Clinical Features and Gut Microbiome of Asymptomatic Entamoeba histolytica Infection

Yasauki Yanagawa,1 Naoyoshi Nagata,2 Kenji Yagita,1 Kazuhiro Watanabe,2 Hidetaka Okubo,2 Yoshimi Kikuchi,1 Hiroyuki Gatanaga,1 Shinichi Oka,1,5 and Koji Watanabe1

1AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, 2Department of Gastroenterology and Hepatology, National Center for Global Health and Medicine, Tokyo, Japan, 3Department of Gastroenterological Endoscopy, Tokyo Medical University, Tokyo, Japan, 4Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan, and 5Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan

Background. Entamoeba histolytica infection is a sexually transmitted disease in some developed countries. Asymptomatic infection often occurs and can be a source of transmission; however, limited data are available regarding the pathogenesis of E. histolytica.

Methods. This was a single-center, cross-sectional study. Specimens were prospectively collected from patients with clinically suspected cases. Entamoeba histolytica infection was defined as a case in which the identification of E. histolytica was confirmed by polymerase chain reaction (PCR) of a clinical specimen. Data from asymptomatic cases were compared with those from symptomatic invasive cases.

Results. Sixty-four E. histolytica–infected cases, including 13 asymptomatic cases, were identified during the study period. Microbiological diagnosis was made by endoscopic sampling in 26.6% of these cases (17/64). Endoscopy identified macroscopically visible lesions in all cases; however, the sensitivity of histopathology on biopsy samples was low (45.5%) compared with PCR (94.7%). In asymptomatic cases, infection sites were limited around the proximal colon; moreover, trophozoites were frequently identified at infection sites whereas cystic forms were commonly detected in stools. Gut microbiome analyses showed more uniform composition in asymptomatic cases than in symptomatic invasive cases, which were represented by a relatively high abundance of Ruminococcaceae, Coriobacteriaceae, and Clostridiaceae, and a low abundance of Streptococcaceae.

Conclusions. These results indicate that the encystation and attenuation of E. histolytica are highly affected by the intestinal contents, including the gut microbiome.

Keywords. parasitology; sexually transmitted infections; amebiasis; microbiome.

Entamoeba histolytica, the causative agent of invasive amebiasis, is the second most common parasitic cause of mortality worldwide [1]. Over the past 2 decades, it was reported that invasive amebiasis is prevalent not only in developing countries where food and water are frequently contaminated by feces, but also in some developed countries in Asia and Europe [2–5]. In these areas, the pathogen spreads as a sexually transmitted infection (STI), especially among men who have sex with men (MSM) and people living with human immunodeficiency virus (HIV) [2, 5, 6]. Furthermore, recent data indicate that this pathogen is spreading among HIV-uninfected men and women in Japan [7, 8].

The severity of E. histolytica infection varies. Only 10% of individuals who are exposed to the pathogen develop “symptomatic” invasive amebiasis; the majority of cases are asymptomatic or display self-limiting mild diarrhea at an early phase [9]. Therefore, E. histolytica infection is often overlooked in clinical settings. Furthermore, it is known that some infections persist asymptptomatically. The half-life of asymptomatic infection is reported to be about 1 year [10], which can result in a transmissible pathogen reservoir among the community, and E. histolytica infection has been unexpectedly diagnosed by endoscopy in developed countries [11–13]. Data from the National Surveillance of Japan indicated an increase in the number of asymptomatic infections from 39 patients in 2010 to 170 patients in 2013 [8]. However, these numbers only accounted for around 10%–20% of all reported cases, indicating that asymptomatic cases of E. histolytica infection are currently underestimated in Japan. For future disease control of E. histolytica infection, it is important to understand the pathogenesis of this microorganism, which involves identifying the determinant factors of disease severity. Previously reported human cohort data indicated that the gut microbiome plays an important role. Cross-sectional studies from India reported that the burden...
of some bacterial species in the gut microbiota was altered by the presence of *E. histolytica* [14], and differences in the gut microbiota between asymptomatic colonization and liver abscess during *E. histolytica* infection have also been reported [15]. The presence of *Prevotella copri* in the gut flora was shown to be associated with susceptibility to *E. histolytica*-induced diarrheal disease in 2 geographically distinct areas [16, 17]. It is of interest to better understand the impact of the gut microbiome on the severity of *E. histolytica* infection.

Herein, we compared the clinical features of asymptomatic *E. histolytica* infection with those of invasive diseases in our cohort and sought to identify the features of the gut microbiota during asymptomatic infection.

**MATERIALS AND METHODS**

**Study Design and Sampling**

This was a single-center, cross-sectional study carried out between 2014 and 2019. Clinical specimens were prospectively collected from patients with suspected *E. histolytica* infection after obtaining written informed consent. The choice of sampling method was completely dependent on the physicians' decision; however, samples were examined not only by approved in vitro diagnostic methods in Japan (stool ova and parasite examinations [O&P] and tissue histopathology) but also by other unapproved laboratory diagnostic methods, as detailed below. Stool samples were examined by O&P, which consisted of direct microscopic examination for trophozoites and formalin-ether sedimentation for cyst forms, and polymerase chain reaction (PCR) using *E. histolytica*-specific primers. For patients undergoing endoscopy, we collected aspirated intestinal fluid samples by washing macroscopically identifiable lesions with 5 mL of saline [18] for O&P and PCR, in addition to tissue biopsy for histopathology with hematoxylin and eosin staining and periodic acid-Schiff staining. Aspirated pus from abscesses was evaluated by O&P and PCR. Residual samples, if any, were immediately frozen at −80°C until further experimental use. This study was approved by the ethics committee of the National Center for Global Health and Medicine (approval number NCGM-G-001566-02 and NCGM-G-003333-00) and was implemented in accordance with the provisions of the Declaration of Helsinki.

**Case Definitions**

In the present study, “*E. histolytica* infection” was defined as a case in which the identification of *E. histolytica* was confirmed by PCR in any clinical specimens (stool, aspirated intestinal fluid, and/or aspirated pus). Clinical forms were defined as follows:

- **Asymptomatic infection:** Abdominal symptoms were not the reason for the hospital visit, and the frequency of defection and the morphology of stools were the same as usual;
- **Liver abscess:** Compatible liver lesions identified by computed tomography and/or sonography that responded to nitroimidazole treatment; and
- **Colitis:** *E. histolytica* infection that was not categorized as an asymptomatic infection or a liver abscess.

**Serum Antibody Testing**

Indirect fluorescent antibody assays using a slide precoated with fixed *E. histolytica* were performed for the detection of anti-*E. histolytica* antibody in serum [19, 20]. The commercial kit, Amoeba-Spot IF* (bioMérieux SA), which was previously (until the end of 2017) approved for the diagnosis of *E. histolytica* infection in Japan, was carried out in accordance with the instructions enclosed with the kit. Seropositivity was defined as a positive response in a serum sample diluted at 1:100, and the anti-*E. histolytica* titer was determined by the highest dilution that produced a positive response.

**Endoscopic Assessment**

The features identified by endoscopy were assessed by lesion site and distinctive macroscopic appearance as follows: aphthae or erosion, slight damage to the mucosa; ulcer, a clear deep mucosal defect; exudate, mucosal epithelial attachment white or yellow in color, accompanied by aphthae, erosion, and an ulcer; bump, edematous swollen mucosa caused by acute inflammation [11].

**Polymerase Chain Reaction**

Clinical specimens (0.2 g) were subjected to DNA extraction using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA (5.0 μL) was subjected to PCR using different groups of primers (N-K2 and R-R) in this study, which were used to amplify *E. histolytica* HM-1:IMSS transfer RNA gene sequences as previously described [21–23].

**Gut Microbiome Analysis**

DNA extraction and 16S ribosomal RNA (rRNA) sequencing were conducted according to a previously described method [24]. The V3–V4 regions of bacterial 16S rRNA were PCR amplified using the 341f/806r primers and the dual-index method [25–27]. Barcoded amplicons were sequenced using the paired-end, 2 × 284-bp cycle run on the MiSeq system with MiSeq Reagent Kit version 3 (600 Cycle) chemistry. Paired-end sequencing reads were merged using the fastq-join program with default settings [28]. Only reads that had quality value scores of 20 for >99% of the sequences were extracted, and chimeric sequences were removed using usearch 6.1 [29]. Nonchimeric reads were identified using the TechnoSuruga Laboratory, Japan database DB-BA 13.0 [30, 31]. Operational taxonomic units (OTUs) were aligned based on open-reference picking using the USEARCH version 6.1.
of QIIME [29, 32]. The OTUs with a 97% similarity level were identified using the Greengenes database version 13.8 [33]. The α- and β-diversity were implemented in the QIIME pipeline [32].

**Statistical Methods**

The patients’ characteristics and the laboratory results from each diagnostic test for E. histolytica infection were compared between cases of asymptomatic infection and symptomatic invasive infection. Analysis of variance tests were used for comparisons of patients’ demographic data and the sensitivities of diagnostic tests. Statistical significance was defined as a 2-sided P value < .05. All statistical analyses were performed using GraphPad Prism 7.0 software (GraphPad, San Diego, California). The microbiome composition of asymptomatic infections was compared with symptomatic invasive infections by performing permutational multivariate analysis of variance. The α-diversity and β-diversity were assessed based on both unweighted and weighted UniFrac distance metrics [32].

**RESULTS**

**Patients’ Characteristics**

During the study period, E. histolytica infection was suspected in 116 patients, for which 125 specimens were evaluated by PCR. Finally, we identified 64 patients with E. histolytica infection. The patients’ characteristics are summarized in Table 1. Forty-five patients (70.3%) were seropositive for at least 1 of the following infections: HIV type 1, hepatitis B virus, hepatitis C virus, or syphilis, indicating that E. histolytica infection is mainly diagnosed as an STI in this study cohort, whereas imported infections from tropical countries were suspected in 20.3% (13/64) of the patients. Based on the clinical forms of E. histolytica infection described in the Methods, 13 (20.3%) and 51 (79.7%) of the E. histolytica–infected patients were categorized as

**Table 1. Characteristics of Patients With Entamoeba histolytica Infection**

| Characteristic | Asymptomatic Infection (n = 13) | Symptomatic Invasive Infection (n = 51)* | P Value |
|---------------|---------------------------------|--------------------------------------------|--------|
| Age, y, median Range | 45 (29–68) | 41 (21–67) | .093 |
| Male sex | 12 (92.3) | 45 (88.2) | >.999 |
| MSM | 7 (53.8) | 39 (76.5) | .165 |
| Positive serology of STIs | | | |
| HIV (4th generation) | 6 (46.2) | 34 (66.7) | .208 |
| With current OIs | 1 (77) | 3 (5.9) | >.999 |
| On ART | 3 (23.1) | 20 (39.2) | .346 |
| Syphilis (TPHA) | 3 (23.1) | 16 (31.4) | .739 |
| Hepatitis B (HBc-Ab) | 5 (38.5) | 22 (43.1) | >.999 |
| Hepatitis C (HCV-Ab) | 2 (15.4) | 2 (3.9) | .181 |
| Travel history to developing countries within 1 y | 4 (30.8) | 9 (17.7) | .439 |
| Clinical symptoms | | | |
| Diarrhea | 0 (0.0) | 32 (62.8) | |
| Fever | 0 (0.0) | 20 (39.2) | |
| Abdominal pain | 0 (0.0) | 18 (35.3) | |
| Bloody stool | 0 (0.0) | 15 (29.4) | |
| No complaint | 13 (100) | 0 (0.0) | |
| Laboratory results, median Range | | | |
| WBC count, ×10^3/μL | 5.38 (2.12–8.05) | 7.94 (2.21–30.38) | <.001 |
| CRP, mg/dL | 0.04 (0.01–3.35) | 2.56 (0.02–24.94) | .008 |
| Eosinophils, /μL | 188 (16–718) | 116 (0–1009) | .266 |
| Anti–Entamoeba histolytica Ab | 8/10 (80.0) | 12/33 (36.4) | .028 |
| CD4 count, cells/μLb | 379 (125–507) | 341 (34–1112) | .683 |
| CD4 percentage, %b | 25.3 (9.7–41.9) | 23.7 (3.2–52.1) | .694 |
| HIV RNA, copies/mLb | 8000 (TND–600 000) | 21 (TND–680 000) | .691 |
| Diagnosis method | | | .265 |
| Stool test | 8 (61.5) | 32 (62.7) | |
| Endoscopy | 5 (38.5) | 12 (23.5) | |
| Aspiration/drainage | 0 (0.0) | 7 (13.7) | |

Data are presented as no. (%) unless otherwise indicated.

Abbreviations: Ab, antibody; ART, antiretroviral therapy; CRP, C-reactive protein; HBc, hepatitis B core; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MSM, men who have sex with men; OI, opportunistic infection; RNA, ribonucleic acid; STI, sexually transmitted infection; TND, target not detected; TPHA, Treponema pallidum hemagglutination assay; WBC, white blood cell.

*Symptomatic invasive infections included 45 patients with colitis and 6 patients with liver abscess. Acute appendicitis (n = 3), perianal abscess (n = 2), and a fulminant colitis case (n = 1) were included in the colitis group.

bThe peripheral blood tests for CD4+ T cells and HIV viral load were performed only for patients with HIV.
having an asymptomatic infection and symptomatic invasive infection (45 cases of colitis and 6 cases of liver abscess), respectively. These epidemiological data were consistent with the national surveillance data in Japan [8]. Clinical presentations at diagnosis were varied from asymptomatic to unstable vital changes requiring immediate surgical intervention. Although diarrhea was the most common symptom among symptomatic invasive infections, 14 patients presented with acute abdomen. From the laboratory test results, the white blood counts and C-reactive protein levels in patients with symptomatic invasive infection were found to be elevated, whereas these inflammatory markers were rarely elevated in asymptomatic infected individuals. Medical treatment of *E. histolytica* infection was performed for all patients according to global guidelines [9]. However, surgical intervention (appendectomy, colectomy, and percutaneous drainage) was required in 6 patients. Death from *E. histolytica* infection was not reported in the study population. Interestingly, serum anti-*E. histolytica* antibody was detected in 80% of the patients with asymptomatic infection, whereas only 38% and 25% of the patients with colitis and liver abscesses, respectively, showed positive serology, which probably resulted from the fact that seropositivity depends on the time interval from infection to blood testing [9, 34, 35]. Microbiological diagnosis was made by endoscopic sampling in 38.5% and 23.5% of asymptomatic infection and symptomatic invasive infections, respectively. These results indicated that asymptomatic *E. histolytica* infection is not a rare comorbidity with STIs, but its diagnosis is sometimes difficult in clinical settings in Japan.

### Clinical Features of Asymptomatic *E. histolytica* Infection

Next, to investigate the clinical features of asymptomatic infection, we summarized the data from 13 patients with asymptomatic *E. histolytica* infection (Table 2). As per the case definition stated above, abdominal symptoms were not the reason for the hospital visit for any of these asymptomatic patients. The major opportunities to diagnose asymptomatic *E. histolytica* infection were at cancer screening in 6 patients (fecal occult blood [FOB] positive: 3 patients and complete medical check: 3 patients), and at screening for other STIs in 4 patients (seropositive result for serum anti-*E. histolytica* antibody). Among 13 patients, stool examination was performed for 9 patients but not for the other 4 patients who were occasionally diagnosed with colonic lesions during endoscopy (2 were FOB positive, 1 underwent a complete medical check, and 1 underwent staging for Kaposi sarcoma). Stool examination identified *Entamoeba* by O&P in 6 patients (67%), whereas stool PCR identified *E. histolytica* in 8 patients (89%). Visible colonic ulcers and/or erosions were identified in all 6 patients who underwent endoscopy, even in 1

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### Table 2. Summary of the Laboratory Data for 13 Patients With Asymptomatic Intestinal *Entamoeba histolytica* Infection

| Case ID | Age, y | Sex | Chief Complaint or Reason for Referral | Stool Examination | Intestinal Fluid Examination | Histopathology of Biopsied Sample | Serum Anti-*Entamoeba histolytica* Ab Titer |
|---------|--------|-----|---------------------------------------|-------------------|-----------------------------|---------------------------------|----------------------------------|
| 11      | 41     | M   | Anti-*E. histolytica* Ab positive      | Negative          | Negative                    | Erosions in the cecum            | Trophozoite Positive            |
| 104     | 40     | F   | FOB test positive                     | Negative          | Negative                    | Ulcer in the cecum               | NA                               |
| 71      | 45     | M   | Extrapulmonary tuberculosis           | Negative          | Positive                    | NA                              | NA                               |
| 77      | 68     | F   | Complete medical examination          | Cyst              | Positive                    | NA                              | NA                               |
| 92      | 45     | M   | Anti-*E. histolytica* Ab positive     | Cyst              | Positive                    | NA                              | NA                               |
| 93      | 42     | M   | Anti-*E. histolytica* Ab positive     | Cyst              | Positive                    | NA                              | NA                               |
| 99      | 29     | M   | Anti-*E. histolytica* Ab positive     | Cyst              | Positive                    | NA                              | NA                               |
| 120     | 45     | M   | LFT elevation                         | Cyst              | Positive                    | NA                              | NA                               |
| 124     | 56     | M   | Complete medical examination          | Cyst              | Positive                    | NA                              | NA                               |
| 53      | 38     | M   | FOB test positive                     | NA                | NA                          | NA                              | NA                               |
| 73      | 58     | M   | FOB test positive                     | NA                | NA                          | NA                              | NA                               |
| 105     | 52     | M   | Complete medical examination          | NA                | NA                          | NA                              | NA                               |
| 54      | 49     | M   | Kaposi sarcoma                        | NA                | NA                          | Multiple ulcers and bumps with    | Negative Positive              |
|         |        |     |                                       |                   |                             | exudates from the cecum to the    |                                   |

Abbreviations: Ab, antibody; F, female; FOB, fecal occult blood; ID, identification number; LFT, liver function test; M, male; NA, not available; O&P, ova and parasite examination; PCR, polymerase chain reaction.

*Anti-*E. histolytica* antibody was measured by an indirect immunofluorescence assay. This commercial antibody test was only available until 2017 in Japan, so it could not be used for the samples from 2018 (cases 104–124).

In case 104, endoscopy was performed by the previous doctor and only a stool test was performed at our hospital.

Bump was defined as edematous swollen mucosa caused by acute inflammation.
patient with a negative result for stool PCR (case identification number 11). Interestingly, compared with symptomatic invasive infection, infection sites were limited around the proximal colon, especially in the cecum (Table 3); moreover, trophozoites were frequently identified at infection sites, whereas cystic forms were commonly detected in stools. Unexpectedly, the sensitivity of histopathologic examination of biopsy samples during endoscopy was significantly lower than that of PCR for the same samples (45.5% [10/22] vs 94.7% [18/19], respectively; P < .001), even though all biopsy samples were obtained from the edge of macroscopically visible lesions (Figure 1 and Supplementary Figure 1). This was probably because biopsy samples were small and Entamoeba often resided only on the surface of the mucous layer. Additionally, the antigen detection test (E. HISTOLYTICA II, Techlab, Blacksburg, Virginia) performed on frozen samples (stool and aspirated intestinal fluid samples) had lower sensitivities for pathogen identification compared with PCR.

**Gut Microbiome Analyses of Asymptomatic *E. histolytica* Infection**

As shown in Table 2, morphological examination by O&P revealed that the cystic form of *Entamoeba* was frequently observed in stools, whereas the trophozoite form was observed in aspirated intestinal fluid samples of asymptatically *E. histolytica*–infected individuals. This result provided a hypothesis that exposure to intestinal contents, such as the gut microbiome, may attenuate the virulence of *E. histolytica* and induce encystation in asymptotically infected individuals. We compared the gut microbiota of asymptotically infected individuals with those of patients who developed symptomatic invasive infections using 37 stool samples (Supplementary Table 1). First, we assessed the α- and β-diversity in the gut microbiota composition of asymptomatic patients, then compared them with symptomatic patients. Regarding α-diversity, there was no significant difference between the 2 groups (Figure 2A). The β-diversity calculated by principle components analysis revealed that a symptomatic infection (red dots in Figure 2B) provided strong separation along the primary axis of variation of the multidimensional scaling plots, whereas an asymptomatic infection (blue dots in Figure 2B) showed more uniform microbiome composition. These results indicated that the diversity of the gut microbiota was consistent between the 2 groups, but its composition was significantly different, with asymptotically infected individuals showing specific features of the gut microbiome. Next, to identify the specific bacterial families in prevalence and abundance during asymptomatic infection, we compared the relative abundance of each bacterial family by OTU analysis (Figure 2C). The proportion of Streptococcaceae (potentially exacerbating bacteria) was significantly lower in asymptotically infected individuals. By contrast, the proportions of Ruminococcaceae, Coriobacteriaceae, and Clostridiaceae (potentially protective bacteria) were significantly higher in asymptotically infected individuals. We also used Vitcomic software (http://vitcomic.org/) to compare the gut microbiome composition between our asymptomatic and symptomatic *E. histolytica*–infected cases and *E. histolytica*–uninfected healthy Japanese individuals, using previously published shotgun sequence data for the healthy group [36]; statistical analyses were not applied to these comparisons because of concerns about the inaccuracy resulting from comparing gut microbiome data that were calculated differently (16S rRNA sequencing and shotgun sequencing) (Supplementary Figure 2). These preliminary analyses revealed that some of the symptomatic and uninfected control cases lack 3 “potentially protective” bacterial families (Ruminococcaceae, Coriobacteriaceae, and Clostridiaceae), whereas all of the asymptomatic cases contain these bacterial families (Supplementary Figure 2B). Moreover, 5 symptomatic cases (NA-95, -118, -59, -23, and -66) and 1 healthy control case (DRR042402-DRR042403) showed no (or extremely low) amounts of each of these potentially protective bacterial families in their stool samples (red arrows in Supplementary Figure 2B). In contrast, Streptococcaceae (potentially exacerbating bacteria) was relatively lower in most of the asymptomatic cases, except NA-104, whereas the abundance of Streptococcaceae varied from low to high among healthy control subjects. Next, we investigated prevalence at the genus and species levels for each of the families in asymptotically infected individuals.

### Table 3. Endoscopic Findings in Patients With *Entamoeba histolytica* Infection

| Finding | Asymptomatic Infections (n = 6)* | Colitis (n = 13)* | P Value |
|---------|---------------------------------|------------------|---------|
| Visible intestinal lesions | 6 (100) | 13 (100) | >.999 |
| Intestinal site of infection | | | |
| Proximal sites | 6 (100) | 12 (92.3) | >.999 |
| Cecum | 6 (100) | 12 (92.3) | |
| Ascending | 1 (16.7) | 7 (53.8) | |
| Transverse | 1 (16.7) | 6 (46.2) | |
| Distal sites | 0 (0.0) | 9 (69.2) | .011 |
| Descending | 0 (0.0) | 4 (30.8) | |
| Sigmoid | 0 (0.0) | 4 (30.8) | |
| Rectum | 0 (0.0) | 8 (61.5) | |
| Macroscopic appearance | | | |
| Erosion | 3 (50.0) | 6 (46.2) | >.999 |
| Ulcer | 3 (50.0) | 7 (53.8) | >.999 |
| Exudate | 3 (50.0) | 12 (92.3) | .071 |
| Bump | 1 (16.7) | 2 (15.4) | >.999 |
| Identification of *Entamoeba* by histopathology | 4 (66.7) | 6 (46.2) | .629 |

Data are presented as no. (%) unless otherwise indicated.

*Each case in both infection groups was diagnosed with *E. histolytica* infection by polymerase chain reaction only of the stool sample, because both cases had been evaluated by endoscopy by a previous doctor. A detailed evaluation of the clinical specimens collected by endoscopy was not available, except for the histopathologic findings.

*Definition of each macroscopic appearance during endoscopy is described in the Methods.
using Metagenome@KIN software (detailed in the Methods). At the species levels for the Streptococcaceae, the proportions of Streptococcus salivarius and Streptococcus sinensis were significantly lower in asymptotically infected individuals. At the species level for Ruminococcaceae, Coriobacteriaceae, and Clostridiaceae, only Collinsella aerofaciens was significantly higher in asymptotically infected individuals (Figure 3). These results suggested that the compositions of the gut microbiome are related to the disease severity of E. histolytica infection, which was represented by low abundance in S. salivarius and S. sinensis and high abundance in C. aerofaciens among asymptotically infected individuals.

DISCUSSION

The disease severity associated with E. histolytica infection varies from asymptomatic chronic infection to life-threatening fulminant diseases; however, the pathogenesis of amebiasis remains unclear. Interestingly, in asymptomatic E. histolytica–infected cases, visible ulcer lesions and/or erosions were limited to around the cecum, whereas conversely, in symptomatic invasive patients, lesions were identified in various sites throughout the colon by endoscopy. Moreover, similar to previous studies [10, 37], most of the asymptotically E. histolytica–infected patients in this study evacuated cyst forms of E. histolytica in their stools. Surprisingly, aspirated intestinal fluid obtained during endoscopy from macroscopic membranous lesions in asymptotically infected individuals frequently contains the trophozoite form of E. histolytica. Furthermore, we examined the gut microbiomes of the study participants. According to the family level analysis, the composition of the microbiome represented by β-diversity analysis in asymptomatic patients showed a relatively uniform microbial community compared with symptomatic invasive patients, with a significant difference being evident between the 2 groups (Supplementary Figure 3). Further assessment of the gut microbiome at the species level revealed that the gene expression levels of 2 bacteria (S. salivarius and S. sinensis) were significantly lower in patients with asymptomatic infection, and the gene expression levels of 1 bacterium (C. aerofaciens) was significantly higher in patients with asymptomatic infection (Figure 3). It was previously reported that S. salivarius has a protective effect on colitis in an animal model due to the inhibitory effect of NF-κB activation [38, 39]. It was also shown that host NF-κB levels were suppressed by E. histolytica in an in vitro infection model [40]. However, no previous reports assessed the direct interaction between these microbiota and E. histolytica during its infection. Taken together,
these findings suggest that the gut microbiota might play an important role in determining the disease pathogenesis of *E. histolytica* infection; however, further studies are needed to elucidate the pathogenesis of asymptomatic/symptomatic *E. histolytica* infection.

Some limitations need to be considered for the present study. First, this study was performed in a single institute with only a limited number of patients with confirmed *E. histolytica* infection. Second, initial recruitment and sampling methods were all dependent on clinical judgments in the present observational...
study design. The frequency of *E. histolytica* infection, especially asymptomatic cases, may have been underestimated, although the ratio of disease forms among the study population was consistent with that of the Japanese national surveillance data. Third, the gut microbiome can be influenced by numerous host conditions, such as food consumption and comorbidities. Gut microbiome comparisons using samples from a single timepoint (eg, upon diagnosis) cannot be conclusively correlated with disease pathogenesis. Finally, no experimental evaluation was performed for elucidating the mechanism that explains the effect of the gut microbiota on the clinical outcome of *E. histolytica* infection. Validation using an experimental model of *E. histolytica* infection should be performed in a future study.

In conclusion, we revealed clinicopathological features of asymptomatic *E. histolytica* infection. Also, our data support the previous reports that the gut microbiome may play an important role. Further investigations will be needed for elucidating its molecular mechanisms.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
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