Title: Blastocystis hominis and colorectal cancer

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Abstract

The protozoan parasite, *Blastocystis hominis*, has been studied by transmission electron microscopy in the stool samples of 320 patients. Various ultrastructural characteristics are noted and the incidence of this parasite infestation studied. The vacuolar form is the most common form. There is a strong correlation between the disease condition and the presence of *Blastocystis hominis* (p<0.0001). The commonest infection was seen in patients having pruritis ani (54.2%) or carcinoma of the colon/rectum confined to the bowel wall or regional lymph nodes (53%) compared with patients having other intestinal diseases (30%). The implications of this finding with respect to colorectal cancer and the aetiology of this condition are discussed.

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1.1 **Blastocystis hominis, the protozoan parasite**

*Blastocystis hominis* is a parasitic protozoan organism whose whole history has proved to be rather perplexing. The difficulties concerning this organism are reflected in the following facts:

- difficulty establishing its parasitic protozoan status
- difficulty in defining its taxonomy
- its mode of transmission is unknown
- its life cycle is uncertain
- its significance is unknown
- its association with disease states is uncertain.

The lack of knowledge with respect to *Blastocystis hominis* makes this organism ideal for study.

What is known about the organism?

*Blastocystis hominis* was initially considered to be a yeast. Although a flagellated cyst was described in the early twentieth century (Prowazek 1904; Alexeieff 1911) subsequent publications continued to call it a yeast (Brumpt 1912) and a fungus (Alexeieff 1917). In fact, for many years it continued to be described as a yeast in textbooks. The advent of electron microscopic techniques together with specific laboratory culture methods has enabled the true position of *Blastocystis hominis* as a protozoan parasite to be appreciated (see Zierdt, Rude and Bull 1967).

The taxonomic position of *Blastocystis hominis* has remained uncertain. These uncertainties are discussed in the review article of Stenzel and Boreham (Stenzel and Boreham 1996).

Although *blastocystis hominis* has been described as being a protozoan parasite causing intestinal disease (Zierdt 1983) the evidence for this has been provided mainly from case reports. The pathogenic potential of *blastocystis hominis* is uncertain. It is unknown whether “*blastocystis hominis* is a truly pathogenic organism or a commensal or perhaps is capable of being a pathogen in specific circumstances” (Stenzel and Boreham 1996).

Epidemiological studies have been hampered by the initial difficulties defining the organism, the identification of the organism and the fact that the stool samples examined have usually been those submitted to a parasitology laboratory for the purpose of excluding an infection. The samples are therefore from a preselected group of patients. This has resulted in limited epidemiological studies. Electron microscopy has enabled the organism to be identified with greater accuracy so that epidemiological studies can now be performed using the electron microscope although such studies are more time consuming. There have been numerous ultrastructural reports which have refined the morphological details of *blastocystis hominis* (Zierdt, Rude and Bull 1967). With these facts in mind it has been decided to study the incidence of *blastocystis hominis* infection as assessed by transmission electron microscopy.

1.2 **Material and Methods**

The patients involved in this study have been referred to hospital with lower gastrointestinal symptoms and seen in an outpatient clinic or as an inpatient.
full and informed consent from the patients the investigation of these patients has included a sigmoidoscopy of an unprepared rectum. During this examination faecal samples have been obtained. Ethical approval has been obtained for the study.

This study has involved 320 patients who presented to a gastroenterological outpatient clinic with a variety of lower gastro-intestinal conditions. The diagnoses of the patients studied and the numbers of patients in each group are as follows:

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemorrhoids (diagnosis 1)</td>
<td>46</td>
</tr>
<tr>
<td>Pruritis ani (diagnosis 2)</td>
<td>24</td>
</tr>
<tr>
<td>Chronic anal fissure (diagnosis 3)</td>
<td>27</td>
</tr>
<tr>
<td>Colo-rectal cancer (diagnosis 4)</td>
<td>83</td>
</tr>
<tr>
<td>Colonic adenoma (diagnosis 5)</td>
<td>35</td>
</tr>
<tr>
<td>Ulcerative colitis (diagnosis 6)</td>
<td>105</td>
</tr>
</tbody>
</table>

**Total** 320

The samples of intestinal content have been processed for transmission electron microscopy.

The transmission electron microscopic study has involved fixing the faecal samples in 3% cacodylate buffered glutaraldehyde (pH 7.3) at 4°C for four to twenty four hours. The samples are then rinsed in cacodylate buffered 10% sucrose (pH 7.3) at 4°C for twenty four hours. Following postfixing in veronal acetate buffered 1% osmium tetroxide (pH 7.3) at 4°C for two hours, the samples are rinsed in chilled tap water at 4°C. Dehydration is carried out in a graded series of ethyl alcohol and the samples are cleared in propylene oxide. The samples are embedded in epoxy resin. Sections are cut 25nm thick and mounted on copper grids prior to being stained with 1% uranyl acetate and Reynolds lead citrate. The sections are examined with a Philips 7000 electron microscope.

2.1 **Ultrastructure of blastocystis hominis**

*Blastocystis hominis* is readily identified with the transmission electron microscope. Many forms of the organism having been described (see Stenzel and Boreham 1996).

In this study of 320 patients three principle forms of *blastocystis hominis* have been observed with the transmission electron microscope. There are some variations within these three basic forms. From this *in vivo* study, there are four ultrastructural observations that will be described, namely:

1. Vacuolar form of *blastocystis hominis* (2.1.1)
2. Amoeboid form of *blastocystis hominis* (2.1.2)
3. Cyst form of *blastocystis hominis* (2.1.3)
4. Phagocytosed *blastocystis hominis* (2.1.4).
2.1.1 Vacuolar form of *blastocystis hominis*

The vacuolar form of *blastocystis hominis* is frequently spherical (figure 1) but may adopt other configurations (figure 2). The organism can be 39µm to 116µm in diameter. This form of *blastocystis hominis* is characterized by a vacuole which can have a variety of ultrastructural appearances (figures 1,3,4 and 5). The organism may be adjacent to surrounding bacteria (figure 3) or separated by a clear zone lacking bacteria or faecal debris (figure 4). The organism may be intimately surrounded by an adjacent surface coat (figure 5) which has also been termed a slime layer or capsule. Some *blastocystis hominis* organisms lack this surface coat (figure 6).

The bacteria adjacent to a *blastocystis hominis* may:

1. abut the surface coat and under such circumstances the surface coat is more electron dense at this site (figure 3 and 8)
2. pass through the surface coat to be adherent to the *blastocystis hominis* proper (figure 9)
3. be adherent to the *blastocystis hominis* proper in those organisms lacking a surface coat (figure 7 and 10).

Some *blastocystis hominis* are in close contact with yeast cells in the faecal content (figure 11 and 12). The central vacuole is frequently single. Commonly, the contents of the vacuole is homogeneous and electron dense. *Blastocystis hominis* contain a number of characteristic structures. The nucleus may be single or multiple, surrounded by a nuclear envelope with nuclear pores. The outer layer of the nuclear envelope (figure 13) is characterized by a typical rough endoplasmic reticulum which has regularly placed large electron dense granules 25nm to 30nm diameter. The nucleus has a crescentic band of electron dense material which has been presumed to be the nucleolus (Dunn, Boreham and Stenzel 1989).

The cytoplasm contains mitochondria which are usually electron dense (figure 5 and 6), rough endoplasmic reticulum, golgi complex (figure 13) and numerous cytoplasmic granules 20nm to 30nm in diameter. These granules are considered to be ribosomes.

2.1.2 Amoeboid form of *blastocystis hominis*

The amoeboid form (figure 14 and 15) has variable shapes depending upon the configuration at the time of fixation of the sample of intestinal content. It may vary in size from 5.6µm to 14.2µm. It has a nucleus similar to that of the vacuolar form of the organism. There is an absence of the surface coat seen in many of the vacuolar forms. The amoeboid form differs from the vacuolar form in a number of respects, namely:

- absence of a surface coat
- no central vacuole
- minimal presence of electron dense mitochondria
- presence of numerous cytoplasmic vacuoles containing phagocytosed bacteria in various stages of destruction
- presence of pseudopodia where there is a paucity of cytoplasmic vacuoles
– the cytoplasmic small dense granules found in the vacuolar form tend to be aggregated together to form what is considered to be the equivalent of polyribosomes
– the surrounding bacteria can be adherent to the plasma membrane of the organism.

2.1.3 Cyst form of *blastocystis hominis*

The cyst form of the organism (figure 16 and 17) is normally round or oval in shape measuring 2.7µm to 3.1µm in diameter. It has a characteristic layered cell wall (figure 18), contains two nuclei and the cytoplasmic organelles include electron dense mitochondria. The cyst form is surrounded by other components of the intestinal content including bacteria but these do not appear to be adherent to the cell wall.

Transitional forms of vacuolar and cyst forms are found. These transitional forms have remnants of a vacuolar form and a cyst form. This includes the remnants of a surface coat (figure 20). This surface coat is sometimes disrupted (figure 19) and residual material is present between the surface coat and the cyst form (figure 19).

2.1.4 Phagocytosed *blastocystis hominis*

When there are significant numbers of polymorphonuclear leucocytes and eosinophils in the intestinal content together with vacuolar forms of *blastocystis hominis* (such as in ulcerative colitis), it is possible to find polymorphonuclear leucocytes having phagocytosed *blastocystis hominis* (figure 21). The phagocytosed *blastocystis hominis* are of the vacuolar form. These phagocytosed *blastocystis hominis* may either resemble normal vacuolar forms of the organism (figure 22) or show evidence of necrosis of the organism (figure 23).

3.1 Analysis of the ultrastructural data and the patient information

Each of the faecal samples from the 320 patients have been examined with the transmission electron microscope and specific attention has been given to the finding of *blastocystis hominis* and the ultrastructural characteristics described. The data has been recorded on each patient and has been compared with the following information:

- Incidence (3.1.1)
- Time of year sample collected (3.1.2)
- Age (3.1.3)
- Disease condition (3.1.4)
- Colorectal cancer (3.1.4.1)

This data has been analysed statistically.

3.1.1 Incidence of *blastocystis hominis*

*Blastocystis hominis* has been found in 111 out of the 320 patients studied giving an overall incidence of 34.7%. Previous studies have shown that the incidence of *blastocystis hominis* infection is higher in developing countries than in developed
countries. The reported incidence in developing countries has been between 30% to 50% (Ashford and Atkinson 1992; Guimaraes and Sogayar 1993; Mercado and Arias 1991; Puga et al 1991; Torres et al 1992) whereas the incidence of the infection in developed countries has been reported as 1.5% to 10% (Doyle et al 1990; Gugliemetti et al 1993; Logar et al 1994; Mai Nguyen and Kreech 1989; Senay and MacPherson 1990; Sun et al 1989; Vickerman 1994; Yamada et al 1987; Zuckerman et al 1990). The current results contradict this view as an incidence of 34.7% would be more in keeping with a developing country.

Could the incidence of blastocystis hominis infection have increased in a so-called developed country? Is transmission electron microscopy a more accurate method of diagnosing blastocystis hominis infection?

What role does the patients’ disease condition have on the incidence of blastocystis hominis infection?

It should be noted that the results from previous studies have been based on samples sent to laboratories to exclude infection. The stool samples in these previous studies are from patients presenting with a loose stool or diarrhoea. There has been no such selection in this study but the study has involved analyzing samples from a defined population of patients referred to a gastrointestinal clinic. In addition, previous examinations involved a light microscopic study of a stool smear. The current study has involved a more detailed and ultrastructural study of fixed stool samples.

The form of blastocystis hominis found in the majority of patients in this current study is the vacuolar form (106 patients, 95.5% of patients with blastocystis hominis), with a lesser number having the amoeboid form (25 patients, 22.5% of patients with blastocystis hominis) and the cyst form (20 patients, 18.0% of patients with blastocystis hominis). A number of patients have more than one form of blastocystis hominis.

<table>
<thead>
<tr>
<th>Form</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuolar form</td>
<td>95.5%</td>
</tr>
<tr>
<td>Amoeboid form</td>
<td>22.5%</td>
</tr>
<tr>
<td>Cyst form</td>
<td>18.0%</td>
</tr>
<tr>
<td>Vacuolar and amoeboid forms</td>
<td>10.8%</td>
</tr>
<tr>
<td>Vacuolar and cyst forms</td>
<td>10.8%</td>
</tr>
<tr>
<td>Vacuolar, amoeboid and cyst forms</td>
<td>5.4%</td>
</tr>
</tbody>
</table>

There are no patients with just the amoeboid and cyst forms together but three patients have the amoeboid form alone and two patients have the cyst form alone. The different forms of blastocystis hominis are distributed amongst all the disease groups studied.

3.1.2 Time of year sample collected

Studies have been carried out on the incidence of blastocystis hominis infection found at different times of year. Some studies suggest that blastocystis hominis is more common during the hot weather (El Masry et al 1990; Knowles and Das Gupta 1924) but this has not been supported by other studies (Cegielski et al 1993; Garavelli and Scaglione 1989; Senay and MacPherson 1990). To address this question the incidence of blastocystis hominis infection in the faecal samples related to their time of collection has been evaluated. The time of collection has been divided into winter (October to March) and summer (April to September). The results are shown in Table 1.
### Table 1.
The number of patients (and percentages) who have *blastocystis hominis* by time of the collection of sample.

<table>
<thead>
<tr>
<th>Time of the year of collection</th>
<th>Blastocystis hominis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Winter</td>
<td>121 (68.36%)</td>
<td>56 (31.64%)</td>
</tr>
<tr>
<td>Summer</td>
<td>88 (61.54%)</td>
<td>55 (38.46%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>209 (65.31%)</td>
<td>111 (34.69%)</td>
</tr>
</tbody>
</table>

Pearson’s Chi-square test: $\chi^2(1) = 1.6254$, $p = 0.202$.

The p-value ($p = 0.202$) from the Chi-square test suggests that although the percentage of cases is greater in the summer months there is no statistically significant relationship between the appearance of *blastocystis hominis* in the samples and the time of year of sample collection.

#### 3.1.3 Age of patient

Fluctuations in the incidence of *blastocystis hominis* infection with age have been noted. Adults have a higher incidence than children (Ashford and Atkinson 1992; Doyle et al 1990; Guimaraes and Sogayar 1993; Hussain Qadri et al 1989; Logar et al 1994; Sanad et al 1991). Young adults have the highest rates of infection (Martin-Sanchez et al 1992; Reinthaler et al 1988). Other studies (Logar et al 1994; Zuckerman et al 1990) have failed to note any difference in incidence between adults and children.

No children or young adults below the age of 18 years have been included in this present study. The patients in the present study have been divided into decades by their age. The incidence of *blastocystis hominis* infection in each of the decades is given in Table 2.

#### Table 2.
The number of patients (and percentages) who have *blastocystis hominis* by age.

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Blastocystis hominis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>≤ 29</td>
<td>16 (76.19%)</td>
<td>5 (23.81%)</td>
</tr>
<tr>
<td>30–39</td>
<td>28 (73.68%)</td>
<td>10 (26.32%)</td>
</tr>
<tr>
<td>40–49</td>
<td>25 (65.79%)</td>
<td>13 (34.21%)</td>
</tr>
<tr>
<td>50–59</td>
<td>45 (64.29%)</td>
<td>25 (35.71%)</td>
</tr>
<tr>
<td>60–69</td>
<td>37 (58.73%)</td>
<td>26 (41.27%)</td>
</tr>
<tr>
<td>70–79</td>
<td>36 (66.67%)</td>
<td>18 (33.33%)</td>
</tr>
<tr>
<td>≥ 80</td>
<td>11 (61.11%)</td>
<td>7 (38.89%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>198 (65.56%)</td>
<td>104 (34.44%)</td>
</tr>
</tbody>
</table>

Pearson’s Chi-square test: $\chi^2(6) = 3.7019$, $p = 0.717$.

The p-value ($p = 0.717$) from the Chi-square test suggests that in this group of patients there is no relationship between the presence of *blastocystis hominis* infection in the age groups studied.

#### 3.1.4 Disease condition

The presence of *blastocystis hominis* in the stool samples has been compared with the six disease conditions suffered by patients in this study. The results are shown in Table 3.
Table 3. The number of patients (and percentages) who have *blastocystis hominis* by disease condition.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Blastocystis hominis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>1. Haemorrhoids</td>
<td>29 (63.04%)</td>
<td>17 (36.96%)</td>
</tr>
<tr>
<td>2. Pruritis Ani</td>
<td>11 (45.83%)</td>
<td>13 (54.17%)</td>
</tr>
<tr>
<td>3. Anal Fissure</td>
<td>18 (66.67%)</td>
<td>9 (33.33%)</td>
</tr>
<tr>
<td>4. Colo-rectal carcinoma</td>
<td>42 (50.60%)</td>
<td>41 (49.40%)</td>
</tr>
<tr>
<td>5. Colonic adenoma</td>
<td>23 (65.71%)</td>
<td>12 (34.29%)</td>
</tr>
<tr>
<td>6. Ulcerative colitis</td>
<td>86 (81.90%)</td>
<td>19 (18.10%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>209 (65.31%)</strong></td>
<td><strong>111 (34.69%)</strong></td>
</tr>
</tbody>
</table>

Pearson's Chi-square test: $\chi^2(5) = 24.8355$, $p < 0.001$.

The p-value ($p<0.001$) from the Chi-squared test shows that there is a strong relationship between the appearance of *blastocystis hominis* in the samples and the disease conditions. If one simply examines the crude figures it will be apparent that there is a higher incidence of *blastocystis hominis* infection in patients with pruritis ani and colo-rectal cancer and a lower incidence in patients with ulcerative colitis.

A logistic regression analysis of the data has been performed in respect of *blastocystis hominis* infection and the disease conditions with adjustment made for age, sex and the time the samples have been collected. Colo-rectal carcinoma has been used as the baseline with the underlying odds ratio of one. The results are shown in Table 4.

Table 4. Logistic regression of having *blastocystis hominis* versus disease condition adjusting for age, sex and time of the collection of sample.

| Odds Ratio | Std. Err. | z   | P>|z| | [95% Conf. Interval] |
|------------|-----------|-----|------|----------------------|
| diag 1     | 0.50442   | 0.22178 | -1.56 | 0.120 0.21307 1.19413 |
| diag 2     | 1.0853    | 0.59338 | 0.15 | 0.881 0.37172 3.16910 |
| diag 3     | 0.51297   | 0.27003 | -1.27 | 0.205 0.18281 1.43939 |
| diag 4     | 0.49988   | 0.22121 | -1.57 | 0.117 0.20998 1.19002 |
| diag 5     | 0.19571   | 0.07876 | -4.05 | 0.000 0.08893 0.43071 |

The greatest difference between the colo-rectal cancer patients and the other disease conditions is with those patients suffering from ulcerative colitis (diagnosis 6). *Blastocystis hominis* is 5.102 ($^{1}_{0.196}$) times more likely in a faecal sample with the disease condition colo-rectal carcinoma than it is in a faecal sample from a patient with ulcerative colitis (diagnosis 6) with the 95% confidence intervals of 2.32 ($^{1}_{0.431}$), 11.11 ($^{1}_{0.089}$).
If the patients with ulcerative colitis are examined in greater detail, they can be subdivided into those patients whose stool sample has been taken at the time of diagnosis (that is, those patients with ulcerative colitis who have not received any treatment) and those patients who have been receiving treatment for ulcerative colitis prior to collection of the stool sample. There are 25 patients in the group who have not received any treatment and of these patients with ulcerative colitis 10 have stool samples with blastocystis hominis present. This gives an incidence of blastocystis hominis infection of 40% in this group. Of the patients with ulcerative colitis who have received treatment 9 out of 85 patients have blastocystis hominis in their stool samples (10.6%). The untreated patients with ulcerative colitis have levels of infection with blastocystis hominis (40%) closer to that found in the disease conditions diagnosis 1, 3 and 5 (see Table 3).

Why is the level of infection with blastocystis hominis so low in those patients with ulcerative colitis who have received treatment? There are a number of possible explanations but it should be remembered that blastocystis hominis can be adversely affected by a number of drugs including metronidazole (see Stenzel and Boreham 1996) and a number of these patients with ulcerative colitis will have received such treatment.

3.1.4.1 Blastocystis hominis and colorectal cancer

With respect to the incidence of blastocystis hominis infection in the various disease conditions, it has been decided to examine in greater detail those results from patients with colonic adenoma (diagnosis 5) and colo-rectal carcinoma (diagnosis 4). Ever since the paper of Jackman and Mayo (1951) evidence has accumulated supporting an adenoma-carcinoma sequence in colo-rectal cancer. In the present study because the faecal samples are obtained at the time of (or immediately prior to) the diagnosis of colo-rectal cancer, before the patients had received any treatment, the results have not been influenced by any specific treatment.

If the incidence of blastocystis hominis infection in patients with colo-rectal carcinoma is compared with the incidence in the remaining patients, the results are as shown in Table 5.

Table 5. The number of patients (and percentages) who have blastocystis hominis by disease condition.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Blastocystis hominis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Colo-rectal carcinoma</td>
<td>42 (50.60%)</td>
<td>41 (49.40%)</td>
</tr>
<tr>
<td>Others</td>
<td>167 (70.46%)</td>
<td>70 (29.54%)</td>
</tr>
<tr>
<td>Total</td>
<td>209 (65.31%)</td>
<td>111 (34.69%)</td>
</tr>
</tbody>
</table>

Pearson’s Chi-square test: $\chi^2(1) = 10.7039, p = 0.001$.

The p-value ($p = 0.001$) from the Chi-square test shows that there is a strong relationship between the appearance of blastocystis hominis in the samples and the dichotomized disease conditions.
If a logistic regression analysis is performed on this data with colo-rectal carcinoma as the baseline having an underlying odds ratio of 1, the results obtained are as shown in Table 6.

**Table 6.** Logistic regression of having *blastocystis hominis* in patients with colo-rectal cancer versus disease other conditions.

<table>
<thead>
<tr>
<th>Logistic regression</th>
<th>Number of obs</th>
<th>LR chi2(1)</th>
<th>Prob &gt; chi2</th>
<th>Log likelihood</th>
<th>Pseudo R2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>320</td>
<td>10.40</td>
<td>0.0013</td>
<td>-201.35581</td>
<td>0.0252</td>
</tr>
</tbody>
</table>

| Odds Ratio  | Std. Err. | z     | P>|z| | [95% Conf. Interval] |
|-------------|-----------|-------|--------|----------------------|
| .4293851    | .112359   | -3.23 | 0.001  | .2571044 .7171078    |

This result would imply that it is 2.33 (1/0.43) times more likely to have *blastocystis hominis* in the samples from patients with colo-rectal carcinoma than it is in the samples from patients with other disease conditions with the 95% confidence interval of 1.39, 3.89. As has previously been stated (page 2) the association with *blastocystis hominis* and disease state is unknown but *blastocystis hominis* has not been implicated in haemorrhoids or anal fissure. It was therefore decided to divide the patients into three groups – group 1, patients with haemorrhoids or anal fissure; group 2, patients with colo-rectal carcinoma; group 3, patients with colonic adenoma.

The numbers of patients and the incidence of *blastocystis hominis* infection in these 191 patients are given in Table 7.

**Table 7.** The number of patients (and percentages) who have *blastocystis hominis* by disease conditions.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Blastocystis hominis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>1. Haemorrhoids &amp; Anal Fissure</td>
<td>47 (64.38%)</td>
<td>26 (35.62%)</td>
</tr>
<tr>
<td>2. Colo-rectal carcinoma</td>
<td>42 (50.60%)</td>
<td>41 (49.40%)</td>
</tr>
<tr>
<td>3. Colonic adenoma</td>
<td>23 (65.71%)</td>
<td>12 (34.29%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>112 (58.64%)</td>
<td>79 (41.36%)</td>
</tr>
</tbody>
</table>

Pearson’s Chi-square test: $\chi^2(2) = 3.9259$, $p = 0.140$.

The p-value ($p = 0.140$) from the Chi-square test suggests that there is no relationship between the presence of *blastocystis hominis* in the faecal samples and the diseased conditions examined. However, if a logistic regression analysis of the data is performed with group 1 (haemorrhoids and anal fissure) as the baseline with the underlying odds ratio of 1, the results obtained adjusting for age, sex and time faecal sample collected are as shown in Table 8.
Table 8. Logistic regression of having *blastocystis hominis* versus disease conditions (haemorrhoids & anal fissure, colo-rectal carcinoma and colonic adenoma) adjusting for age, sex and time of the collection of sample.

| Odds Ratio | Std. Err. | z | P>|z| | [95% Conf. Interval] |
|------------|-----------|---|------|------------------|
| diag 2     | 2.3779    | 1.00968 | 2.04 | 0.041 | 1.034584, 5.465443 |
| diag 3     | 1.0582    | 0.52999 | 0.11 | 0.910 | 0.3965151, 2.824179 |

This indicates that the odds ratio with the 95% confidence interval of having *blastocystis hominis* in faecal samples from patients with colo-rectal carcinoma relative to haemorrhoids and anal fissure is 2.38 with the 95% confidence interval (1.03, 5.47). This is significant at the 4.1% level implying that there may be a relationship but that it is not too strong.

The data from patients with colo-rectal carcinoma and colonic adenoma was further evaluated dividing the colo-rectal carcinoma patients according to the staging of their carcinoma following investigation, surgical excision of the tumour and histopathological staging of the resected tumour. For the purpose of staging the tumour the classical Cuthbert Dukes staging has been used. The incidence of *blastocystis hominis* infection in the faecal samples from these groups of patients is shown in Table 9.

Table 9. The number of patients (and percentages) who have *blastocystis hominis* by the Dukes’ staging.

<table>
<thead>
<tr>
<th>Dukes’ staging</th>
<th>No</th>
<th>Yes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukes A</td>
<td>10 (52.63%)</td>
<td>9 (47.37%)</td>
<td>19 (100.00%)</td>
</tr>
<tr>
<td>Dukes B</td>
<td>10 (50.00%)</td>
<td>10 (50.00%)</td>
<td>20 (100.00%)</td>
</tr>
<tr>
<td>Dukes C</td>
<td>11 (40.74%)</td>
<td>16 (59.26%)</td>
<td>27 (100.00%)</td>
</tr>
<tr>
<td>Disseminated</td>
<td>7 (63.64%)</td>
<td>4 (36.36%)</td>
<td>11 (100.00%)</td>
</tr>
<tr>
<td>Colonic adenoma</td>
<td>23 (65.71%)</td>
<td>12 (34.29%)</td>
<td>35 (100.00%)</td>
</tr>
<tr>
<td>Total</td>
<td>61 (54.46%)</td>
<td>51 (45.54%)</td>
<td>112 (100.00%)</td>
</tr>
</tbody>
</table>

From table 9 it can be seen that the percentage incidence of *blastocystis hominis* infection in the faecal samples of patients with carcinoma of the colo-rectum disseminated beyond the regional lymph nodes is similar to that of patients with colonic adenoma. These patients can therefore be divided into those patients with Dukes A, B and C colo-rectal carcinoma, those patients with colo-rectal carcinoma disseminated beyond the regional lymph nodes and those patients with colonic adenoma. The data on the percentage incidence of *blastocystis hominis* infection in the faecal samples from these groups is shown in Table 10.
Table 10. The number of patients (and percentages) who have *blastocystis hominis* by the Dukes’ staging.

<table>
<thead>
<tr>
<th>Dukes’ staging</th>
<th>Blastocystis hominis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>1. Dukes A, B and C</td>
<td>31 (46.97%)</td>
<td>35 (53.03%)</td>
</tr>
<tr>
<td>2. Disseminated</td>
<td>7 (63.64%)</td>
<td>4 (36.36%)</td>
</tr>
<tr>
<td>3. Colonic adenoma</td>
<td>23 (65.71%)</td>
<td>12 (34.29%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>61 (54.46%)</td>
<td>51 (45.54%)</td>
</tr>
</tbody>
</table>

Pearson’s Chi-square test: $\chi^2(1) = 3.6540, \ p = 0.161$.

The p-value ($p = 0.161$) from the Chi-square test suggests that there is no relationship between the appearance of *blastocystis hominis* in the faecal samples and the merged Dukes’ staging.

The logistic regression analysis of this data when adjusted for age, sex and the time the sample is collected and using the colonic adenoma group as the baseline with the underlying odds ratio as 1 is as shown in Table 11.

Table 11. Logistic regression of having *blastocystis hominis* versus the Dukes’ staging adjusting for age, sex and time of the collection of sample.

<table>
<thead>
<tr>
<th>Logistic regression</th>
<th>Number of obs</th>
<th>= 109</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LR chi2(9)</td>
<td>= 14.07</td>
</tr>
<tr>
<td>Prob &gt; chi2</td>
<td>= 0.1198</td>
<td></td>
</tr>
<tr>
<td>Log likelihood</td>
<td>= -67.960843</td>
<td></td>
</tr>
<tr>
<td>Pseudo R2</td>
<td>= 0.0938</td>
<td></td>
</tr>
</tbody>
</table>

| | Odds Ratio | Std. Err. | z | P>|z|   | [95% Conf. Interval] |
|-------------------------|-----------|--------|---|--------|---------------------|
| dukessgp 1 | 3.6804    | 1.9857 | 2.42 | 0.016 | 1.278338, 10.59648 |
| dissgp 2   | 2.0421    | 1.6469 | 0.89 | 0.376 | .420359, 9.92091   |

This analysis indicates that it is 3.68 times more likely to have *blastocystis hominis* in the faecal sample from patients with Dukes’ A, B and C colo-rectal carcinoma relative to colonic adenoma with the 95% confidence interval as 1.28, 10.60. This means that there is 95% certainty that the true value lies between 1.28 and 10.60 which is significant at the 1.6% level.

4.1 Is such a result relevant to colo-rectal cancer?

**Can this result be supported by any existing evidence?**

There is undoubted evidence for a genetic predisposition to the development of colo-rectal polyps and colo-rectal carcinoma. However, this genetic predisposition is not absolute and environmental factors are also involved. The concept of an adenoma – carcinoma sequence for colo-rectal carcinoma has arisen from work such as that of Jackman and Mayo (1951). In fact, it could be argued that little has changed over the last 50 years to add to the initial statement of Jackman and Mayo in their paper, namely “in our opinion polyps (adenomas) of the large intestine, if given sufficient time, develop into carcinomas. Whether all carcinomas of the colon have their origin in polyps is a
debatably point, and the mechanism by which a polyp becomes transformed into cells which assume independent growth is unknown”. This having been stated there has been an advancement of knowledge with respect to the genetic predisposition of the development of polyps and colorectal cancer. Although advances have been made in the understanding of the molecular genetics of colorectal cancer, these advances have yet to answer the questions posed by Jackman and Mayo over 50 years ago.

Colo-rectal cancer is a significant problem which in the United Kingdom and Ireland in the 1990’s accounted for approximately 1 in 8 newly diagnosed cancers and 1 in 9 deaths from cancer (Rowan and Brewster 2005). In the 1990’s there were approximately 33,900 cases of colorectal cancer newly diagnosed annually in the United Kingdom and Ireland with the annual death rate of approximately 18,600. There is a geographic variation with colorectal cancer being common in North America, Western Europe, Australia and New Zealand but there is a low incidence reported in India and Africa. The incidence of colorectal cancer is increasing in Japan and Eastern Europe. Geographic variations in the incidence of colorectal cancer and variation in the mortality from the disease is apparent within individual countries. This is highlighted in the study of patients from the United Kingdom and Ireland in the 1990’s (Rowan and Brewster 2005).

In addition to genetic factors, a number of other factors have been implicated in colorectal cancer. These other factors include a high fibre diet and a high vegetable intake which are associated with a lower risk of colorectal cancer (see Rowan and Brewster 2005). The importance of environmental factors can be best illustrated by noting the changes in the rates of colorectal cancer amongst migrant populations. The incidence of colorectal cancer in American Negroes compared with rates in Africa (Burkitt 1971) and the incidence in Japanese migrants to California (Wynder and Shigamatsu 1967) and to Hawaii (Stemmermann 1970).

Changes in diet can have a significant effect on intestinal transit time. The transit time of the intestinal content is shorter with increasing dietary fibre (see Burkitt 1971). The role of such changes in the aetiology of colorectal cancer is uncertain but changes in transit time will affect the duration of exposure of the colon and rectum to any factors in the intestinal content which may be implicated in the aetiology of colorectal cancer.

With genetic and environmental factors being important in the development of colorectal cancer, what is the relative significance of these factors? It is difficult to be certain. The best estimate has been gained from a large study of twins in Sweden, Denmark and Finland (Lichtenstein et al 2000). This study estimated that genetic factors accounted for 35% of the risk in colorectal cancers so that environmental factors would account for a significant proportion of the risk.

5.1 How can the environmental factors influence the adenoma – carcinoma sequence resulting in the development of colorectal cancer?

There are many possible explanations which can be broadly divided into three groups, namely:

1. factors directly influencing cells resulting in the development of malignancy
2. factors which inhibit those mechanisms of the body which suppress/remove any malignant potential
3. An environment conducive to the survival and propagation of organisms that may produce their effect through either (1) or (2).
If *blastocystis hominis* does have a role in colo-rectal cancer because of its increased presence as shown in the present study, it would seem to be consistent with the principles laid down in (3).

Another difficulty when evaluating any role for *blastocystis hominis* in colorectal malignancy relates to the *lag time* between the infection and the development of overt colo-rectal malignancy. With this lag time in mind it is interesting to note the length of time taken for untreated colonic adenomatous polyps to develop carcinoma. This is illustrated by the study of Stryker et al (1987). Stryker and colleagues evaluated the colon and rectum radiologically in 226 patients who had polyps larger than 10mm diameter and in whom surgery was not deemed appropriate. This study took place before the advent and widespread use of colonoscopy. During the period of the study, Stryker and colleagues found that 32 colo-rectal malignancies developed. Actuarial analysis of the data revealed that the risk of individual polyps becoming malignant are 24% at 20 years, with the cumulative risk of invasive carcinoma at any site in the large intestine being 35% at 20 years. This study indicates that not all polyps become malignant and that it may take a significant length of time before the polyp becomes malignant. Interestingly, the cumulative risk at 20 years involves approximately one third of patients developing cancer of the colon and in the present study approximately one third of patients with a benign colonic adenoma are infected with *blastocystis hominis*. Are those patients whose adenoma progress to developing into a colorectal cancer the patients with *Blastocystis hominis* infection?

6.1 Conclusion

The significance of *blastocystis hominis* in the stool has been the subject of debate. It has been suggested that it is a commensal organism (see Senay and MacPherson 1990).

**Commensal**

| Com/cum | – | with, together, together with, in company with. |
| Mensa | – | a table |
| Commensal (noun) | – | “any of a company eating at the same table” |
| Commensalism (noun) | – | “an association between two species in which one benefits and the other is neither harmed nor benefited” |


*Blastocystis hominis* has also been regarded as a pathogen. The results of the present study concerning the incidence of *blastocystis hominis* infection in various disease states suggests that *blastocystis hominis* ought to be regarded as a potential pathogen. There is an increased incidence of *blastocystis hominis* in colo-rectal cancer and it may be an environmental factor in the aetiology of colo-rectal cancer.

Acknowledgements

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Figure 1. Transmission electron micrograph.

Intestinal contents
Female, 53 years old
Colonic adenoma.

A vacuolar form of *blastocystis hominis* with vacuole (V), nucleus (N), electron dense mitochondria (M) and adjacent bacteria (B) shown.
Magnification x 28,100
Figure 2.

Transmission electron micrograph.

Intestinal contents
Female, 40 years old
Ulcerative colitis.

Two vacuolar forms of *blastocystis hominis* (BH) with surrounding faecal debris and bacteria (B). The space (S) between the *blastocystis hominis* and the surrounding faecal content is shown.
Magnification x 7,775
Figure 3.

Transmission electron micrograph.

Intestinal contents
Male, 57 years old
Ulcerative colitis.

A vacuolar form of *blastocystis hominis* (BH), with the surface coat (SC) and surrounding bacteria (B) shown.
Magnification x 26,700
Figure 4. Transmission electron micrograph.

Intestinal contents
Male, 27 years old
Ulcerative colitis.

A vacuolar form of *blastocystis hominis* (BH) is shown. The bacteria and faecal debris are separated from the *blastocystis hominis* by a space (S).

Magnification  x 10,495
Figure 5. Transmission electron micrograph.

Intestinal contents
Female, 67 years old
Diverticular disease of the colon.

A vacuolar form of *blastocystis hominis* with the vacuole (V), nucleus (N), electron dense mitochondrion (M) and surface coat (SC) shown.
Magnification x 22,005
Figure 6. Transmission electron micrograph.

Intestinal contents
Male, 49 years old
Carcinoma of the rectum.

A vacuolar form of *blastocystis hominis* with the nucleus (N) and electron dense mitochondrion (M). There is no surface coat.
Magnification x 35,250
Figure 7.

Transmission electron micrograph.

Intestinal contents  
Male, 81 years old  
Haemorrhoids.

A vacuolar form of *blastocystis hominis* with the vacuole (V) and adherent bacteria (B) shown. There is no surface coat.  
Magnification x 80,310
Figure 8. Transmission electron micrograph.

Intestinal contents
Female, 64 years old
Ulcerative colitis.

A vacuolar form of *blastocystis hominis* (BH) with the surface coat (SC) and adjacent bacteria (B) shown.
Magnification x 67,230
Figure 9. Transmission electron micrograph.

Intestinal contents
Male, 57 years old
Ulcerative colitis.

A vacuolar form of *blastocystis hominis* (BH) with the surrounding surface coat (SC) and adjacent bacteria (B) shown.
Magnification x 64,080
Figure 10. Transmission electron micrograph.

Intestinal contents
Female, 53 years old
Colonic adenoma.

A vacuolar form of *blastocystis hominis* with the vacuole (V) and adjacent adherent bacteria (B) shown.
Magnification x 22,510
Figure 11. Transmission electron micrograph.

Intestinal contents
Male, 53 years old
Normal

A vacuolar form of *blastocystis hominis* (BH) adjacent to a yeast cell (YC).
Magnification x 25,095
Figure 12. Transmission electron micrograph.

Intestinal contents
Male, 53 years old
Normal.

Site of contact of the *blastocystis hominis* surface coat (SC) and the cell wall (CW) of the yeast cell. An electron dense mitochondrion (M) is shown.
Magnification $x\, 70,550$
Figure 13. Transmission electron micrograph.

Intestinal contents
Male, 47 years old
Haemorrhoids.

A vacuolar form of *blastocystis hominis* with the vacuole (V), nucleus (N), mitochondria (M), golgi complex (GC) and surface coat (SC) shown.
Magnification x 29,295
Figure 14. Transmission electron micrograph.

Intestinal contents
Male, 57 years old
Ulcerative colitis.

The amoeboid form of *blastocystis hominis* showing the nucleus (N) and numerous cytoplasmic vacuoles (CV) containing phagocytosed bacteria. Numerous bacteria (B) are found in the intestinal content surrounding the amoeboid *blastocystis hominis*. Magnification x 16,965
Figure 15. Transmission electron micrograph.

Intestinal contents
Male, 57 years old
Ulcerative colitis

An amoeboid form of *blastocystis hominis* (BH) with the nucleus (N) and cytoplasmic vacuoles (CV) containing phagocytosed bacteria shown.
Magnification x 9,530
Figure 16.

Transmission electron micrograph.

Intestinal contents
Male, 57 years old
Ulcerative colitis.

Two cyst forms of *blastocystis hominis* (BH) with the remnants of the vacuolar form of *blastocystis hominis* and the surface coat.

Magnification x 12,965
Figure 17.

Transmission electron micrograph.

Intestinal contents
Male, 66 years old
Haemorrhoids.

Cyst form of blastocystis hominis with the nuclei (N) and layered cyst wall (CW) shown.
Magnification x 45,755
Figure 18. Transmission electron micrograph.

Intestinal contents
Male, 66 years old
Haemorrhoids.

Detailed of the cell wall (CW) of the cyst form of *blastocystis hominis.*
Magnification x 114,385
Figure 19. Transmission electron micrograph.

Intestinal contents
Female, 61 years old
Haemorrhoids and diarrhoea of unknown cause.

A cyst form of *blastocystis hominis* (BH) with the disrupting remnants (R) of the vacuolar *blastocystis hominis* and the surface coat (SC).
Magnification  x 20,660
Figure 20. Transmission electron micrograph.

Intestinal contents  
Female, 36 years old  
Pruritis ani.

A cyst form of *blastocystis hominis* (BH) with the surrounding remnants (R) of the vacuolar *blastocystis hominis* and the surface coat (SC).  
Magnification  x 22,200
Transmission electron micrograph.

Intestinal contents
Female, 73 years old
Ulcerative colitis.

A phagocytosed *blastocystis hominis* (BH) and polymorphonuclear leucocytes (PNL). Magnification x 10,475
Figure 22.

Transmission electron micrograph.

Intestinal contents
Female, 65 years old
Ulcerative colitis.

A phagocytosed vacuolar form of *blastocystis hominis* (BH) together with phagocytosed bacteria (B).
Magnification x 12,530
Figure 23. Transmission electron micrograph.

Intestinal contents
Male, 71 years old
Ulcerative colitis.

A necrosing phagocyted *blastocystis hominis* (BH). The phagocyting polymorphonuclear leucocytes (PNL) are shown.

Magnification x 11,620.