

## Value of microscopy in the diagnosis of dysentery associated with invasive *Entamoeba histolytica*

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### Abstract

**Aims**—To assess the reliability of the detection of erythrophagocytic amoebic trophozoites in stool samples in the diagnosis of dysentery associated with invasive *Entamoeba histolytica*.

**Methods**—Amoebic culture was carried out on single stool samples collected from patients from Mexico, Colombia, and Bangladesh. The stools had been examined by light microscopy. Amoebic dysentery was diagnosed when erythrophagocytic *E histolytica* trophozoites were observed in a case of bloody diarrhoea. *E histolytica* isolates were characterised by isoenzyme electrophoresis and results correlated with microscopical findings in stools. Statistical analysis was performed using the  $\chi^2$  test.

**Results**—Where erythrophagocytic amoebae had been observed in dysenteric stool specimens the *E histolytica* phenotype was invariably invasive ( $p < 0.0001$ ). Observation of erythrophagocytic amoebae in dysentery is 100% specific and predictive of infection with invasive *E histolytica*. When amoebic culture-positive cases only are considered it is 96% sensitive. In this study *E histolytica* of zymodeme XIV was more commonly associated with amoebic dysentery than zymodeme II. There was no significant difference between the carriage rate of invasive and non-invasive *E histolytica* in non-dysenteric diarrhoea. Asymptomatic subjects carried non-invasive *E histolytica* more frequently than invasive *E histolytica*. Patients with non-amoebic dysentery, when shown to be infected with *E histolytica*, carried non-invasive strains (12%).

**Conclusions**—Sensitivity and specificity of microscopical examination of a single stool specimen for diagnosing amoebic dysentery is very high; intestinal carriage of invasive *E histolytica* detected by culture is not necessarily an indication of active disease as patients with diarrhoea and asymptomatic subjects shed invasive and non-invasive *E histolytica*. There are possibly two subpopulations of invasive *E histolytica* with different pathogenic potential which can be differentiated by zymodeme analysis.

When Löscher discovered and described amoebae in dysenteric stools, he did not consider them to be the primary agent of amoebic dysentery, even though his detailed microscopical description noted that trophozoites ingested particulate material which included red blood cells.<sup>1</sup> Later, in their exhaustive pathological description of different forms of invasive amoebiasis, Councilman and Lafleur also commented on the presence of red blood cells inside trophozoites in a case of amoebic dysentery, although they did not consider the finding a diagnostic hallmark of the disease.<sup>2</sup> Quincke and Roos used the erythrophagocytic ability of *Entamoeba histolytica* to distinguish it from *Entamoeba coli* and observed the disappearance of erythrophagocytic trophozoites from dysenteric stool specimens after quinine treatment.<sup>3</sup> Walker and Sellards saw *E histolytica* trophozoites with phagocytosed red blood cells in their cases of experimental human amoebiasis<sup>4</sup> and were the first to recommend direct microscopical examination of smears from freshly passed stools, avoiding disintegration of the trophozoites, and identification of the parasite by its size, nuclear characteristics, and the presence of ingested red blood cells for the diagnosis of amoebic dysentery. Clinical experience gained with British recruits overseas supported these recommendations,<sup>5</sup> and with time the microscopical finding of red blood cells ingested by *E histolytica* trophozoites became firmly established as a diagnostic criterion for amoebic dysentery.<sup>6</sup> This criterion is still used<sup>7,8</sup> although we know of no systematic re-evaluation of its validity. Most studies on the sensitivity of microscopical examination of stools for diagnosis of amoebiasis concern detection of *E histolytica* cysts<sup>9-11</sup> with concentration methods that are inappropriate for observing the trophozoites. Kershaw found that around half of 300 amoebic infections diagnosed by multiple stool examination presented with overt dysentery but did not report the diagnostic sensitivity of searching for trophozoites containing red blood cells.<sup>12</sup> Pathogenicity of *E histolytica* correlates with rate of erythrophagocytosis in vitro,<sup>13</sup> yet reports of the ability of non-pathogenic amoebae, such as *E coli* and *E moshkovski* to ingest red blood cells both in vitro<sup>13,14</sup> and in vivo,<sup>15</sup> have challenged the specificity of this criterion for diagnosing amoebic dysentery. Similarly, the existence of two genotypically different but morphologically indistinguishable parasites within the

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species *E histolytica*,<sup>16-18</sup> one of which is invasive and the other of which behaves as a commensal,<sup>19, 20</sup> challenges the ability of the microscopist to diagnose amoebic dysentery by finding ingested red blood cells. Failure to search adequately could give a false negative result, and occurrence of non-pathogenic *Entamoeba* species or non-invasive strains of *E histolytica* with ingested red blood cells could yield a false positive diagnosis of amoebic dysentery. Haque *et al* found that ingested red blood cells were associated with invasive *E histolytica*, but the number of non-invasive isolates in that study was too small to be conclusive.<sup>21</sup> Here we report on the efficacy of microscopy for the detection of invasive *E histolytica* in dysentery.

### Methods

The patients selected for this study produced stools which gave positive cultures for *E histolytica*. Single stool samples were collected at the following laboratories: International Centre for Diarrhoeal Diseases, Bangladesh; Departamento de Infectología, Instituto Nacional de la Nutrición "Salvador Zubirán", Mexico City; and Laboratorio de Microbiología y Parasitología, Departamento de Ciencias Biológicas, Universidad de los Andes, Santa Fé de Bogotá, Colombia.

Stool samples were examined macroscopically for the presence of blood and mucus, and for consistency. A smear of faeces in 0.9% saline was examined microscopically at low and high magnifications for the presence

of *E histolytica* trophozoites, and free and ingested red blood cells. Faeces were inoculated into Robinson's medium within 6 hours of collection and *E histolytica* positive cultures were subcultured every 48 hours.<sup>22</sup>

*E histolytica* isolates were identified by zymodeme analysis, considered to be the gold standard for this purpose.<sup>23, 24</sup> This was performed in the country where the isolates were made, as described before.<sup>21, 25</sup> Results of zymodeme analysis were compared retrospectively with microscopical and macroscopical examination of stool specimens without reference to other clinical and serological information.<sup>26-29</sup>

To assess the association between stool criteria and zymodeme analysis the presence of diarrhoeal disease was defined on the basis of stool examination by microscopy (table 1). Amoebic dysentery was defined as a case of bloody diarrhoea associated with erythrophagocytic trophozoites identified at microscopy; dysentery of other aetiology was a case of bloody diarrhoea without erythrophagocytic trophozoites; diarrhoea and asymptomatic carriers were defined as cases of amoebic infection associated with non-dysenteric, loose, or formed stools, respectively.

The correlation between results of stool examination and zymodeme characterisation of *E histolytica* isolates was analysed by using the  $\chi^2$  test and the Nanostat statistics program developed at the London School of Hygiene and Tropical Medicine. Significance was defined at the 5% level.

### Results

Table 2 shows that erythrophagocytic trophozoites were seen in 53% of the stools from which invasive zymodemes of *E histolytica* were isolated. These stools represent all the cases of amoebic dysentery in this study. Erythrophagocytic trophozoites were seen in 27 out of 28 cases associated with invasive *E histolytica*. The remaining case was classified as dysentery of unknown aetiology (table 3). Trophozoites with ingested red blood cells were not seen in any stools from which non-invasive *E histolytica* was isolated. These results were highly significant ( $p < 0.0001$ ). The sensitivity and specificity of the detection of erythrophagocytic trophozoites as diagnostic of dysentery associated with invasive *E histolytica* in this study were therefore 96% and 100%, respectively (table 4). Interestingly, the difference between numbers of invasive and non-invasive isolates from the stools that had *E histolytica* trophozoites without ingested red blood cells was not significant ( $p = 0.8$ ); the failure to see trophozoites at all occurred more frequently in stools from which non-invasive amoebae were isolated ( $p < 0.001$ ).

Zymodeme analysis discriminated between two groups of invasive *E histolytica*: those characterised as zymodeme II, which was associated with several diagnostic categories; and those identified as zymodeme XIV, which was almost invariably associated with cases of amoebic dysentery (table 3).

Table 1 Definitions used for diarrhoeal disease

Diagnosis	Microscopical examination of stools
Amoebic dysentery	Free red blood cells, erythrophagocytic trophozoites
Dysentery of unknown aetiology	Free red blood cells, non-erythrophagocytic trophozoites could also be present
Diarrhoea	No red blood cells observed, non-erythrophagocytic trophozoites could also be present
Asymptomatic carrier	No abnormalities found, but cysts or non-erythrophagocytic trophozoites may be present

Table 2 Correlation between *E histolytica* phenotype analysed (No/%) by zymodeme and microscopy of stool samples

Microscopical findings	<i>E histolytica</i> phenotype		
	Invasive	Non-invasive	Total
Trophozoites with ingested red blood cells	27 (53)	0 (0)	27 (25)
Trophozoites without ingested red blood cells	18 (35)	24 (41)	42 (38)
No trophozoites seen	6 (12)	34 (59)	40 (37)
Total	51 (100)	58 (100)	109 (100)

Table 3 Correlation between *E histolytica* zymodemes (No/%) and diagnosis of diarrhoeal disease

Diagnosis	<i>E histolytica</i> zymodeme*			
	I	II	XIV	Total
Amoebic dysentery	0 (0)	14 (39)	13 (86)	27 (25)
Dysentery of other aetiology	7 (12)	0 (0)	1 (7)	8 (7)
Diarrhoea	33 (57)	20 (55)	1 (7)	54 (50)
Asymptomatic carrier	18 (31)	2 (6)	0 (0)	20 (18)
Total	58 (100)	36 (100)	15 (100)	109 (100)

\*Zymodeme I, non-invasive; zymodemes II and XIV, invasive.

Table 4 Efficiency of microscopy in diagnosis of amoebic dysentery\*

Erythrophagocytic trophozoites	Isolation of invasive <i>E histolytica</i>			Sensitivity	Specificity
	Positive	Negative	Total		
Observed	27	0	27	96%	100%
Not observed	1	7	8		
Total	28	7	35		

\*Amoebic culture positive cases only.

The difference between the number of cases classified as "diarrhoea" harbouring invasive *E histolytica* (zymodemes II and XIV) and those with non-invasive *E histolytica* (zymodeme I) was not significant ( $p = 0.4$ ). Non-invasive *E histolytica* (zymodeme I) isolates were more common than invasive *E histolytica* isolates (zymodemes II and XIV), in cases classified as "dysentery of unknown aetiology," but this was also not significant ( $p = 0.9$ ). We found two asymptomatic carriers of invasive *E histolytica* (table 3). The more frequent asymptomatic carriage of non-invasive *E histolytica* (zymodeme I), compared with asymptomatic carriers, among invasive *E histolytica* (zymodeme II, or zymodemes II and XIV) was significant ( $p = 0.03$  and  $0.005$ ) but not when compared with zymodeme XIV alone ( $p = 0.08$ ), probably because of the small number of isolates in the latter category.

### Discussion

A diagnostic criterion of amoebic dysentery—namely, erythrophagocytic trophozoites—was first described, although not recognised as such, in 1875 when *E histolytica* was discovered by Lösch.<sup>1</sup> As far as we are aware the accuracy of this microscopical finding has not been checked because most studies concern carriers of cysts. Although this is not a prospective study of dysentery cases, because the samples studied were initially selected by culture, the high diagnostic sensitivity of this feature (96%) is encouraging considering the erratic parasite excretion, the fragility of trophozoites, the relatively short time spent on microscopic examination of each specimen and the microscopical expertise required.<sup>30</sup> Furthermore, no fixed and stained preparations were examined to increase detection of erythrophagocytic *E histolytica*.<sup>31,32</sup> The conditions that applied in this study reflect those of a busy routine diagnostic laboratory in an area endemic for amoebic infection where the chances of examining more than one stool specimen are rare, especially in dysentery cases in which the amount of clinical material generally is small and a therapeutic decision has to be made as soon as possible.

The absence of erythrophagocytic trophozoites in stools of carriers of non-invasive *E histolytica* gave a specificity of 100% for this microscopical criterion. Therefore, there is no evidence here of erythrophagocytic non-invasive *E histolytica* or confusion with amoeba-like cells such as macrophages.<sup>33</sup> This high specificity does not support worries about non-specificity of the erythrophagocytosis cri-

terion.<sup>13-15</sup> Although the question of direct correlation between the rate of erythrophagocytosis activity and pathogenicity of *E histolytica* is still open to debate,<sup>13,34</sup> our findings support the microscopical observation of erythrophagocytic *E histolytica* trophozoites as the most reliable early diagnostic indication of amoebic dysentery in the clinical laboratory.

We found that both non-invasive and invasive *E histolytica* can be carried asymptotically<sup>35</sup> and also that trophozoites without red blood cells can be observed in cases of diarrhoea where patients are shedding either invasive or non-invasive *E histolytica*. The presence of *E histolytica* trophozoites without ingested red blood cells is not a diagnostic indication of active invasive amoebiasis and may reflect the excretion of trophozoites in a patient with diarrhoea of another aetiology. This is presumably so even when the zymodeme isolated is invasive. Failure to observe trophozoites was more common in cases excreting non-invasive *E histolytica*. This could be the result of a smaller number of parasites being shed compared to the invasive strains, but studies are needed to clarify this.

Zymodeme analysis has provided the gold standard for the development of other phenotypic and genetic methods to distinguish invasive and non invasive *E histolytica*.<sup>16-18</sup> As far as we are aware, only zymodeme analysis has discriminated between subpopulations of invasive *E histolytica*,<sup>18</sup> and interestingly, one of them, zymodeme XIV, was almost invariably associated with amoebic dysentery. This raises the question as to whether there are more than two genotypically different parasites within the *E histolytica* species, one non-invasive and at least two with different degrees of invasiveness.

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