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# Morphological Diversity of *Blastocystis hominis* in Sodium Acetate-Acetic Acid-Formalin-Preserved Stool Samples Stained with Iron Hematoxylin

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**The objective of this investigation was to study the morphological characteristics of *Blastocystis hominis* in sodium acetate-acetic acid-Formalin-preserved stool samples. Routinely processed samples were examined for morphological detail, including size, shape, nuclear detail, and central body characteristics. Morphological findings revealing the importance of recognizing *B. hominis* in the diagnostic laboratory are described.**

*Blastocystis hominis* is a protozoan that inhabits the human intestinal tract (10). Three forms of *B. hominis* have been described: the central body (CB) form, a granular form, and an amoeba form (11). The last two are identified by culture techniques (11) and electron microscopy (transmission electron microscopy and scanning electron microscopy) (11). As these techniques are not readily available in most laboratories, the objective of our study was to evaluate the varied morphological characteristics of the CB form of *B. hominis* in sodium acetate-acetic acid-Formalin-preserved (9) stool samples submitted for routine parasitological investigation to the Regional Parasitology Laboratory, Hamilton, Canada.

**Parasitological investigation.** Sodium acetate-acetic acid-Formalin-preserved stool samples were concentrated by the formalin-ether technique (5). A direct examination and an iron hematoxylin stain (6) were performed. One hundred ten samples with a concentration of ≥1 *B. hominis* organism per low-power field (magnification of ×125) were selected, and 25 to 50 parasites per slide were examined in consecutive oil immersion fields (magnification of ×1,250). A total of 3,200 parasites were examined. The samples were evaluated for distinct morphological detail, including size (<5, 5 to 15, or >15 μm), shape (spherical or ameboid), nuclear detail (number, position, and density), and CB characteristics (percentage of the cell volume, appearance, and staining properties). CB characteristics were determined through direct observation of the appearance of the contents (reported as homogeneous or granular) and of the staining properties. The percentage of the cell volume was determined by measuring the CB mass and comparing it with the diameter of the protozoan. These calculations were then converted to a percent ratio format. All morphological findings were quantitated and are described below.

**Microscopic descriptive analysis.** As seen in Table 1, >90% of the parasites examined were spherical, and these were usually in the 5- to 15-μm range. It is not unusual to find smaller *B. hominis* organisms, and forms measuring <5 μm were found 18% of the time. As illustrated, 66% of the *B. hominis* cells studied contained two nuclei, which were almost exclusively (98%) in a bipolar arrangement. Uninucleated forms were found in 32% of the samples and multinucleated (>2 nuclei) forms were rare (2%). Nuclear detail varied, with nuclei appearing as undefined fragmented nuclear material (73%) or elongating to appear as a solid mass (27%). Data in Table 1 demonstrate that the CB occupied >70% of the volume of the cell in 93% of the parasites examined, with 48% of the CBs occupying >90% of the total cell volume. The CB contents appeared homogeneous 68% of the time, with the remainder being granular in appearance. Of the *B. hominis* organisms examined, 53% exhibited the classical yellow-brown CB staining properties while 47% either took up little stain or turned pale blue. Tinctorial variation within the CB can be related directly to synthetic

TABLE 1. Microscopic analysis of *B. hominis*

| Parameter | Observation | No. of parasites[a] (%) |
|---|---|---|
| Size (μm) | <5 | 590 (18.4) |
| | 5–15 | 2,586 (80.8) |
| | >15 | 26 (0.8) |
| Shape | Spherical | 2,925 (91.4) |
| | Ameboid | 275 (8.6) |
| No. of nuclei | 1 | 1,016 (31.8) |
| | 2 | 2,132 (66.6) |
| | >2 | 52 (1.6) |
| Position[b] | Bipolar | 2,132 (97.6) |
| Nuclear density | Fragmented | 2,319 (72.5) |
| | Solid | 881 (27.5) |
| Relative CB vol of cell (%) | >90 | 1,541 (48.2) |
| | 70–90 | 1,448 (45.2) |
| | <70 | 211 (6.6) |
| CB content appearance | Granular | 1,014 (31.7) |
| | Homogeneous | 2,186 (68.3) |
| CB staining properties | Yellow-brown | 1,682 (52.6) |
| | Blue or absent | 1,518 (47.4) |

[a] The total number of parasites tested was 3,200.
[b] Applies only to the 2,184 organisms that have more than one nucleus.

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activity within this reproductive organelle. A darkly stained CB indicates closely packed particles and limited staining indicates decreased or no particle synthetic activity.

Currently there is a great deal of variance in the prevalence statistics for *B. hominis* reported across North America. Numbers as low as 0.2% and as high as 23% have been documented (1–3, 7). In our population we found *B. hominis* in 16.5% of the samples. One postulation is that variations in morphology and staining characteristics of *B. hominis* may be partially responsible for the wide range in reported prevalence. Laboratory staff may not be recognizing *B. hominis* in all its diverse forms. As *B. hominis* is now recognized as a protozoan (10), it is important for parasitology technical staff to successfully differentiate it from other protozoa and report it to the physician as they would other parasites of equal importance. A standard description of *B. hominis* is essential to facilitate identification of the parasite and thus promote greater accuracy in reporting. Such a description may help to clarify the organism's potential for pathogenicity by enabling further studies to be conducted (1, 2, 4, 7, 8). Only by recognizing and reporting *B. hominis* accurately will it be possible to address the controversy regarding its potential to cause disease.

In conclusion, our results demonstrate the importance of recognizing the morphological diversity of *B. hominis* for identification purposes in the parasitology laboratory.

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