JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1993, p. 2706–2708
0095-1137/93/102706-03$02.00/0

Vol. 31, No. 10

Evidence of an Epidemic of *Blastocystis hominis* Infections in Preschool Children in Northern Jordan

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Received 26 April 1993/Returned for modification 10 June 1993/Accepted 7 July 1993

***Blastocystis hominis* is now gaining acceptance as an agent of human intestinal disease. A case-control study of the cause of gastroenteritis in children less than 6 years old was conducted. A total of 500 stool specimens were examined by wet mount preparation, formalin-ether concentration, Sheather's sugar flotation technique, and permanent stains when necessary. *B. hominis* was found in 63 (25%) of 250 stool specimens of the cases examined; 38 (15%) of these specimens contained this parasite alone. The appearance of severe symptoms was associated with increased numbers of the parasite in the diarrheic specimens (more than five parasites per field at a magnification of ×400). The most common symptoms were abdominal pain, recurrent diarrhea, cramps, anorexia, and fatigue. Contaminated water was suspected to be the major source of infection, since several cases were associated with *Giardia* infection. These findings support the concept of *B. hominis* pathogenicity in children with gastroenteritis.**

Blastocystis hominis, previously known as a nonpathogenic yeast, now is classified as a protozoan (18). The association of *B. hominis* with human disease has been controversial (4, 10, 11, 19). Studies reporting its association with diarrhea and clinical symptoms in both immunocompetent and immunocompromised patients are increasing (2, 9, 12, 14, 15). The Centers for Disease Control and Prevention also considers it to be a pathogenic protozoan.

This project was designed to study the organisms (including rotavirus, enteropathogenic bacteria, and parasites) that cause diarrhea in preschool children. Frequent identification of *B. hominis* in loose-to-watery stools from children with gastroenteritis, in the absence of other pathogens, raised the question about its direct involvement as a cause of the disease. The severity of clinical symptoms increased whenever the number of parasites was more than five trophozoites per field at a magnification of ×400 (referred to hereafter as an ×400 field).

We present the results of this study together with conclusions on the potential source of infection and probable mode of transmission.

MATERIALS AND METHODS

This project is a case-control study conducted from July 1992 through March 1993. A single diarrheic stool specimen was collected from 250 children 6 months to 6 years of age who came to the health care centers with complaints of diarrhea and other clinical symptoms indicating gastroenteritis. The health care centers included the private clinics, hospitals, maternal and child health care centers, and the United Nations Refugee World Aid clinics in Irbed, Jordan, a city which is the health care center for many other villages around northern Jordan. Diarrhea was defined as four or more soft-to-watery stool specimens per day. Control specimens matched by the donor's age and sex were collected from nurseries and day-care centers and from children who came to health centers in Irbed for reasons other than diarrhea. A total of 500 fresh stool specimens were examined immediately upon arrival and were processed for intestinal pathogens.

A questionnaire covering demographic information about each subject, such as age and sex, and information about the residency area, breast feeding, foods, the source of drinking water, the presence of animals in or around the houses, and the health status of each subject was completed and later correlated with laboratory findings.

Rotavirus. Part of each specimen was kept in an Eppendorf tube at −20°C until all specimens were collected. An enzyme-linked immunosorbent assay was used for the detection of rotavirus antigens.

Bacteriology. Standard culture and biochemical techniques were used for the isolation and identification of *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, and *Vibrio cholerae*.

Parasitology. Wet mount preparations with physiologic saline and iodine were prepared. The formalin-ether concentration technique was used for the detection of helminth eggs and protozoan cysts, and Sheather's flotation technique was used and cold acid-fast stained smears were prepared for the detection of *Cryptosporidium* spp. Trichrome-stained slides were prepared from soft-to-watery stool specimens whenever the presence of a pathogenic protozoan was suspected in one of the wet mount preparations.

RESULTS

B. hominis was found in 63 (25%) of the 250 stool specimens of the case group. Of these, 38 specimens (15%) contained *B. hominis* in the absence of other pathogens. The other 25 (10%) had other pathogenic parasites, bacteria, or rotavirus in the same specimen (Table 1).

For the control group, this parasite was detected in 10 (4%) of 250 specimens but in low numbers (fewer than three organisms per ×400 field), with no enteric symptoms. The male-to-female ratio was almost 2:1 in the case group. Most cases were in the younger age group (<2 years), with an infection rate of 52%. The infection rate decreased with age, while the infection rate in the control group was higher in the older age group. Those >2 years old had an infection rate of 70% (Table 2).

B. hominis was the second most frequent parasite found,

TABLE 1. Parasitic infections in cases and controls grouped by sex

Parasite	No. of infections in:					
	Cases			Controls		
	M	F	Total	M	F	Total
B. hominis alone	28	10	38	8	2	10
B. hominis with:						
Giardia lamblia	10	5	15	0	0	0
Enterobius vermicularis	2	3	5	0	0	0
Rotavirus	2	0	2	0	0	0
Enteric bacterial pathogen	2	1	3	0	0	0
Total	44	19	63	8	2	10

with *Giardia* infections being the most frequent. *B. hominis* was detected easily in iodine wet mounts before concentration techniques were done to reveal other parasites in the specimen. Trophozoites appeared in the same stool specimen in two sizes, small and large, with granular cytoplasm and the large central body characteristic of this parasite.

DISCUSSION

Recent interest in the role of *B. hominis* in human diseases has resulted in an increasing number of reports in the last few years (5–7, 13). The pathogenic potential of *B. hominis* has been reported in the literature since 1899 (19), and studies reporting its association with human disease have been increasing (4, 8, 13, 17). The incidence rate has ranged from 10% in Nepal (10) to 89% in Saudi Arabia (12). In the present study, the incidence rate in children <6 years old with gastroenteritis was 25%, in 15% of which *B. hominis* was detected in the absence of other pathogens. These patients had soft-to-diarrheic stools with more than five parasites per ×400 field.

Pathogenicity seems to depend on the number of parasites. However, reports of symptomatic patients with fewer than five parasites per ×400 field have also been documented, and more-severe symptoms have been noticed with an increase in number of parasites (5, 7). The stool specimens were examined for other pathogens (rotavirus, bacteria, and other parasites). As in other studies, the symptoms consisted mainly of mild diarrhea, abdominal pain, cramps, nausea, and weakness. Fever was not frequently reported. The duration of symptoms in these children was 2 to 10 days. Rotavirus infections usually cause more-severe diarrhea than that observed in these cases. Treatment of some patients with broad-spectrum antibiotics did not alleviate the diarrhea or the symptoms, indicating that the cause was not bacterial. Bacterial cultures of these specimens gave negative results, except in a very few cases in which *Salmonella* or *Shigella* spp. were isolated. These few cases were excluded from the study.

TABLE 2. Infection rate with *B. hominis* for cases and controls grouped by age

Age group (yr)	Infection rate (%) for:	
	Cases (symptomatic)	Controls (asymptomatic)
<1.9	20	0
2–3.9	14	3
4–6	4	7
Total	38	10

The observed reduction of infections with age in the case group (Table 2) was also reported for other parasitic infections, such as *Giardia* infections, which are known to be acquired early in life. Unknown factors related to age influence host susceptibility to clinical disease after infection is acquired. Infants and children under 10 years of age are more susceptible to *Giardia* infections, and acute illness from these infections is more common in children than in adults. Infected adults are more often asymptomatic (1). There is no explanation for the variability of clinical illness during this infection. However, protective immunity is suggested by the self-limiting nature of *Blastocystis* infections. *B. hominis* organisms were found in the control group (>2 years) but in small numbers (i.e., one to three parasites per ×400 field). The increased incidence with age in the asymptomatic control group might reflect previous infections, with the development of immunity when the patients became carriers.

The male-to-female ratio of the symptomatic cases was 2:1. It could not be explained on the basis of the information available from the questionnaire. The more frequent association of *B. hominis* with *Giardia* spp. than with other pathogens in the specimens examined (Table 1) might imply similar modes of transmission. Contaminated water was suspected to be the source of infection because the higher incidence rate in children was in the age group <2 years. These children were fed formula milk in addition to mother's milk. The questionnaire revealed that the water used to prepare the milk was not always boiled. The source of drinking water in the Irbed area is spring water pumped through pipes, but when water is scarce during the summer people use untreated water stored in wells or tanks. A few use mineral water. Outbreaks due to *B. hominis* have been reported in subtropical countries (2, 3, 16), and case reports in the literature support the possibility of direct involvement of this organism in diarrheic illnesses (19).

On the basis of our findings we recommend that *B. hominis* be considered a potential pathogen in symptomatic people, especially children when the number of organisms in a stool specimen exceeds five organisms per field. Awareness on the part of clinicians and laboratory technicians is essential for proper diagnosis and treatment of cases.

ACKNOWLEDGMENTS

I am grateful to all the staff members in the health centers at which the specimens were collected for their kind help and cooperation. I also thank Kasem ElKhlouf and Nedal Sa'aed for technical help.

This work was supported by research grant no. 2/92 from the Jordan University of Science and Technology.

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