only risk factor identified among the persons reported here was older age. Unlike persons in other reports, persons in our report were all men, and 2 reported GI symptoms. The mechanism for UTI in these cases is unclear but could have included ascending and hematogeneous spread.

We calculated incubation periods for GI symptoms for 6 persons as the time between onset of GI symptoms and the May 14 wedding (5 persons) or last travel date (1 person). The incubation period was 5–7 days (average 5.5 days). The incubation period for UTI, which could be calculated for 2 persons, was an average of 25.5 days. Long incubation periods for *Salmonella* spp. infections have been reported (*6*–*9*); reasons include exposure to a low dose of bacteria, specific populations (e.g., young children, child day care attendees), and method of food preparation (*6*–*9*). The age of persons in our investigation did not affect the length of the incubation period. The amount of food eaten was not collected during the interview; however, most persons in our investigation reported eating a wide variety of foods, and 1 reported eating small portions. All food was prepared during the week before the wedding and served cold. This length of time and the potential for temperature abuse could have increased the infectious dose and decreased the incubation period (*6*). In addition, the 1 person with travel-related infection was not exposed to these food items. We found no literature on the incubation period for *S. enterica* ser. Agbeni. The reason for the long incubation period in this investigation is unclear and could be due to host-specific factors, the implicated serotype, or the food source.

The 3-day time frame for exposures was not sufficient to identify appropriate exposures. Expanding the period for collecting exposure information about *Salmonella* spp. infections and the reporting and investigation of persons with *Salmonella* spp. identified in urine to public health authorities might be needed to help identify and solve outbreaks.

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Entamoeba bangladeshi nov. sp., Bangladesh

To the Editor: Diarrheal diseases have a major effect on global health, particularly the health of malnourished children (*1*). The enteric parasites *Entamoeba histolytica* and *E. moshkovskii* are potential causes of diarrheal disease in children (*2*). For the past 20 years, we have been studying *Entamoeba* infections in children from the urban slum of Mirpur in Dhaka, Bangladesh (*3*).

E. histolytica infections can be detected through fecal microscopy, culture, PCR, and antigen detection. Microscopy and culture have limited specificity because several species of *Entamoeba,* which vary in their pathogenic potential, have morphologically similar cysts and trophozoites (*4*). In 2010–2011,

during analysis of feces positive for *Entamoeba* organisms by microscopy or culture but negative for *E. histolytica*, *E. dispar*, and *E. moshkovskii* by PCR, a new species was identified, which we have named *Entamoeba bangladeshi* nov. sp. in recognition of the support of the Bangladesh community for this research.

Feces from both diarrheal and surveillance specimens were collected from a cohort of children living in Mirpur (*3*). A total of 2,039 fecal samples were examined microscopically (0.9% saline smear) and/or by fecal culture for amebic trophozoites and cysts (*4*). One hundred forty-nine (7%) of the samples were positive by microscopy and/or culture for an *Entamoeba* parasite with both cysts and trophozoites that closely resembled those of *E. histolytica*, *E. moshkovskii*, and *E. dispar*.

DNA was extracted directly from fecal samples by using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA from positive fecal cultures was isolated by using the cetyl-trimethylammonium bromide extraction method (*5*). PCR was conducted to detect *E. histolytica*, *E. dispar*, and *E. moshkovskii*, all of which are morphologically indistinguishable by microscopy and are endemic to Bangladesh (Table) (*6*–*9*). An antigen detection test (TechLab Inc., Blacksburg, VA, USA) was also used to identify fecal samples positive for *E. histolytica*.

Fecal samples (129) and cultures derived from fecal material (20) were tested by PCR. Forty-four fecal samples were positive for *E. histolytica*, 42 for *E. dispar*, and 7 for *E. moshkovskii*. PCR results for 48 samples were negative for all 3 parasites (mixed infections account for the total being >129); 5 cultures also were negative for all 3 parasites.

ENTAGEN-F and ENTAGEN-R primers, which exhibit a broad specificity for the small subunit ribosomal RNA (SSU rRNA) gene sequences of *Entamoeba*, were used in PCR to amplify DNA fragments from 43 of the samples that were negative by PCR for the 3 *Entamoeba* species; amplification conditions were adapted from Stensvold et al. (*10*). The amplified DNA was separated by electrophoresis by using a 2% agarose gel. Bands of the size predicted for the *Entamoeba* spp. SSU rRNA gene amplicon were detected in 15 samples (online Technical Appendix Table, wwwnc.cdc.gov/EID/pdfs/12-0122-Techapp.pdf). The PCR products were extracted by using the QIAquick Gel Extraction Kit (QIAGEN) and cloned by using the Zero Blunt TOPO Cloning Kit (Invitrogen, Carlsbad, CA, USA). The sequenced clones from 2 different isolates, 1 diarrheal and 1 surveillance specimen, were completely novel when compared with the SSU rRNA gene sequences from other organisms and did not match any previously sequenced *Entamoeba* species. These isolates represent a new species of *Entamoeba* (GenBank accession nos. JQ412861 and JQ412862), here named *E. bangladeshi* (online Technical Appendix)

We examined the phylogenetic relationship between *E. bangladeshi* and other *Entamoeba* parasites by using maximum-likelihood analysis as implemented in MEGA 5 (online Technical Appendix Figure, panel A). *E. bangladeshi*, although distinct, clearly grouped with the clade of *Entamoeba* infecting humans, including *E. histolytica*. *E. bangladeshi*, however, appeared more distantly related than the noninvasive *E. dispar*, but closer than *E. moshkovskii,* to *E. histolytica*.

To further characterize *E. bangladeshi,* we established it in xenic culture, and it displayed the ability to grow at 37°C and 25°C, a characteristic shared with *E. moshkovskii* and *E. ecuadoriensis* but that distinguishes it from *E. histolytica* and *E. dispar*. Cultured trophozoites were evaluated through light and transmission electron microscopy (online Technical Appendix Figure, panel B). By light microscopy, we detected no apparent differences between *E. bangladeshi* and *E. histolytica*. The physical resemblance between *E. histolytica* and *E. bangladeshi* is notable because direct microscopic examination of fecal samples is still used as a diagnostic tool in areas to which these species are endemic to detect *E. histolytica* parasites.

Table. Oligonucleotide primers used for screening and sequencing of *Entamoeba bangladeshi* nov. sp., Bangladesh*

Target organism	Primer name	Primer sequence, 5′ → 3′	Reference
Broad specificity *Entamoeba* sp.	Entagen-F	ACT TCA GGG GGA GTA TGG TCA C	(*6*)
	Entagen-R	CAA GAT GTC TAA GGG CAT CAC AG	(*6*)
E. histolytica	Eh-F	AAC AGT AAT AGT TTC TTT GGT TAG TAA AA	(*8*)
	Eh-R	CTT AGA ATG TCA TTT CTC AAT TCA T	(*8*)
	Eh-YYT Probe	**YYT-**ATT AGT ACA AAA TGG CCA ATT CAT TCA**-Dark Quencher**	(*8*)
E. moshkovskii	Em-1	CTC TTC ACG GGG AGT GCG	(*7*)
	Em-2	TCG TTA GTT TCA TTA CCT	(*7*)
	nEm-1	GAA TAA GGA TGG TAT GAC	(*7*)
	nEm-2	AAG TGG AGT TAA CCA CCT	(*7*)
E. dispar	E-1	TTT GTA TTA GTA CAA A	(*9*)
	E-2	GTA [A/G]TA TTG ATA TAC T	(*9*)
	Ed-1	AGT GGC CAA TTT ATG TAA GT	(*9*)
	Ed-2	TTT AGA AAC AAT GTT TCT TC	(*9*)

*__Boldface__ indicates the probe fluorophore and quencher.

Our findings add to the diversity of *Entamoeba* species found in humans. The incidence and effect of infection in infants by the newly recognized species *E. bangladeshi* await future epidemiologic studies.

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Autochthonous *Leishmania siamensis* in Horse, Florida, USA

To the Editor: *Leishmania siamensis*, a recently described species, was identified as the cause of autochthonous visceral leishmaniasis in 2 men in southern Thailand (*1*,*2*). Cutaneous leishmaniasis has been reported in horses in Europe and South America. Lesions in horses are solitary or multiple nodules that are often ulcerated and most commonly occur on the head, pinnae, legs, and neck. Other clinical signs are usually absent. In South America, biochemical characterization has identified *L. braziliensis* in horses (*3*). Leishmaniasis has been reported in horses in Puerto Rico (*4*), and equine leishmaniasis has been described, but no reports have been published, in the United States. *L. infantum* has been reported in equine cutaneous leishmaniasis in Europe (*5*). A report from central Europe recently identified an organism with 98% nucleotide identity over the ITS (internal transcribed spacer) 1 region to *L. siamensis* as the cause of cutaneous leishmaniasis in 4 horses (*6*). *L. siamensis* was also identified in a case of cutaneous bovine leishmaniasis in Switzerland (*7*).

In August 2011, a 10-year-old, 505-kg Morgan horse mare in Florida, USA, with no history of travel outside the eastern United States was evaluated at the University of Florida for an ulcerated mass in the left pinna. When, 6 months earlier, the owner had noticed the mass, it was ≈1 cm in diameter, firm, raised, and covered with hair. Three months later, the ear was unchanged, and the mare was successfully impregnated. Over the subsequent 2 months, the mass began to grow and ulcerate. At that time, veterinary consultation was obtained and a biopsy performed. Histologic

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Entamoeba bangladeshi nov. sp., Bangladesh

Technical Appendix

Table. Sequencing outcomes for remaining 13 of 15 samples that were negative by PCR for *Entamoeba histolytica*, *E. dispar*, and *E. moshkovskii* and that were not the novel species *E. bangladeshi*

Specimen no.	Sequencing outcome
1	97% identity to *E. bangladeshi*, but insufficient sequence length led to ambiguous assignment, conservatively removed from further analysis
2	No priming upon sequencing
3	No priming upon sequencing
4	98% identity to *E. hartmanni,* 99% similar to specimen AM 385
5	Poor priming, produced sequence of insufficient length for analysis
6	98% identity to *E. hartmanni,* 99% similar to specimen AM 288
7	No priming upon sequencing
8	No priming upon sequencing
9	No priming upon sequencing
10	100% identity to *E. moshkovskii;* this was likely not picked up by species-specific qPCR secondary to degradation of the sample and loss of specific primer locus
11	No priming upon sequencing
12	No priming upon sequencing
13	No priming upon sequencing

Taxonomic Summary *Entamoeba bangladeshi*

Diagnosis. Microscopically indistinguishable from *Entamoeba histolytica* in cyst and trophozoite stages (*1*). In xenic culture, has the ability to grow at 37°C and room temperature, a characteristic shared with *E. moshkovskii* and *E. ecuadoriensis* but which distinguishes it from *E. histolytica* and *E. dispar*. Tests negative in *E. histolytica* ELISA and in species-specific PCRs. Currently only identifiable by its small subunit ribosomal RNA gene sequence.

Host/Type locality. Obtained from infant feces in Mirpur, Dhaka, Bangladesh, N 23°47′34,” E 90°21′38”

Etymology. Species name reflects the geographic origin of the specimen and recognizes the contribution of the Bangladeshi people to amebiasis research.

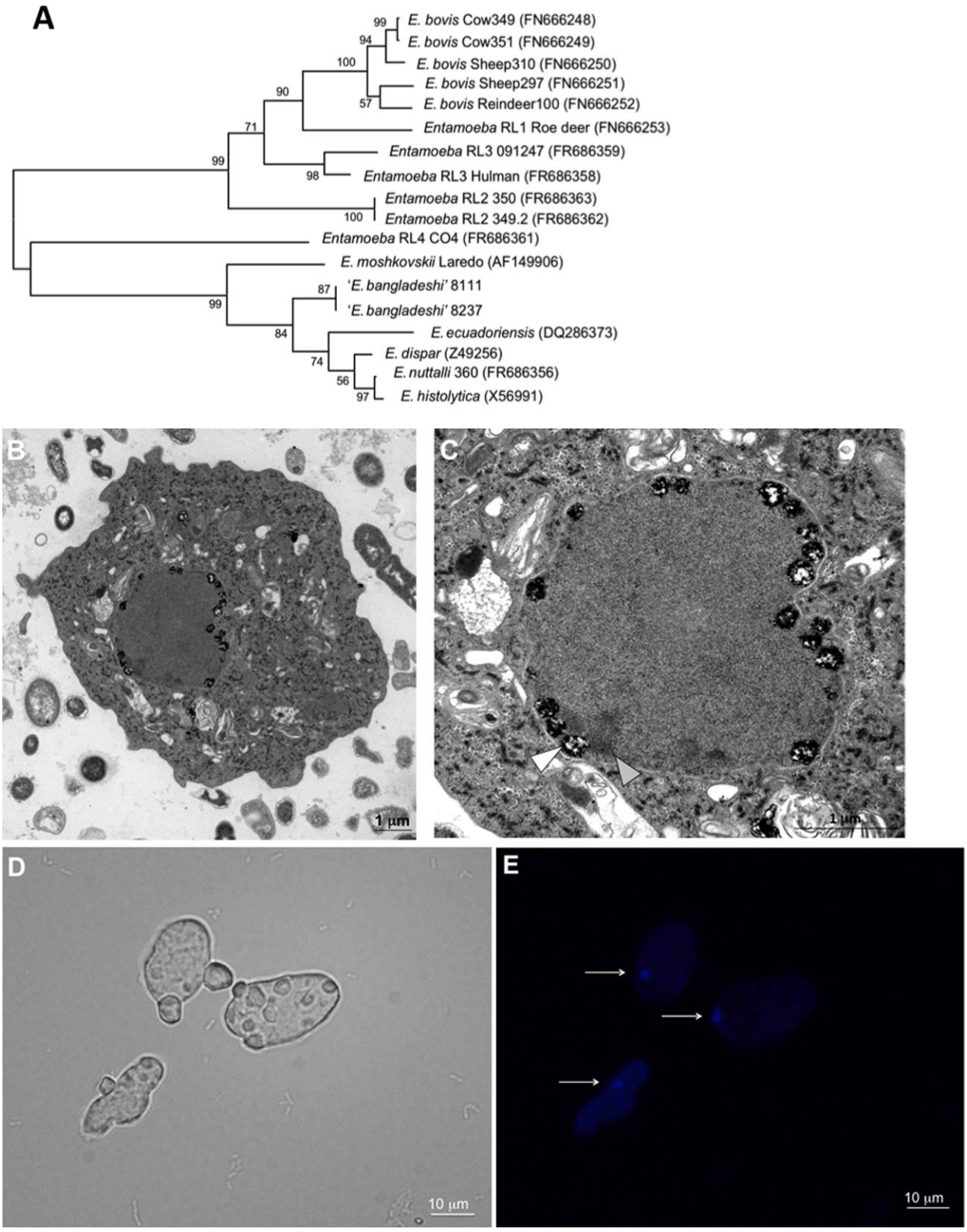

Figure. Phylogenetic relationships of *Entamoeba bangladeshi*. A) Phylogenetic analyses were performed by using maximum likelihood (MEGA 5 [*2*]) and a general time-reversible model of nucleotide substitution

with 4 γ-distributed categories of among-site rate variation and the proportion of invariant sites, selected by ModelTest (as implemented in MEGA 5). Statistical support was evaluated by using bootstrapping (1,000 replicates). Although unrooted, the tree is shown with the topology found by Stensvold et al. (*3*). The sequence alignment was derived from that used in Figure 2 of Stensvold et al. (*3*), with the remainder of the *E. bangladeshi* small subunit ribosomal gene sequence classified as missing data. Numbers in parentheses after species names are GenBank accession numbers. B) Transmission electron microscopy (TEM) demonstrating the ultrastructure of an *E. bangladeshi* trophozoite. Original magnification ×4,000. Scale bar = 1 μm. C) Higher magnification (×10,000) view of the nucleus of the *E. bangladeshi* trophozoite from panel B, illustrating the peripheral chromatin (gray arrowhead). *E. bangladeshi* and *E. histolytica* trophozoites have similar ultrastructure; however, the nucleus of *E. bangladeshi* presents more dark-staining structures (white arrowhead) than the typical *E. histolytica* nucleus. Scale bar = 1 μm. TEM samples were fixed in 2% paraformaldehyde and 2% glutaraldehyde in phosphate buffered saline and then postfixed with 1% osmium tetroxide plus 0.1% potassium ferrocyanide. Dehydration through an ethanol gradient was performed, followed by infiltration and embedment in epon. Sections were cut on a Leica Ultracut Ultramicrotome (Leica Microsystems Inc., Buffalo Grove, IL, USA), poststained with uranyl acetate and lead citrate, and imaged by using a JEOL 1230 transmission electron microscope (JEOL, Tokyo, Japan). D and E) Light microscopy analysis of *E. bangladeshi* trophozoites. Light microscopy samples were fixed with 4% paraformaldehyde and stained with DAPI (Invitrogen, Carlsbad, CA, USA) to visualize nuclei. Shown is a phase contrast image containing 3 amoebae (D) and the corresponding DAPI fluorescence (E). Nuclei are indicated with arrows. Scale bar = 10 μm.

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