of organisms encountered on trichrome stain. Data were available in 100 cases. In 5 cases, organisms were less than 6 μm in diameter; in 66 cases, they were between 6 and 10 μm in diameter; in 23 cases, they were between 11 and 15 μm in diameter; and in 6 cases, they were larger than 15 μm in diameter. There were no significant differences between the different clinical-pathological groups in the size distribution of organisms.

Among the 101 evaluable patients, the presence of polymorphonuclear leukocytes was documented in 6. There was no statistically significant difference between the presence of polymorphonuclear leukocytes in patients with acute gastroenteritis and asymptomatic carriers.

DISCUSSION

In this study, we prospectively evaluated the epidemiology and the clinical-pathological significance of *B. hominis* in patients from whom no other pathogen was identified.

The early literature from 1912 through the 1930s contained numerous case reports incriminating *B. hominis* as a cause of gastrointestinal tract infection and others disputing those claims (13, 18). These articles also introduced evidence for an association between the number of organisms present and the clinical presentation. In 1917, Wenyon and O'Connor reported the presence of *B. hominis* in 25% of stools, with large numbers of organisms associated with acute diarrhea (18). They monitored one case sequentially and found a correlation between the number of organisms present and symptoms. Their report suggested the "possible pathogenicity for blastocystis when present in any quantity."

In 1930, Sangiorgi demonstrated the possible pathogenicity of *B. hominis* (13). Sangiorgi found that the incidence of *B. hominis* varied from 0.7% in Venice to 20% in Albania. In a study of 2,000 soldiers, 263 had active intestinal disorders, and of these, 11 had "blastocysts alone." Results of emetine therapy "disillusioned" them. In 4 of these 11 cases, patients had acute dysentery with abdominal pain lasting approximately 1 week. They had 6 to 10 liquid stools per

day, associated with leukocytes and large numbers of *B. hominis* cells. In 7 of the 11 cases, patients were afebrile, with two to three stools per day and slow improvement.

In reviewing the recent literature, it was found that four studies have examined the role of *B. hominis* as a pathogen. Miller and Minshew assessed a small number of hospitalized patients (10). Most of these patients had other diseases which could have explained the gastrointestinal tract problems or had recently received antibiotics. The authors concluded that *B. hominis* was not a pathogen.

Kain et al. retrospectively evaluated a larger number of patients and had 37 patients in whom *B. hominis* was the only pathogen and in whom no other "underlying illness" was identified (6). The authors had a control population consisting of patients with other gastrointestinal tract pathogens who were negative for *B. hominis*. In 35 of the 37 patients, there were signs and symptoms of gastrointestinal disease. They concluded that *B. hominis* had "some pathogenic role" in these patients. They also concluded that the number of organisms present in the stool was not predictive of symptomatic gastrointestinal disease. They found, however, that patients with high numbers of organisms were significantly more likely to have acute symptoms than patients with low numbers. They found that patients with *B. hominis* were significantly more likely to have traveled and to have consumed untreated water than controls. They concluded that this finding "suggests that consumption of fecal-contaminated water may be a method of transmission" of *B. hominis*.

In the study by Markell and Udkow, *B. hominis* was identified in 148 patients (8). In 32 of the patients, at least six stool specimens were examined. In 27 of these 32 patients, other pathogens were found, and in 5 only *B. hominis* was found. We are not given data on how many stool specimens were required to detect these other pathogens. We are also not given details of the data on the other 116 patients, except that they were excluded because they did not have at least six stool specimens. The symptomatic patients with *B. hominis* as their only pathogen were treated with iodoquinol,

with persistence of the symptoms and organism. The authors concluded that *B. hominis* "is not a pathogen."

In the study by Sheehan et al., *B. hominis* was found in 62 patients, with large numbers (more than five per high-power field) in 43 patients, and was the only intestinal parasite in 23 of the 43 patients (15). Sheehan et al. found an association of symptomatic patients with large numbers of *B. hominis* cells in their stools and also an association of symptomatic patients with increased eosinophil counts. They concluded that their study "suggests that *B. hominis* is a human pathogen."

In our study, we prospectively investigated a large number of patients in whom *B. hominis* was the only detectable potential pathogen. Most of the other studies in the literature, as noted above, assessed only small numbers of patients.

Our series of patients also differs from those of other studies in that virtually all of our patients were seen as outpatients in a general practitioner's office. A study of this patient population should more accurately reflect the spectrum of diseases seen in our general population than do hospital-based studies.

Our series of patients demonstrated a spectrum of clinical presentations, which we have classified into clinical-pathological groups. A similar range of clinical presentations can be seen for recognized pathogens, such as *Campylobacter* spp. and *Giardia lamblia* (5, 9). We have demonstrated an asymptomatic carrier state for *B. hominis*. Our findings are consistent with a role for *B. hominis* in acute and chronic gastroenteritis. From the results of this study, however, we cannot determine whether that role is one of association or causation, and this would require a separate evaluation. Our statisticians feel that the number of stool specimens per patient and the organisms we screened for were sufficient and that further data using this study design would not achieve different results or conclusions. We emphasize that, as for many other potential stool pathogens, the existence of a carrier state does not rule out a pathogenic role in other situations.

Patients classified as postdiarrheal carriers and as having persistent blastocytosis have to be considered incompletely evaluated.

The distribution of symptoms is similar to that seen in patients with *G. lamblia* (5), with predominantly watery diarrhea, gas, and abdominal pain present in our study in more than 42% of symptomatic patients.

This study was not set up as a randomized trial to determine the efficacy of therapy in treating *B. hominis*, and we have made no attempt to determine efficacy of therapy. In this study, treated and untreated patients were evaluated separately, and we do not feel that the medication given had a significant effect on our results.

There is discussion in the literature, as noted above, with regards to the association of the number of organisms and clinical presentation. We did not find a statistically significant association between the number of organisms present and the disease state. We also did not find a statistically significant association between the number of polymorphonuclear leukocytes and the disease state.

We found no association between the size of the *B. hominis* organisms and a particular clinical-pathological group, suggesting that size is not a determinant of pathogenicity.

In our study, we found that it was not necessary to travel to acquire the organism; however, without an appropriate

control population, we cannot conclude whether travel is a risk factor.

In assessing gastrointestinal tract pathogenicity, other possible causes for diarrhea, such as enteric viruses, pseudomembranous colitis, postinfectious disaccharidase deficiency, noninfectious etiologies, and unknown causes, must be taken into account. One must also remember that a postdiarrheal carrier state, as can be seen in salmonellosis, is a possibility. It is very difficult to prove that an organism is a pathogen in a particular patient. Comparing results from patients with *B. hominis* alone and controls without any stool pathogen, including *B. hominis*, may be useful. Immunology studies could also help elucidate these cases. Investigation of an epidemic of *B. hominis* gastroenteritis in a community would also provide very valuable epidemiological evidence for pathogenicity. We know of no such outbreak. It is interesting, in reviewing some of the papers from the early part of this century, that many of the questions posed have remained largely unanswered.

ACKNOWLEDGMENTS

We thank all the staff at Metro-McNair Clinical Laboratories who helped produce this research paper.

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