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Entamoeba Species in South Africa: Correlations With the Host Microbiome, Parasite Burdens, and First Description of *Entamoeba bangladeshi* Outside of Asia

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Background. Diarrhea is frequent in communities without clean water, which include low-income South African populations in Giyani and Pretoria. In these populations, the amount of diarrhea caused by *Entamoeba histolytica*, inclusive of all ages, sexes, and human immunodeficiency virus status, is uncertain. Infection with *E. histolytica* can modulate the host microbiota, and a key species indicative of this is the *Prevotella copri* pathobiont.

Methods. A cross-sectional study of patients attending gastroenterology clinics was conducted to determine the frequency and burden of 4 *Entamoeba* species and *P. copri*.

Results. *Entamoeba* species were present in 27% of patients (129/484), with *E. histolytica* detected in 8.5% (41), *E. dispar* in 8% (38), *E. bangladeshi* in 4.75% (23), and *E. moshkovskii* in 0%. This is the first description of *E. bangladeshi* outside Bangladesh. In *E. histolytica*–positive samples, the levels of both the parasite and *P. copri* were lower in nondiarrheal samples, validating the results of a study in Bangladesh ($P = .0034$). By contrast, in *E. histolytica*–negative samples positive for either of the nonpathogenic species *E. dispar* or *E. bangladeshi*, neither *P. copri* nor *Entamoeba* levels were linked to gastrointestinal status.

Conclusions. Nonmorphologic identification of this parasite is essential. In South Africa, 3 morphologically identical *Entamoeba* were common, but only *E. histolytica* was linked to both disease and changes in the microbiota.

Keywords. *Entamoeba bangladeshi*; *Entamoeba histolytica*; *Protozoa*; microbiome; *Prevotella copri*.

Entamoeba species are a group of unicellular, anaerobic, parasitic organisms found in humans, nonhuman primates, and other vertebrate and invertebrate species [1]. *Entamoeba* infections in humans can result in asymptomatic carriage or a wide range of symptomatic diseases. Of the subset of individuals developing symptoms, diarrhea and dysentery are the most common manifestations. Extraintestinal complications occur less frequently but can be associated with high mortality [2].

Species of *Entamoeba* that can infect and be found in the intestinal lumen of humans include *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, *Entamoeba coli*, *Entamoeba polecki*, *Entamoeba hartmanni*, and *Entamoeba bangladeshi* [3]. Of these, *E. bangladeshi* is the most recent to be described, with the species name reflecting the geographic origin of the first patient it was isolated from. *E. bangladeshi* is indistinguishable by microscopy from *E. histolytica*, the prototypical pathogenic *Entamoeba* species, but it can be differentiated from other known *Entamoeba* species by immunologic and molecular techniques.

A recent study involving stool samples collected from infants (age range, 0–24 months) residing in Vhembe District, Limpopo, South Africa, failed, however, to detect *E. histolytica* by polymerase chain reaction (PCR) analysis [4]. However *E. histolytica* was common (18%) in an earlier 2006 study involving participants of all ages (ie, from 0 to >60 years) [5]. The cause for this discrepancy is not fully understood. In preliminary work, samples collected from patients of all ages visiting a gastroenterology clinic between November 2013 and June 2015 in both rural Giyani (Mopani District, Limpopo) and urban Pretoria (Soshanguve District, Gauteng, South Africa) were evaluated by microscopy for the presence of ameboid organisms, and 50% of the samples were *Entamoeba* positive. These findings might be due to the presence of other morphologically identical non–*E. histolytica* species of *Entamoeba,* such as *E. dispar* and *E. bangladeshi,* to geographical heterogeneity in the frequency of *E. histolytica* in South African populations, or to a much lower frequency of *E. histolytica* in the community-based surveys of enteric disease than in patients requiring clinical care [3, 6, 7].

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To test these hypotheses, DNA was extracted from the Giyani and Pretoria samples, and a multiplex quantitative PCR (qPCR) assay was used to detect both *E. histolytica* and the other morphologically identical *Entamoeba* species, such as *E. dispar*, *E. moshkovskii*, and *E. bangladeshi*. This assay was also modified to include a general *Entamoeba* probe to capture data on the presence of novel *Entamoeba* species genetically similar to the pathogenic species *E. histolytica* that may be present in the South African population.

The qPCR assay data captured quantitative information, and this permitted us to examine the correlation between the parasite burden in these samples and the outcome of infection. A link between parasite burden and symptomatic disease has been found in previous studies [4, 8, 9]. Recent studies have also highlighted the relationship between *Entamoeba* species and the bacterial communities of the gut. The presence of *Entamoeba* organisms was associated with a decrease in the abundance of *Prevotella copri* in farmers and fishermen from Southwest Cameroon [10], and the abundance of *P. copri* increased in diarrheal *E. histolytica* cases [9]. Hence, we also sought to quantify this bacterium in the microbiome of *Entamoeba*-positive samples in our study population.

METHODS

Ethics Statement

The research and ethics committee of the University of Venda granted institutional approval. The study received ethical clearance from the Department of Health and Welfare, Polokwane, Limpopo Province, South Africa. We also obtained permission from the ethics committee of participating hospitals and clinics to collect samples. The objectives and concepts of the study were clearly explained in the language understood by the potential participants (ie, English, Sepedi, Xitsonga, and Tshivenda). A written, informed consent form was signed prior to study enrollment. In cases where the participant was either a non-English speaker or illiterate, a witness also signed the consent form.

Study Area and Population

The tested stool samples were predominantly from urban and rural populations of moderate-to-low socioeconomic status [11, 12]. They were collected between November 2013 and June 2015 from diarrheal and nondiarrheal patients in the rural Nkomo clinic (Giyani) and the urban clinic within the Dr George Mukhari Hospital (Soshanguve District).

The catchment area for the Dr Georges Mukhari Hospital includes Soshanguve, Ga-Rankuwa, Mabopane, and parts of Madibeng District. The Nkomo clinic serves households within the Greater Giyani Local Municipality, Mopani District. Household water and sewage access is summarized in Table 1 [11, 12].

Both adults and children of all ages were eligible for participation. A questionnaire was used to collect sociodemographic information, such as the age, sex, and origin of the study participants.

Table 1. Water and Sewage Arrangements Among Households, by Gastrointestinal Clinic and Districts

Clinic, District	Piped Water, %	Flush Toilet Connected to Sewage System, %
Dr Georges Mukhari Hospital		
Soshanguve	58.7	85.3
Ga-Rankuwa	73.6	90.1
Mabopane	62.5	85.4
Madibeng[a]	22.2	27.2
Nkomo clinic		
Greater Giyani Municipality[b]	13.4	11.9

Data are from the South African Government STATS SA Community Survey of 2016 [12].

[a]Only part of Madibeng District is considered to be in the Dr Georges Mukhari Hospital catchment area.

[b]Greater Giyani Municipality lies within Mopani District.

Sample Collection

After the patients were given a clear explanation of the stool sample collection process, they received screw-cap bottles into which they placed their samples. Stool samples were classified as diarrheal or nondiarrheal on the basis of the physical presentation of the sample, as defined by the Bristol stool form scale (diarrheal specimens, types 6 and 7; nondiarrheal specimens, types 1–5) [13]. The bottles were labelled with unique participant identifiers and then placed in a cooler box and transported to the University of Venda microbiology laboratory for further processing. Upon arrival to the laboratory, samples were aliquoted in 2-mL tubes and stored frozen at −20°C. The aliquoted samples were shipped to the University of Virginia Infectious Diseases Research laboratory for analysis.

Genomic DNA Purification

Genomic DNA from each patient's sample was extracted using a QIAamp DNA Stool Mini Kit (Qiagen) according to the manufacturer's recommended procedures, using approximately 200 mg of stool samples with the modifications described by Liu et al [14]. One stool sample from a healthy US child whose stool had previously been tested and found to be negative for all *Entamoeba* species was included in each batch, to monitor for the occurrence of contamination during extraction. The DNA was eluted in 200 µL of elution buffer (Qiagen) and stored at −80°C until further analysis.

Multiplex qPCR Assay for Detection of *Entamoeba* Species

A multiplex qPCR assay was used for the amplification and detection of all *Entamoeba* species. Genus-specific primers were used in combination with a 42-nucleotide probe that should hybridize to *E. bangladeshi*, *E. dispar*, *E. histolytica*, and *E. moshkovskii* amplicons. Owing to the length required to generate this probe in these A/T-rich genomes, a double quencher was included in the design of the probe (Biosearch Technologies; Figure 1). This probe recognizes *E. histolytica*, *E. moshkovskii*, *E. dispar, E. bangladeshi*, and *E. hartmanni* amplicons but was not similar to the ribosomal RNA (rRNA) region in

Figure 1. Alignment of the 18S ribosomal DNA sequences of the *Entamoeba* species that are known to infect humans. *A*, Quantitative polymerase chain reaction assay designs for detection of *Entamoeba histolytica* (GenBank accession no. KX528461.1), *Entamoeba dispar* (KP722600.1), *Entamoeba moshkovskii* (AF149906.1), and *Entamoeba bangladeshi* (KR025412.1) Sequences were aligned using the Clustal Omega program [41]. The target of the *Entamoeba* genus probe is highlighted in gray, and the sequences of the species-specific probes and the genus-specific primers are underlined.

the nonpathogenic species *E. coli, E. polecki*, *Endolimax nana*, *Iodamoeba bütschlii* and *Entamoeba gingivalis*. The PCR was performed with 25-μL reaction mixture containing Bio-Rad iQ powermix, 0.4 μM of primers, and 0.2 μM for each probe. Probes, primers, and reaction conditions are shown in Table 2.

Analysis of qPCR *Entamoeba*-Positive Samples Uncharacterized at the Species Level by the qPCR Assay

Any sample that gave a positive signal with the *Entamoeba* probe but was negative for all 4 *Entamoeba* species of interest (ie, *E. histolytica*, *E. moshkovskii*, *E. dispar*, and *E. bangladeshi*) was characterized further by amplifying additional 18S regions and determining their sequences [3, 15]. This DNA was amplified by primers Ehd-88R and EM-RT-F2 (Table 2), using the high-fidelity Phusion polymerase as described by Royer et al (98°C for 30 seconds; 40 cycles at 98°C for 20 seconds, 56°C for 30 seconds, and 72°C for 30 seconds; and a final extension at 72°C for 10 minutes) [3]. A 2% agarose gel stained with 3 μL of ethidium bromide was used to separate the amplified DNA. The PCR products were extracted from the agarose gel by using the Qiagen QIAquick Gel Extraction Kit, and the purified amplicons were sequenced using the Sanger method (Genewiz).

Sequence and Phylogenetic Analysis

After sequence results were obtained, the ABI files were downloaded and trimmed using Geneious (version 7.0.6). A phylogenetic tree was created to examine the phylogenetic relationship between novel *Entamoeba* species and the known *Entamoeba* parasites by use of the neighbor-joining algorithm included in Geneious (Biomatters) [16].

P. copri qPCR Assay

To improve the specificity of qPCR detection of *P. copri* in clinical fecal samples, a new TaqMan assay was designed, using the National Center for Biotechnology Information (NCBI) reference sequence NR_113411.1. Optimal qPCR assay conditions were initially determined using DNA amplified from cultured *P. copri* (CB7, DSMZ; a gift from D. Littman). Assay specificity was determined by purification and sequencing of the amplicons from selected fecal samples and by comparison with both the NCBI reference sequence NR_113411.1 and the sequence obtained from the cultured *P. copri* DNA. The PCR was performed with a 25-μL reaction mixture containing Bio-Rad iQ powermix, 0.4 μM of primers, and 0.2 μM for each probe. Probes, primers, and reaction conditions are shown in Table 2. The previously described Enterobacteriaceae assay was used to normalize both the *P. copri* levels and as a measure of extracted bacterial DNA quality [9]. Enterobacteriaceae- and *P. copri*–negative samples were omitted from the quantitative analysis of *P. copri* (Supplementary Table 1).

Statistical Analysis

The Fisher exact test was used to analyze contingency tables. The D'Agostino and Pearson omnibus normality test and the

Table 2. Entamoeba Species and Prevotella copri Probes, Primers, and Cycling Conditions

Oligonucleotide (Dye)	Probe/Primer Sequence[a]	Cycling Conditions
E. histolytica probe (FAM)	TCATT+GAATGAATTGGCCATTT[b]	95°C for 3 min, then 40 cycles at 95°C for 10 s and 61°C for 20 s
E. bangladeshi probe (Texas Red)	CCTTACAGAG+TATGGCCAATTT[b]	95°C for 3 min, then 40 cycles at 95°C for 10 s and 61°C for 20 s
E. dispar probe (HEX)	ACTTA+CATAAATTGGCCAACTTT[b]	95°C for 3 min, then 40 cycles at 95°C for 10 s and 61°C for 20 s
E. moshkovskii probe (Quasar 670)	CCGTGAAGAGAGTGGCCGA[b]	95°C for 3 min, then 40 cycles at 95°C for 10 s and 61°C for 20 s
Entamoeba genus probe (Quasar 705)	GGATAGCTT[I-X]TGTGAATGATAAAGATAATACTT GAGACGATCC[c]	95°C for 3 min, then 40 cycles at 95°C for 10 s and 61°C for 20 s
Ehd-88R	GCGGACGGCTCATTATAACA[d]	95°C for 3 min, then 40 cycles at 95°C for 10 s and 61°C for 20 s
EM-RT-F2	GTCCTCGATACTACCAAC[e]	95°C for 3 min, then 40 cycles at 95°C for 10 s and 61°C for 20 s
P. copri probe (FAM)	TGCCCACCACT+TGG	95°C for 3 min, then 40 cycles at 95°C for 10 s and 60°C for 1 min
P. copri F	AAGCTTGCTTTTGATGGGCG	95°C for 3 min, then 40 cycles at 95°C for 10 s and 60°C for 1 min
P. copri R	TGATCGT+CGCCTTGG	95°C for 3 min, then 40 cycles at 95°C for 10 s and 60°C for 1 min

[a]Each "+" indicates the location of a "locked" nucleotide [43], and "[I-X]" indicates the location of the internal quencher.

[b]Probe from Arju and Gilchrist (unpublished data).

[c]Probe specific to this study.

[d]Primer Ehd-88R from Verweij et al [44].

[e]Primer EM-RT-F2 from Lau et al [22].

nonparametric Mann-Whitney comparisons test were used to analyze and compare qualitative data. Tests were performed using GraphPad Prism, version 6. The differences were considered significant if the *P* value was <.05.

RESULTS

Demographic and Clinical Features

A total of 484 participants were recruited in this study, of whom 227 (47%) were from Giyani (a rural setting) and 257 (53%) were from Pretoria (an urban setting). Table 3 summarizes the demographic data of the study population.

Rural Setting

Participants with recorded ages ranged between 2 and 73 years old, with the majority (41%) aged 6–64 years. Thirty-seven percent (83) were male, and 46% (105) were female; for 17% (39), data on sex were not recorded. Of the 227 stool samples collected, 61 (27%) were diarrheal and 166 (73%) were nondiarrheal.

Table 3. Demographic and Clinical Features of the Study Population

Characteristic	Rural Setting, Giyani (n = 227)	Urban Setting, Pretoria (n = 257)
Sex (n = 395)		
Male	83 (37)	104 (40)
Female	105 (46)	103 (40)
Not recorded	39 (17)	50 (19)
Age, y (n = 346)		
Range	2–73	1–90
Mean ± SD	19.2 ± 17.71	41.7 ± 22.13
<5	37 (16.3)	19 (7.4)
6–64	93 (41)	154 (60)
>65	6 (3)	37 (14.4)
Not recorded	91 (40)	47 (19)
Stool type[a] (n = 484)		
Diarrheal (type 6 and 7)	61 (27)	125 (49)
Nondiarrheal (types 1–5)	166 (73)	132 (51)

Data are no. (%) of participants, unless otherwise stated.

[a]Based on the Bristol stool scale.

Urban Setting

Participants with recorded ages ranged between 1 and 90 years old, with the majority (60%) aged 6–64 years. Male sex was recorded for 40% (104), and 40% (103) were female; data on sex were not recorded for 19.5% (50). Of the 257 stool samples collected, 125 (49%) were diarrheal and 132 (51%) were nondiarrheal.

Prevalence and Distribution of *Entamoeba* Species, by qPCR Analysis

Of the 484 samples tested by qPCR analysis, 29% (140) were positive for *Entamoeba* species. The total frequency of *E. dispar* detected by qPCR in the study population was 8% (38), with *E. histolytica* detected in 6.4% (31), *E. bangladeshi* detected in 4.5% (22), and unknown *Entamoeba* species detected in 10% (49). In 11 samples, coinfections with different *Entamoeba* species were observed (7 were coinfected with *E. histolytica* and *E. bangladeshi*, 1 was coinfected with *E. bangladeshi* and *E. dispar*, and 3 were coinfected with *E. histolytica* and *E. dispar*). In line with previous results, *E. moshkovskii* was not identified in the South African populations studied (Figure 2).

Confirmatory Testing

To confirm the *E. bangladeshi* qPCR assay results, we sequenced the amplicon from 4 positive samples and compared the sequences to the *E. bangladeshi* sequence deposited in the NCBI GenBank (accession number KR025412.1). The South African sequences were identical to that of *E. bangladeshi*.

Characterization of *Entamoeba* Samples Not Identified by Species-Specific Probes

Entamoeba primers (Ehd-88R; EM-RT-F2) were used to amplify DNA fragments from the 49 samples that were qPCR

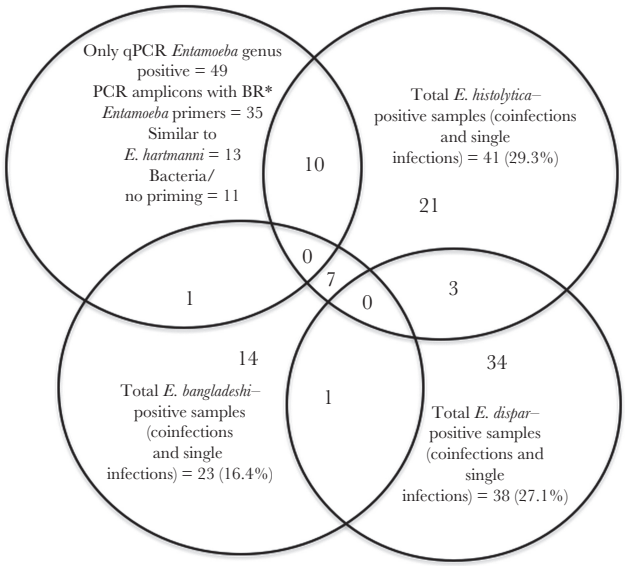

Figure 2. *Entamoeba* species found in South African populations. A total of 140 *Entamoeba*-positive samples were identified by quantitative polymerase chain reaction (qPCR) analysis. In 11 samples, the infecting species could not be identified. Thirty-eight samples were positive for *Entamoeba dispar,* 41 were positive for *Entamoeba histolytica*, 23 were positive for *Entamoeba bangladeshi,* and 0 were positive for *Entamoeba moshkovskii*. In 11 samples, coinfections with different *Entamoeba* species were observed (7 were coinfected with *E. histolytica* and *E. bangladeshi*, 1 was coinfected with *E. bangladeshi* and *E. dispar*, and 3 were coinfected with *E. histolytica* and *E. dispar*). *E. hartmanni*, *Entamoeba hartmanni*. *Broad range (BR).

positive for the broad range *Entamoeba* but negative for all the species-specific probes. The amplified DNA was separated by electrophoresis, and in the 35 cases where the bands of the size predicted for the *Entamoeba* species were identified, it was purified from the agarose by using the QIAquick Gel Extraction Kit (Qiagen). The SSU rRNA gene amplicon was detected in 35 samples. Sequencing of the purified amplicon identified 10 additional *E. histolytica*–positive samples (n = 41), with an adjusted frequency *Entamoeba* qPCR-positive samples of 29.3%, and 1 additional *E. bangladeshi* sample (n = 23), with an adjusted positivity frequency of 16.4% (Figure 2). This result suggested that in these samples the parasite level had simply fallen below the detection limit of the species-specific qPCR assay. These samples were not included in the later analysis. In 13 cases, the 18S rRNA amplicon sequences were similar to those of the nonpathogenic species *E. hartmanni* (all sequences were deposited in GenBank under accession numbers MF471201–MF471217), and in the remaining 11 cases either no useful sequence data were obtained or findings were similar to sequences from bacteria and had no significant similarity to any *Entamoeba* reference sequence in the NCBI database.

Parasite Burden in South African Samples

Other enteropathogens are common in this South African population, and in diarrheal samples coinfections can make it challenging to identify the causal organism [4]. *Entamoeba* were no more frequent in diarrheal samples than in controls (data not shown). No differences in *Entamoeba* frequency was observed between the rural and urban populations. The cycle value at which the (baseline-corrected) amplification curve exceeds the background fluorescence (Cq) is closely related to the amount of input DNA. The Cq data provided by the qPCR assay can therefore be used to determine whether the *Entamoeba* species burden was different in diarrheal and control fecal samples [4, 13, 17]. As the distribution of Cq values were non-Gaussian, significance was determined using the Mann-Whitney test. As expected, a significant difference in *Entamoeba* levels was observed in cases of *E. histolytica*–associated diarrhea ($P = .0072$; Figure 3A), but the level of the nonpathogenic species *E. dispar* was unchanged in control and diarrheal samples (Figure 3C). Interestingly, the level of *E. bangladeshi* was also unchanged (Figure 3B). Again, no significant differences were observed in the parasite burden in rural and urban samples.

Quantity of *P. copri* in *Entamoeba*-Positive Samples.

In a Bangladesh study, elevated levels of the pathobiont *P. copri* were associated with *E. histolytica*–associated diarrhea [9, 18]. The level of *P. copri* in *Entamoeba*-positive diarrheal and control samples was measured and, to control for variations in fecal bacterial numbers, was normalized using an Enterobacteriaceae bacterial reference [9, 19]. A fecal DNA standard was used to control for any differences in amplification efficiency in the *P. copri* and Enterobacteriaceae qPCR assays. Samples negative for either *P. copri* or Enterobacteriaceae were omitted from the quantitative analysis towing to concerns about sample quality (Supplementary Table 1). To convert the qPCR results to bacteria concentrations, DNA was extracted from a known amount of *E. coli* (ATCC 25922) and assayed. The relative level of *P. copri* was 1 log lower in *E. histolytica*–colonized samples as compared to the level in *E. histolytica* diarrheal samples (Figure 4A) but was unchanged in *E. dispar* or *E. bangladeshi* infections when diarrheal and nondiarrheal cases were compared (Figure 4B and 4C).

DISCUSSION

The present study reports an overall frequency of *Entamoeba* species in our samples collected from gastrointestinal clinics as 27% (129/484), with *E. histolytica* being present in 6.4% of the cases (31/484). Differences in the assay used, as well as in age, geographic location, and the fact that these samples were collected from gastrointestinal clinics, make it difficult to compare these results to those obtained from previous population-based studies [4, 8]. A weakness in the current study was that information on human immunodeficiency virus status (expected to increase with age) was not available. In addition, the study was not adequately powered to analyze the susceptibility to *Entamoeba* among participants stratified by age [20, 21].

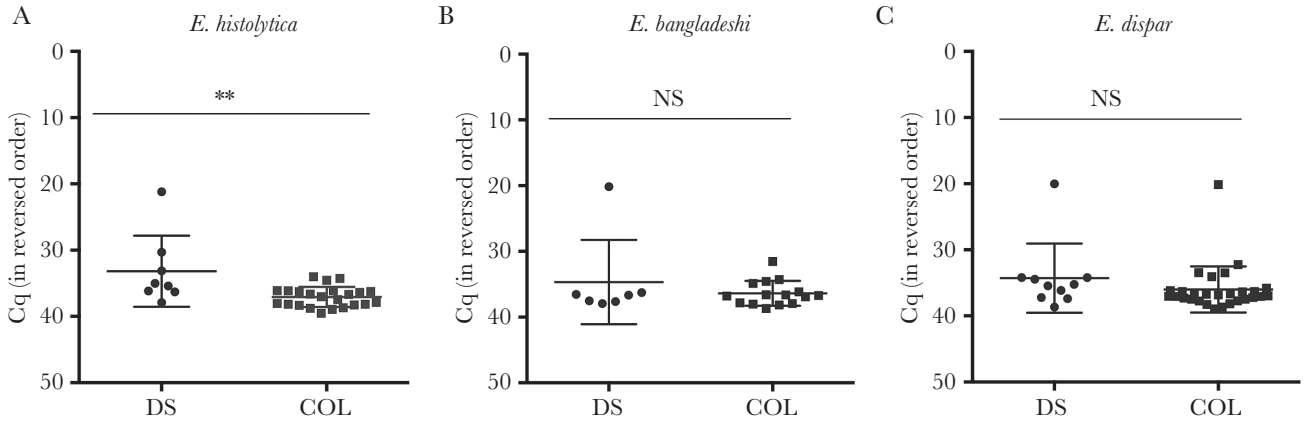

Figure 3. High parasite burden was associated with diarrhea due to *Entamoeba histolytica* (*A*) but not *Entamoeba bangladeshi* (*B*) and *Entamoeba dispar* (*C*). The *y*-axes indicate threshold values of quantitative polymerase chain reaction analyses positive for each parasite. Horizontal lines indicate mean values, and vertical lines indicate standard deviations. $^{**}P \leq .0072$, by the Mann-Whitney test. Cq, cycle value at which the (baseline-corrected) amplification curve exceeds the background fluorescence; NS, not significant.

The Cq of the majority of our *Entamoeba*-positive asymptomatic samples was ≥35 and would have been missed by a less sensitive assay. Assay specificity at high Cq values was confirmed by amplicon sequencing of select samples (data not shown). In the work reported here, the assay included an *Entamoeba* general probe that acted as an independent control to identify any potential closely related novel South African *Entamoeba* species present in these samples (an in-depth surveillance of the *Entamoeba* species in the Mopani district of South Africa had not previously been done). All assay results were analyzed to be certain that the species-specific signal remained at a constant ratio to the result obtained from the broad range probe. The probe would have recognized any *Entamoeba* species similar to the pathogenic species *E. histolytica*, *E. moshkovskii*, and *E. bangladeshi* or the nonpathogenic species *E. dispar* (Figure 1A). The sequences of the closely related species also blocked nonspecific hybridization, allowing the higher assay Cq cutoff of ≤40 and increased assay sensitivity (Figure 1A) [22].

A higher *E. histolytica* parasite burden in samples increases the probability that the *E. histolytica* strain detected is responsible for diarrheal symptoms [9, 17]. In agreement with the previous studies, our results showed a statistically significant increase in the *E. histolytica* parasite load in South African diarrheal samples. The level of the nonpathogenic species *E. dispar* did not significantly change. This suggested that diarrhea coincident with *E. dispar* infections was due to other pathogens. The

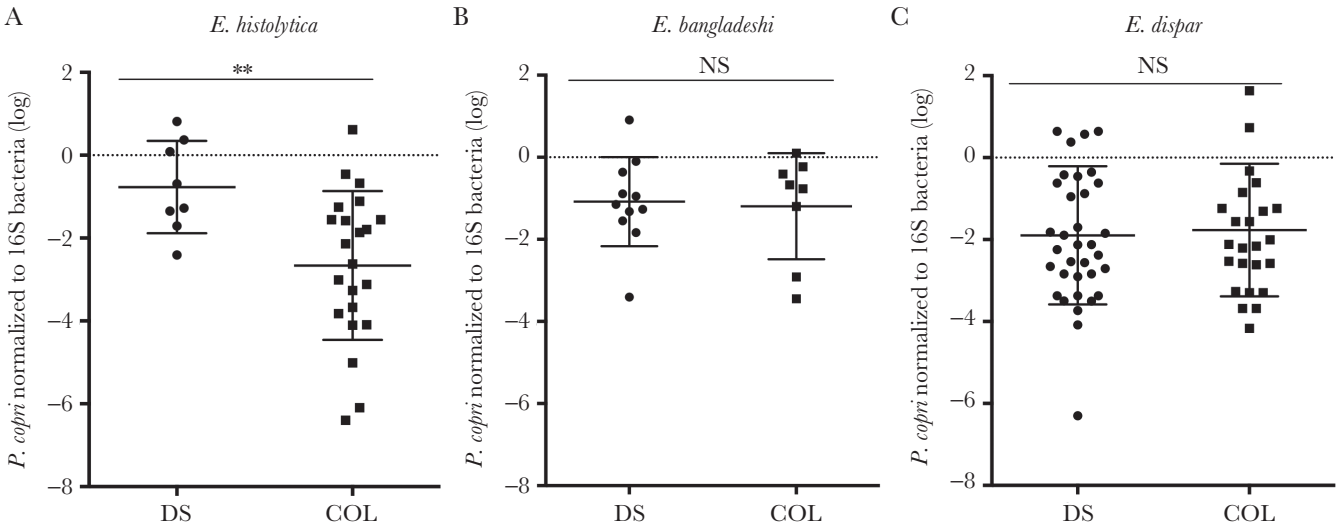

Figure 4. Increased levels of *Prevotella copri* were associated with diarrhea due to *Entamoeba histolytica* (*A*) but not *Entamoeba bangladeshi* (*B*) and *Entamoeba dispar* (*C*). In the *y*-axes, the cycle values at which the (baseline-corrected) amplification curve exceed the background fluorescence were converted to bacteria numbers by use of a calibration curve and normalized to the Enterobacteriaceae levels. Horizontal lines indicate mean values and vertical lines indicate the standard deviations. $^{**}P = .0034$, by the Mann-Whitney test. COL, asymptomatic colonizer samples; DS, diarrheal samples; NS, not significant.

pathogenicity of the recently identified *E. bangladeshi* is still uncertain, but the level of *E. bangladeshi* was also the same in both diarrheal and nondiarrheal South African samples. Additional work is planned to identify whether other coinfecting enteric pathogens are present in these samples.

Novel *Entamoeba* species have been identified in different geographical contexts. Therefore, samples that were positive with the *Entamoeba* general probe but negative with the species-specific probes were characterized by amplicon sequencing (Figure 3) [1, 3, 23, 24]. While novel South African *Entamoeb*a species were not identified, to our knowledge this study is the first to describe the presence of *E. bangladeshi* in samples collected outside Bangladesh. This species was first described in Bangladesh in 2011, but our results suggest that *E. bangladeshi* may actually have a broad geographical range and is prevalent in both Asian and African continents [3]. This finding also suggests that other members of the *Entamoeba* genus not identified in previous surveys may also be common in South Africa [5, 25–28]

In addition to the parasite burden, predisposition to diarrheal disease is thought to be influenced by the parasite environment [29–32]. Moreover, it has been suggested that specific components of the microbiota might be associated with symptomatic or asymptomatic *E. histolytica* colonization [33–35]. We examined the level of *P. copri* in the South African samples positive for *Entamoeba* species. Consistent with previous studies, the level of this bacterium was lower in asymptomatic *E. histolytica*–positive samples when compared to the level in *E. histolytica*–associated diarrheal samples [9]. Future work is needed to determine the significance of *E. histolytica*–associated changes in the microbiota. The gut *Prevotella* species are anaerobic bacilli predominant in the lumen of the colon [36]. Recent studies, however, suggest that disruption of the host mucosa can result in an increase in *Prevotella* species at mucosal sites and a subsequent increase in host inflammatory responses [18, 37]. Additional studies are needed to determine whether low *P. copri* levels could mitigate the host immune response occurring during amoebic colitis. It is possible that *E. histolytica*, unlike nonpathogenic *Entamoeba* species, disrupts the protective mucosal layer and exposes the host epithelium to the luminal microorganisms. This could expose the epithelium to high *P. copri* levels, as well as to *E. histolytica*, and result in an excessive inflammatory response with subsequent diarrhea [38–40]. In samples positive for the commensal *E. dispar* (which is not known to induce an inflammatory response or diarrhea), *P. copri* levels were not significantly different in either diarrheal or nondiarrheal samples [39].

In summary, an increase in the commensal bacterium *P. copri* was associated with diarrhea due to *E. histolytica*. The interplay between the pathogen, host, and host microbiota may be of importance in the development of symptomatic disease.

Supplementary Data

Supplementary materials are available at *The Journal of 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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