MAJOR ARTICLE

A Possible Cluster of Sexually Transmitted *Entamoeba histolytica:* Genetic Analysis of a Highly Virulent Strain

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Background. Transmission of *Entamoeba histolytica* generally occurs by fecal excretion of cysts followed by oral ingestion of contaminated food or water. However, fecal-oral transmission may occur within households and long-term care institutions, and sexual transmission occurs among men who have sex with men. Epidemiologically linked clusters of *E. histolytica* infection are rare in industrialized countries. We report such a sexually linked cluster in Canada.

Methods. An index case involving a young female with an amebic liver abscess led to an epidemiological investigation of sexual contacts. Anti-amebic serological analysis, stool specimen examinations, and abdominal ultrasounds were done for the contacts. Enzyme-linked immunosorbent assay was done for stool antigen specific to *E. histolytica.* Genotyping and phylogenetic analysis was performed on 1 stool isolate.

Results. By tracing sexual contacts related to the index case, we uncovered a cluster of 7 cases of amebiasis (3 with liver abscesses). Oral-anal sex was common in the group; the 5 female individuals were bisexual (4) or homosexual (1). The outbreak strain was genotyped, and cluster analysis indicated that this virulent strain differed substantially from asymptomatic or diarrheal *E. histolytica* isolates.

Conclusions. *E. histolytica* can be transmitted by heterosexual activity as well as male and female homosexual activity. Patients with amebiasis should be counselled about possible sexual transmission.

Entamoeba histolytica and the avirulent morphologically identical variant *Entamoeba dispar* are widespread throughout developing countries, with prevalence rates of 20%–30% [1]. Transmission generally occurs by fecal excretion of cysts followed by oral ingestion of contaminated food or water. Similarly, fecal-oral transmission may occur within households [2], in institutions housing developmentally delayed persons [3], and as part of male homosexual activity [4, 5]. The prevalence of *E. histolytica* and *E. dispar* infections among men who have sex with men (MSM) is enhanced by having multiple partners and by immune deficiency in those who are infected with human immunodeficiency virus (HIV). Aside from the above situations, *E. histolytica* is uncommonly transmitted in industrialized countries, and outbreaks are very rare in such places. Invasive disease is associated with production of anti-amebic antibodies [6]. We report a cluster of a highly virulent form of *E. histolytica* that was transmitted within Canada; the transmission was driven by heterosexual and female homosexual activity.

METHODS

All stool specimens were processed for microscopy using the formalin-ether concentration procedure. Six patients each had 3 stool specimens examined for ova and parasites (Table 1). In brief, for each specimen, the stool was thoroughly mixed and was suspended in the sodium acetate acetic acid formalin fixative with wooden applicator sticks, and ~5 mL of suspension was filtered through gauze into a 15-mL centrifuge tube. The suspensions were sedimented at 1500–1600 *g* for 3 min and were washed in saline 3 times. The pellets were

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Table 1. Epidemiological Information of Patients in This Cluster of Sexually Transmitted Amebiasis

Patient	Age, years; sex	Disease	Date of diagnosis	Microscopic analysis of stool	Antigen detection ELISA	Serological titer	Travel history (date)	Sexual contacts	Sexual preference
A	40; F	Liver abscess	Feb 2008	ND	ND	3200	UK, Germany, Italy (Aug–Sep 2007)	C, G, E	Bisexual
B	28; F	Asymptomatic; RLQ abdomen lymphadenopathy	Apr 2008	Entamoeba	ND	ND	UK, Germany, Italy (Aug–Sep 2007)	C, D, G	Bisexual
C	28; M	Liver abscess	May 2008	ND	ND	12,800	UK, Germany, Italy (Aug–Sep 2007)	A, B, D	Heterosexual
D	29; F	Asymptomatic	Jun 2008	Entamoeba	ND	800	None	C, B, G	Bisexual
E	59; M	Liver abscess	Jun 2008	Entamoeba	ND	200	Iraq, Africa (May 2008)	A	Heterosexual
F	32; F	Asymptomatic	Oct 2008	Entamoeba	ND	400	None	G	Homosexual
G	30; F	Asymptomatic	Sep 2008	Entamoeba	Yes	1600	Italy (Dec 2007)	A, B, D, F, H	Bisexual
H	30; M	Asymptomatic	Sep 2008	Negative[a]	ND	ND	None	G	Heterosexual

NOTE. ELISA, enzyme-linked immunosorbent asssay; ND, not done; RLQ, right lower quadrant; UK, United Kingdom.

[a] Three specimens tested negative.

each resuspended in 10 mL of 10% buffered formalin, and then 3 mL of diethyl ether was added, after which the capped specimens were mechanically shaken for 1 min. Each sample was pelleted at 1500–1600 *g* for 3 min. The solvent layer and supernatant were then decanted. A wet preparation from each specimen concentrate was scanned for 10–15 min under a compound microscope using 10× and 40× objectives.

Detection of* E. histolytica sensu stricto *by stool antigen enzyme-linked immunosorbent assay. Unpreserved stool samples were independently processed by commercial enzyme-linked immunosorbent assay (ELISA) for stool antigen specific to *E. histolytica* (*E. histolytica II* test; Techlab) as described elsewhere [7].

Detection of immunoglobulins M and G directed against* E. histolytica *in human serum samples. Serum samples from patients were collected and evaluated at the reference laboratory for the Province of Ontario by using a validated in-house ELISA method that detects immunoglobulin M (IgM) and IgG antibodies against *E. histolytica*. In brief, *E. histolytica* lysate is coated on the surface of a standard ELISA plate. Antisera are then added to the coated well and are detected using a secondary anti-human antibody coupled to alkaline phosphatase. The cutoff was an optical density (OD) of 0.100. The OD was calculated as follows: OD value = actual value − (blank + negative control serum mean).

***Genotyping of* E. histolytica.** DNA from the stool specimen was extracted using QIAamp DNA Stool Mini Kit (Qiagen). A nested polymerase chain reaction (PCR) was performed first with transfer RNA (tRNA)–specific primers and then with the *E. histolytica*–specific primer pairs targeting the tRNA-linked polymorphic short tandem repeats (STR) loci, with use of AccuPrime Pfx Supermix (Invitrogen) and under the conditions described elsewhere [8]. The PCR product of each tRNA STR loci was sequenced on an ABI 3130XL Genetic Analyzer (Applied Biosystems) in both forward and reverse directions with respective *E. histolytica*–specific PCR primers in our laboratory. The tRNA STR typing for the 3 tRNA loci RR, SD, and NK was done in accordance with the schema of Tawari et al [9]. In addition, tRNA STR typing for the other 3 tRNA loci, AL, DA, and SQ, was done in accordance with the unpublished schema generously provided by Dr. Graham Clark (C. G. Clark, personal communication).

Phylogenetic analysis of sequence types. The parsimony analysis was performed using Phylip, version 3.68 [11]. An alphanumeric designation system was designed to encode the data from the tRNA-linked polymorphic STR loci blocks, because it is more faithful to the tRNA STR genotyping schema. In brief, a unique alphanumeric code was designated to each block in the tRNA STR loci; a tabular representation of the coding system is available in the Appendix, which appears only in the online version of the journal. Because the alphanumeric data usually is not well supported by the bootstrap analysis, a DNA parsimony analysis was also performed on the Clustal-aligned raw nucleotide-sequence data from the tRNA-linked polymorphic STR loci. The DNA parsimony analysis was performed on raw sequence data under the assumption that the tRNA STR blocks are homologous. The Pars parsimony was performed to generate a hypothetical relationship among the different *E. histolytica* isolates. A bootstrap analysis was performed with 100 sampling replicates on the alphanumeric data and also on raw nucleotide-sequence data to determine the robustness of the fit of the data to the generated tree.

RESULTS

Index case. A 39-year-old, previously healthy, bisexual woman (hereafter, referred to as patient A) traveled with a female companion (patient B) and a male companion (patient C) to England, Germany, and, finally, southern Italy in August 2007; they returned to Canada on 11 September 2007 (Table 1). They were well during the trip except for continuing symptoms of gastroenteritis experienced by patients B and C. On 22 February 2008, patient A developed anorexia, right upper quadrant pain, chills, and fever (temperature, 39.5°C). After 5 days of illness, she was admitted to the hospital. Transaminase and alkaline phosphatase levels were elevated; blood culture results were negative. A computed tomography scan noted an $8 \times 8 \times 8$-cm liver abscess in the right lobe (segment 7). A percutaneous liver aspirate specimen was noted to resemble anchovy paste; no bacteria were seen on Gram stain, and the specimen was sterile, but she had been receiving ceftriaxone and metronidazole. She was presumed to have a pyogenic liver abscess; a drainage catheter was left in place for 5 days, and she was discharged to home and given amoxicillin–clavulanic acid for 3 weeks. She improved quickly during the first week, and the abscess diminished in size during the next 8 weeks. Stool examination was not done, but *E. histolytica* serological analysis showed the titer was 1:3200. She was treated again with metronidazole, followed by iodoquinol. Right lower quadrant discomfort persisted for 5 months, cecal pole thickening was noted on repeated abdominal imaging, and cecal and ileo-cecal valve ulcers were visualized by colonoscopy, but no organisms were seen by histopathological analysis.

Additional cases. In late May 2008, the 28-year-old male companion (patient C) presented to the same hospital with right shoulder tip pain, severe right upper quadrant tenderness, and fever (temperature, >39°C). Abnormal results of liver tests and a computed tomography scan confirmed the presence of 2 liver abscesses in the right lobe (segments 6 and 7). Because of the travel history and the presence of amebiasis in his contact, he was treated only with metronidazole (followed by iodoquinol), and he improved during the next 10 days, with an accompanying decrease in the size of the liver abscess. Blood

culture results were negative, and serological analysis confirmed *E. histolytica* infection. The third travel companion (patient B) and the partner of patient C (patient D), who had no history of foreign travel, also had *E. histolytica* in their stool specimens and had elevated serological titers. The abdominal ultrasound for patient B showed right lower quadrant lymphadenopathy. Patients B and D received treatment with metronidazole and iodoquinol.

Patient A indicated that a 58-year-old male sexual partner (patient E; Fig. 1) had been admitted to another hospital on 21 June 2008; he had a 5.8 × 5.5 × 5.6-cm liver abscess in segment 7. This was confirmed to be an amebic liver abscess on the basis of stool examination, serological analysis (titer, 1:200), and the absence of bacterial infection in the blood and abscess material. Patient E had traveled to Iraq and North Africa in April and May. He also had severe cecal thickening and ulceration.

Because of concern about transmission, patients A, B, and C alerted their sexual partners (Table 1 and Fig. 1). Three more asymptomatic amebic infections accompanied by high serological titers were discovered among 4 persons who were investigated; results of abdominal ultrasounds and liver tests were normal. Three individuals (patients D, F, and G) were female homosexual and bisexual individuals who were sexually linked to patients A, B, and C. They had engaged in oral-anal sexual activity. Patients D and F had not traveled outside Canada and the United States in the previous year. All patients were treated with metronidazole and iodoquinol. Stool specimens were then documented to be free of amebas.

Genotyping of outbreak strain. Table 2 summarizes the genotyping profile of the outbreak strain (in specimens from patient G), compared with those of nonoutbreak asymptomatic and diarrheal isolates collected during the same period. The parsimony analysis generated trees of similar topology with both alphanumeric data (Fig. 2) and raw nucleotide-sequence data (Fig. 3). The result of bootstrap analysis with 100 sampling replicates using the alphanumeric data and raw nucleotide-sequence data showed lower bootstrap values for the nodes for the alphanumeric data and higher bootstrap values for the nodes for the raw nucleotide-sequence data. This indicates that the tree generated from the alphanumeric data is not as strongly supported by the data set, compared with the tree generated from the raw nucleotide-sequence data. Nevertheless, the alphanumeric analysis fits well with the present tRNA STR

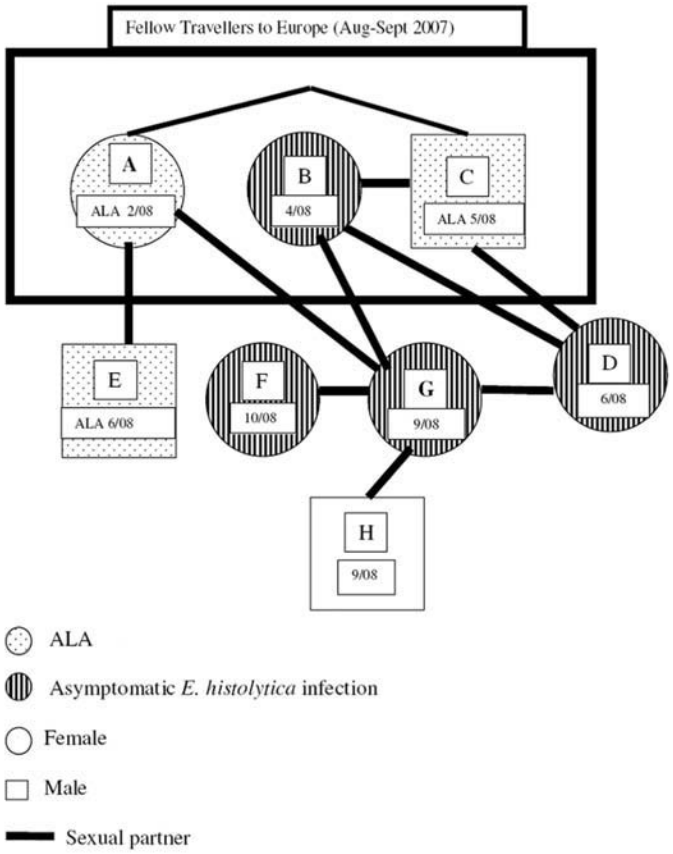

Figure 1. Relationship between patients A–H in the cluster of *Entamoeba histolytica* infections. The numbers in the unshaded boxes indicate the month and year of diagnosis for each individual. ALA, amebic liver abscess.

Table 2. Results of *Entamoeba histolytica* Genotyping

Patient symptoms	Isolate	Transfer RNA short tandem repeats					
		AL	DA	NK	RR	SD	SQ
Asymptomatic	1	Novel A	5 DA	10 NK	5 RR	7 SD	6 SQ
Asymptomatic	2	Novel A	5 DA	10 NK	5 RR	7 SD	6 SQ
Diarrhea	3	4 AL	6 DA	11 NK	Novel A	7 SD	4 SQ
Diarrhea	4	4 AL	6 DA	Novel A	10 RR	12 SD	6 SQ
Diarrhea	5	Novel B	Novel A	Novel B	6 RR	9 SD	Novel A
Diarrhea	6	4 AL	6 DA	10 NK	3 RR	12 SD	4 SQ
Various[a]	7	4 AL	10 DA	3 NK	6 RR	15 SD	2 SQ
Colitis[b]	ATCC HM1: IMSS	4 AL	6 DA	18 NK	1 RR	15 SD	4 SQ

NOTE. Genotyping schema based on the method of Tawari et al [9] with modification by Graham Clark (C. G. Clark, personal communication). ATCC, American Type Culture Collection.

[a] Refer to the text of the present article for the constellation of symptoms this strain caused in different individuals.

[b] Reference strain was originally obtained from a patient in 1967 with colitis (from a colonic ulcer biopsy specimen) in Mexico [10].

schema described by Tawari et al [9] and Graham Clark (C. G. Clark, personal communication), in which genotyping is based on the STR repeats in each block of the respective tRNA loci sequence.

DISCUSSION

In the current cluster, amebiasis was presumably acquired in Europe (likely southern Italy); patient A is the index case who presented with cecitis and a liver abscess 5 months after her return to Canada (Fig. 1). Patient C may have acquired amebiasis from sexual partner A or B within Canada, because he presented with a liver abscess 8 months after returning from their European trip. Amebic liver abscesses usually occur within 3 months after travel and rarely, if ever, after 6 months [6]. Because patient A had a late presentation, she also may have acquired the infection within Canada from one of her fellow travelers and sexual partners. Similarly, patient E may have acquired the disease as a result of his recent travels to Africa and Iraq, but it seems much more likely that it was sexually acquired from patient A, because she had an amebic liver abscess first, they were sexually linked, and it would be unlikely for 2 sexual partners to develop liver abscesses from recent travels to 2 different geographic locations. The other 4 asymptomatic infections were only discovered by tracing the named sexual partners of the initial case patients. All these contacts were proven to have *E. histolytica* infection on the basis of the

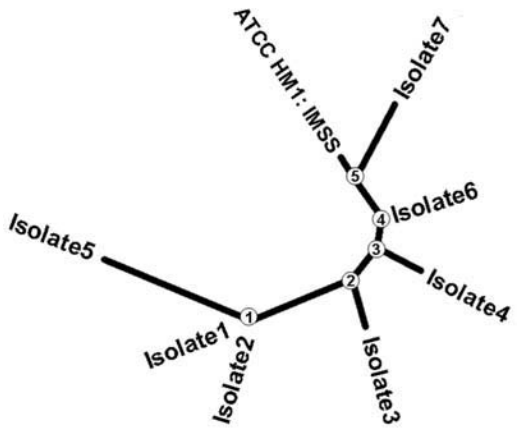

Figure 2. Unrooted parsimony tree ("Pars" parsimony algorithm) based on alphanumeric values of the transfer RNA–linked polymorphic short tandem repeats loci. The circles on the nodes indicate the level of bootstrap support based on 100 replicates, with the following node numbers: node 1, 45.5%; node 2, 71.4%; node 3, 43.5%; node 4, 35.9%; and node 5, 28.3%. Branch lengths are proportional to the averages of the number of reconstructed changes of state over all sites over all possible most parsimonious placements of the changes of state among the branches. That is, longer branches indicate a greater number of changes from the taxon to the hypothesized ancestral node. The 7 *Entamoeba histolytica* stool isolates from our investigation came from different patients; the American Type Culture Collection (ATCC) *E. histolytica* HM1: IMSS reference strain was also included for comparison.

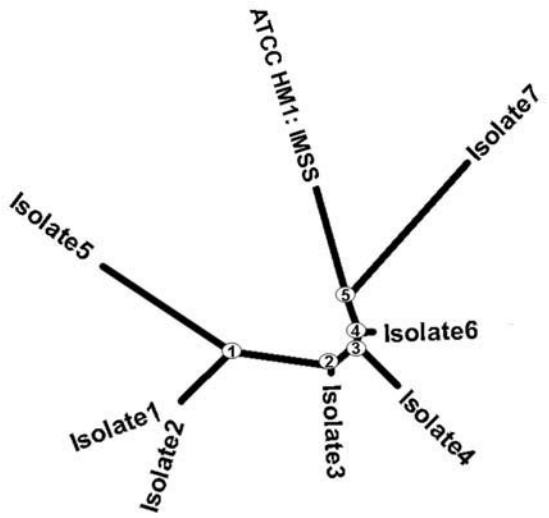

Figure 3. Unrooted parsimony tree ("Pars" parsimony algorithm) based on raw sequence data of the transfer RNA–linked polymorphic short tandem repeats loci. The circles on the nodes indicate the level of bootstrap support based on 100 replicates, with the following node numbers: node 1, 100%; node 2, 86%; node 3, 79%; node 4, 77.5%; and node 5, 86%. Branch lengths are proportional to the averages of the number of reconstructed changes of state over all sites over all possible most parsimonious placements of the changes of state among the branches. That is, longer branches indicate a greater number of changes from the taxon to the hypothesized ancestral node. The 7 *Entamoeba histolytica* stool isolates from our investigation came from different patients; the American Type Culture Collection (ATCC) *E. histolytica* HM1: IMSS reference strain was also included for comparison.

presence of amebas in stool specimens, stool antigen detection, and/or high amebic serological titers. Only 1 of the investigated contacts did not have evidence of *E. histolytica* infection. There was almost certainly female-female transmission, because 1 woman, patient F, was strictly homosexual, and 4 women were bisexual (Table 1). Several individuals (patients A, D, F, and G) confirmed that they engaged in oral-anal sexual activity.

Amebic infection occurs equally often among male and female individuals, but invasive disease is ~10 times more frequent among male individuals; similarly, in this cluster, both infected men had liver abscesses, and only 1 of 5 infected women had a liver abscess.

E. histolytica is potentially very transmissible. Although the infectious dose is not known, in theory, 1 viable cyst is sufficient to establish infection. Anal-oral transmission occurs readily in MSM, but it is not widely appreciated that heterosexual and female homosexual transmission may also occur. Usually, transmission occurs via cysts, which are hardy and can survive in the environment, but close personal contact, as documented here, may involve transmission of trophozoites as well; however, they are not likely to survive gastric acid after ingestion.

It is clear that *E. histolytica* is transmissible person-to-person and that transmission may be more likely within households [2]. In studies in Brazilian slums, 10% of the sample population was colonized with *E. histolytica* [12]. However, household contacts of an index case were much more likely to be colonized: in 27 of the 28 households, at least 1 additional case of *E. histolytica* infection was identified within families, and 73% of the household contacts were also colonized. Of participants, 85% had cleared the infection by 30–45 days [13]. Higher rates of infection have been observed among contacts of patients with an amebic liver abscess or amebic dysentery or in asymptomatic carriers, compared with control subjects [14, 15]. A study in Mexico also found that 40% of contacts of carriers of *E. histolytica* and *E. dispar* were also infected [16].

Household transmission has also been noted in industrialized countries. In one cluster in the Netherlands, the index case was the mother who had acquired infection in India [17]. Two other family members then developed symptomatic disease. All 5 family members had elevated amebic serological titers, and all had *E. histolytica* identified in stool specimens; none had traveled outside Western Europe [17]. Another report noted the transmission of *E. histolytica* within an Italian household in which the Philippine maid was the likely source [2].

Sexual transmission of amebiasis has been most commonly recognized among MSM [4, 5]; intestinal amebiasis in MSM is assumed to be acquired primarily via oral-anal sex either directly or secondarily from sex toys or fellatio. Most MSM with amebiasis are colonized with *E. dispar,* but *E. histolytica*–associated disease does occur [18]. Heterosexual transmission of *E. histolytica* is rarely described, but a small cluster of amebiasis occurred in Italy in which 2 male individuals were likely the index case patients. They then infected their female partners (2 were asymptomatic, and 2 had liver and colon infections) [19]. Seroepidemiological analysis of Japanese women attending a sexually transmitted disease clinic suggested increasing

rates of *E. histolytica* infection from 2003 to 2006, which may have been sexually acquired [20]. However, Phillips et al [21] did not find enteric protozoa in heterosexual men or women attending a sexually transmitted disease clinic.

Recent studies suggest that only a minority of all *E. histolytica* infections progress to the development of clinical symptoms in the host, and population level differences exist between the *E. histolytica* strains isolated from asymptomatic and symptomatic individuals. The strain identified here appears to be particularly virulent, because it was associated with severe hepatic and cecal disease in 3 of 8 contacts and because 7 of 8 contacts had amebic infection and high amebic serological titers. The transmission was amplified within this cluster because of the multiple partners, oral-anal contact, and mixed sexual preferences (female homosexual, bisexual, and heterosexual). To our knowledge, female homosexual transmission of amebiasis has not been previously described.

Genotyping analysis using the schema published by Tawari et al [9] with modifications made in accordance with Graham Clark (C. G. Clark, personal communication) revealed that the outbreak strain (isolate 7 in Table 2) is genetically distinct from *E. histolytica sensu stricto* isolates obtained from patients either with diarrhea or who were asymptomatic. Isolates 1 and 2 (Table 2) were from individuals residing at the same household and therefore are epidemiologically linked. Moreover, with either an alphanumeric annotation or the raw sequence data within the tRNA loci, phylogenetic analysis confirmed that the outbreak strain was genetically distinct, compared with less virulent isolates. With both the alphanumeric data (Fig. 2) and the raw sequence data (Fig. 3), the virulent laboratory strain, American Type Culture Collection HM1: IMSS (which originally caused cecal ulcers and colitis [10]), appeared to be genetically most similar to the outbreak strain, suggesting that the genotyping schema may be able to provide information on the potential virulence of a particular isolate. More isolates with associated clinical data are needed for the genotyping schema to be fully corroborated. One of the limitations of this study is that we were unable to recover *E. histolytica* DNA from every patient in the outbreak. However, the epidemiological investigation strongly suggests that transmission occurred via direct spread. The branch lengths associated with the outbreak strain suggest that it has the most reconstructed changes of state over all sites over all possible most parsimonious placements of the changes of state among the branches.

In summary, even in industrialized countries, amebiasis should be considered to be potentially transmissible within households, between heterosexual partners, and especially between same-sex partners. Patients with amebiasis should be counselled about enteric precautions, and specific discussions are required about the potential for sexual transmission. Although the number of isolates in this study does not permit statistical analyses with sufficient power, the data suggest that the genotyping schema used here may be useful to distinguish virulent strains from less virulent strains of *E. histolytica sensu stricto*.

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