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Cross-species Transmission Potential of *Entamoeba histolytica* among Humans, Chimpanzees, and Baboons in the Greater Gombe Ecosystem, Tanzania

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Abstract

Cross-species Transmission Potential of *Entamoeba histolytica* among Humans, Chimpanzees, and Baboons in the Greater Gombe Ecosystem, Tanzania

By: Jessica Deere

Amoebiasis, the diarrheal disease caused by the protozoan *Entamoeba histolytica*, infects approximately 50 million people worldwide and results in up to 100,000 deaths annually. Amoebiasis is particularly problematic in developing nations where poverty and poor sanitation contribute to the contamination of food and water. Although *E. histolytica* is a zoonotic parasite that has the potential to infect non-human primates, the potential for such transmission in rural communities that overlap with wild primates remains unknown. Consequently, this study examined the cross-species transmission potential of *E. histolytica* among humans, chimpanzees, and baboons living in the Greater Gombe Ecosystem, Kigoma District, Tanzania. Risk factors for infection were analyzed using a cross-sectional survey designed for this rural, tropical system that is characterized by high rates of overlap among humans and non-human primates. Five hundred and eighty-seven fecal samples were screened for *Entamoeba* spp. and *E. histolytica*. Of the fecal samples examined, 60.3% of human samples, 65.6% of chimpanzee samples, and 88.6% of baboon samples were positive for *Entamoeba* spp. Further diagnostic PCR revealed *E. histolytica* infection rates of 12.1% in humans, 34.2% in chimpanzees, and 10.9% in baboons. Chimpanzees had a significantly higher frequency of infection than both humans and baboons. Humans that experienced gastrointestinal symptoms, such as diarrhea and stomach cramps, had greater odds of infection than humans who did not experience gastrointestinal symptoms, when controlling for age and sex (OR = 2.2723; 95% CI 1.0318-5.0043; $p = 0.0416$). Season, age, and sex were not reliable predictors of *E. histolytica* infection in humans, chimpanzees, or baboons. The high infection rate of *E. histolytica* in three sympatric primates – humans, chimpanzees, and baboons – suggests that zoonotic transmission is likely occurring and stresses the need for further phylogenetic studies. Interventions targeting better sanitation and hygiene practices for humans living in and around Gombe National Park can help prevent amoebiasis in humans, while also protecting the endangered chimpanzees and other primates in this region.

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1. Introduction

Entamoeba histolytica is an enteric protozoan parasite that infects approximately 50 million people worldwide, causing widespread morbidity and mortality (*1*). Amoebiasis, the diarrheal disease caused by this invasive parasite, is the second leading cause of death from intestinal parasitic disease worldwide, following cryptosporidiosis (*2*), killing approximately 40,000 to 100,000 people annually (*3*). Infection occurs through fecal-oral transmission, wherein the mature cyst of *E. histolytica* from fecal-contaminated food or water is ingested. *E. histolytica* infection is particularly problematic in developing nations due to poor sanitation and hygiene practices (*4*).

Prevention and control of amoebiasis is further complicated by the zoonotic potential of *E. histolytica*, which is known to infect both human and non-human primates (NHPs). Cysts and trophozoites of the genus *Entamoeba* have previously been identified in NHPs through standard microscopic examination (*5*,*6*,*7*,*8*). However, the nonpathogenic *Entamoeba dispar* is morphologically indistinguishable from *E. histolytica*; therefore, molecular identification is necessary (*9*). Despite the clinical significance of *E. histolytica*, few studies have been conducted on the risks zoonotic transmission pose to public health (*10*).

The life cycle of *E. histolytica*, displayed in **Figure 1**, begins with ingestion of an infectious cyst through fecal-contaminated food, water, or hands (*11*). After ingestion and passage through the stomach, the organism excysts in the small intestine and active trophozoites are released and emerge in the large intestine. Trophozoites multiply and produce cysts and both stages are passed in feces. While trophozoites do not survive outside the body, cysts may survive months in a moist environment (*11*). Trophozoites

may remain in the intestinal lumen, creating noninvasive colonization; invade the intestinal mucosa, becoming intestinal disease; or invade through the bloodstream, becoming extra-intestinal disease (*12*). The passage of cysts continues the life cycle.

Understanding the risks of zoonotic disease transmission is crucial to both human and animal health, especially in systems characterized by high rates of human-animal overlap (*13*,*14*). Gombe National Park, Tanzania is home to many NHPs, including baboons and chimpanzees. Many of the primates are habituated and live in close proximity to humans, thus human-primate interaction is common (*15*,*16*). As *E. histolytica* has been identified in chimpanzees and baboons, there is a potential for transmission of this parasite to humans in the Greater Gombe Ecosystem (GGE) (*17*,*5*).

Anthropogenic habitat change causes humans and NHPs to come into closer and more frequent contact, which leads to an increase in risk of zoonotic disease transmission (*19*,*20*). Additionally, habituation of primates to human presence for research and tourism in Gombe National Park provides opportunities for primates to be exposed to new diseases (*21*,*22*). Gombe National Park, established in 1968, is a small (35 km^2) forest reserve located on a narrow strip of land between Lake Tanganyika and a rift escarpment that rises from the lakeshore (*21*). Since establishment of the national park following Jane Goodall's research on the Gombe chimpanzees in 1960 (*23*), the woody vegetation and forest cover have increased inside the park (*21*). However, rapid human population growth, and the dependence of the Tanzanian economy on agriculture (*24*), has resulted in deforestation for conversion to farmland outside the park (*21*). Furthermore, desertification and soil degradation from droughts has caused natural resources to decline, leading to fragmented landscapes that increase human-wildlife contact (*15*,*16*).

Gombe National Park is home to seven species of nonhuman primates, including endangered eastern chimpanzees (*Pan troglodytes schweinfurthii*) and olive baboons (*Papio anubis*) (*17*). The wild chimpanzee population has been studied continuously for over 50 years and provides to the national economy of Tanzania through tourism (*16*). There are three chimpanzee communities: Mitumba, Kasekela, and Kalande (**Figure 2**); with Kalande being the only unhabituated community (*25*). This study focused on the two habituated communities of chimpanzees that experience different degrees of human encroachment. Members of the communities often overlap in habitat range; therefore, there is opportunity for contact among different communities. Mitumba, the smaller northern community, is located in proximity to Mwamgongo, a village of approximately 5000 human inhabitants and their livestock. Whereas, Kasekela, the larger central chimpanzee community, is located in less disturbed forest (*16*). Agricultural fields are raided by chimpanzees (T. Gillespie, Personal Communication). Researchers, tourists, park management staff, and local field assistants are allowed inside the park and the park border is not fenced; therefore, local villagers and their animals have access to the park (*16*). Unlike the densely populated northern and southern borders of the park, the eastern border is less settled because of the high elevation and soil depletion (*25*). Therefore, the GGE provides a unique setting to study disease transmission among a dense, isolated human population and the NHPs they come into contact with, both directly and indirectly.

E. histolytica has been identified microscopically in Gombe National Park (*17*), but the morphological similarity to other *Entamoeba* species mandates the molecular confirmation of *E. histolytica*. While fewer than 10% of those infected with *E. histolytica*

develop invasive amoebiasis (*26*), infection with this intestinal parasite in both humans and NHPs may cause diarrhea, hemorrhagic dysentery, liver abscesses and death (*27*,*28*). The clinical complications *E. histolytica* may cause for both humans and NHPs magnify its impact on this human population located in one of the world's poorest economies (*24*) and on the endangered chimpanzee population. To investigate the potential of zoonotic transmission of *E. histolytica* in this system and contribute to the long-term research in Gombe National Park, we examined patterns of infection with the parasite and assessed risk factors for infection among humans, chimpanzees, and baboons in the GGE, Tanzania.

2. Materials and Methods

2.1. Ethics statement

This project was reviewed and approved by the Emory University Institutional Review Board (approval #: IRB00018856) under the Expedited review process per 45 CFR 46.110(3), Title 45 CFR Subpart D section 46.404, one parent consent, and 21 CFR 56.110 and the Tanzanian National Institute for Medical Research Institute, Dar es Salaam, Tanzania, which approved oral consent due to low literacy rates. All adult subjects provided informed consent, and a parent or guardian of any child participant provided informed consent on their behalf. Oral informed consent was obtained by trained local field assistants and documented by witnessed notation on IRB-approved enrollment forms. All animal use followed the guidelines of the Weatherall Report and the NIH Guide for the Care and Use of Laboratory Animals on the use of nonhuman primates in research, and was approved by the Tanzania Wildlife Research Institute

and Tanzania Commission for Science and Technology (permit number 2009-279-NA-2009-184), and the Emory University Animal Care and Use Committee (protocol ID 087-2009). Approval was also obtained from Tanzania National Parks (Permit number TNP/HQ/C10/13) to collect samples from wild chimpanzees. The researchers did not have any interactions with the chimpanzees in the park.

2.2. Study site and sample collection

This study was conducted in the Greater Gombe Ecosystem, Kigoma District, Tanzania. Specifically, in Gombe National Park (4°40'S, 29°38'E) and the village of Mwamgongo (4°40'S, 29°34'60'E). Fecal samples were collected between March 2010 and February 2011. Human, chimpanzee, and baboon paired fecal samples were collected during the dry (July 1 – August 15) and wet (November 1 – December 15) seasons. Residents of Mwamgongo village (estimated population size ~5000) and residents of Gombe National Park (estimated population size ~100) comprised the human subjects. Residents of Gombe National Park consist of park staff, Jane Goodall Institute (JGI) researchers, and members of their families who reside at the park headquarters (Mitumba) or at the JGI headquarters (Kasekela). As part of routine observational monitoring (*38*), chimpanzees (n ~ 90) were sampled in Mitumba and Kasekela at quarterly intervals. Baboons (n ~ 198) were opportunistically sampled during the two collection periods.

Consenting human participants received specimen cups and instructions on how to collect the sample. Chimpanzee and baboon specimens were non-invasively collected from identified individuals immediately after defecation and transferred to a screw cap

plastic vial containing a 2.5% potassium dichromate solution (Fisher Scientific, Pittsburgh, PA). For chimpanzee and baboon samples, care was taken to avoid contamination from the ground by transferring only the interior and top most portion of feces to the vial using a sterile wooden spatula or swab and avoiding the collection of soil, foliage, or water contaminants. Each vial was labeled with a unique identification number and date of collection. Information such as name of the observer, location, and animal name was also recorded. Samples were sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL), stored at 4°C, and shipped in ice to Atlanta, GA, United States.

2.3. DNA extraction and molecular detection

Nucleic acid was extracted from all human, chimpanzee, and baboon fecal samples (n= 587) preserved in 2.5% potassium dichromate solution using the FastDNA® SPIN Kit for Soil (MP Biomedicals, LLC, Solon, OH) following the protocol outlined in da Silva et al. (*29*). DNA extracts were subsequently tested using a polymerase chain reaction (PCR) assay adapted from Foo et al. (*30*). First, a segment (~748 bp) of the *Entamoeba* spp. ribosomal small subunit (SSU-rRNA) gene was amplified by PCR. For specimens positive for *Entamoeba* spp., a segment (~301 bp) of *E. histolytica* SSU-rRNA gene was also amplified by PCR. PCR was performed by addition of 2 μl DNA template to a tube containing 18 μl of mastermix (10 μl of *Taq* DNA Polymerase (QIAGEN, Germantown, MD), 6 μl of distilled water, and 1 μl of each primer). For detection of *Entamoeba* spp., *Entamoeba* spp. forward and *Entamoeba* common reverse primers were used. For detection of *E. histolytica*, *E. histolytica* forward conserved and *Entamoeba* common reverse primers were used. All primers used are summarized in **Table 1**. PCR

amplification cycles were performed in an Eppendorf Mastercycler pro thermal cycler. For *Entamoeba* spp., thermal cycler settings were 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 55.8°C for 30 seconds, and 72°C for 30 seconds; 72°C for 10 minutes, and 4°C ∞. For *E. histolytica*., thermal cycler settings were 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 52.1°C for 30 seconds, and 72°C for 30 seconds; 72°C for 10 minutes, and 4°C ∞. The amplified PCR products were resolved by 1.0% agarose gel electrophoresis and stained with Invitrogen™ UltraPure™ Ethidium Bromide (Fisher Scientific, Pittsburgh, PA). All samples were run for 30 minutes at 80 volts. The DNA bands were visualized under UV illumination and photographed using a Molecular Imager Gel Doc™ XR System (BIO-RAD, Hercules, CA).

2.4. Human risk factor survey

A cross-sectional survey was administered by trained local field assistants in the national language (Swahili) to minimize response bias. Human subjects were selected randomly and enrollment was facilitated by a health officer. Each human subject was surveyed and the topics focused on demography, gastrointestinal symptoms, medication usage, and water usage. Data were first recorded on paper forms and then entered into spreadsheets in Microsoft Excel (Redmond, WA) and reviewed for accuracy.

2.5. Statistical analyses and control for sample bias

PCR results were manually recorded, entered into Microsoft Excel (Redmond, WA), and subsequently reviewed for accuracy. Chimpanzee and baboon individuals can have uneven sampling because of degree of habituation, distance of travel after

defecation, or other factors, thus we calculated infection rate using the number of individuals instead of the number of samples (*17*). To control for sample bias, we calculated infection rate as the proportion of individuals in each group positive for *E. histolytica* divided by the total number of individuals in each group examined. If a sample was negative for *Entamoeba* spp., the sample was considered negative for *E. histolytica*. Statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC). Associations between human survey responses and infection status were compared using logistic regression and odds ratios (OR) with 95% confidence intervals (CI) were calculated. A generalized estimated equation (GEE) with exchangeable working correlation structure was used to account for repeat sampling of individuals. Associations between available chimpanzee and baboon demographic and observational health data and infection status were also compared using the same statistical methods.

3. Results

Five hundred and eighty-seven fecal samples were screened for *Entamoeba* spp. including 267 human, 241 chimpanzee, and 79 baboon specimens (**Table 2**). *Entamoeba* spp. were detected by PCR from 389 (66.3%) fecal samples. *E. histolytica* was detected by PCR from 69 (11.8%) fecal samples. The infection rate of *E. histolytica* was highest among chimpanzees (34.1%), compared to humans (12.1%) and baboons (10.9%). Of the 23 detections of *E. histolytica* in humans, 14 (60.9%) resided in Mwamgongo village and nine (39.1%) lived inside of the park (seven in Kasekela camp and two in Mitumba camp) (**Table 3**).

Chimpanzees had a significantly higher frequency of *E. histolytica* than both

humans and baboons (Fisher's exact test $p = 0.0326$ and $p = 0.0369$, respectively). No significant differences in frequency were observed between humans and baboons (Fisher's exact test $p = 0.4989$) or between the two chimpanzee communities (Fisher's exact test $p = 0.4433$). There were also no significant differences in frequency observed between humans living outside the park and humans living inside the park (Fisher's exact test $p = 0.8337$) or between the two camps located inside the park (Fisher's exact test $p = 0.4907$).

Data from the cross-sectional survey were used to identify potential risk factors for *E. histolytica* infection. After checking for confounding effects, age and sex were controlled for in some of the models. Therefore, adjusted odds ratios are reported (**Table 4**). Persons who experienced gastrointestinal symptoms had approximately twice the odds of *E. histolytica* infection, when controlling for age and sex (OR = 2.2723; 95% CI 1.0318-5.0043; $p = 0.0416$). When reviewing survey data for *E. histolytica* positive persons, 7/23 reported cramping; 4 reported having diarrhea; 1 sought treatment at the village clinic (Metronidazole); and 3 had watery or bloody stool. Seven of the 23 individuals positive for *E. histolytica* lived in a household with at least one other *E. histolytica* positive person. Twenty-six percent of study respondents reported consuming water from an open source, although the association was not statistically significant (OR = 1.0724; 95% CI 0.3791-3.0337; $p = 0.8951$). Chimpanzee and baboon demographic factors such as age and sex were not risk factors for *E. histolytica* infection. Evidence of diarrhea was not a reliable predictor of *E. histolytica* infection in chimpanzees. No significant association with season was observed in humans, chimpanzees, or baboons (**Tables 4, 5, and 6**). Human samples showed a smaller frequency of infected samples

during the wet season (36%); whereas, chimpanzee and baboon samples both contained a higher frequency of infection during the wet season (72% and 80%, respectively).

As part of other studies, the samples screened for *E. histolytica* were also screened for other enteric pathogens such as *Cryptosporidium*, heat-labile and heat-stable enterotoxigenic *Escherichia coli*, *Salmonella*, and *Shigella*. When comparing presence of *E. histolytica* with presence or absence of other enteric pathogens, Fisher's exact tests show that infection with other enteric pathogens is random for humans, chimpanzees, and baboons (p = 0.321; 0.272; 1.000, respectively).

4. Discussion

This was the first study to molecularly confirm presence of *E. histolytica* in Gombe National Park. Infection rates ranging from 6-15% in humans and 30-36% in chimpanzees suggest high zoonotic potential for *E. histolytica* in areas with frequent human-NHP interaction. There was a higher than 60% frequency of *Entamoeba* spp. in all three primate species, further indicating the importance of understanding the distribution of *Entamoeba* in this system.

While a recent study detected no *E. histolytica* in a group of chimpanzees living in the Issa Valley in Tanzania (*9*), approximately 100 km east of the Gombe chimpanzee population, the Gombe chimpanzees showed a significantly higher frequency of *E. histolytica* infection than both humans and baboons. Unlike the Gombe chimpanzees, the Issa Valley chimpanzees do not come into regular contact with humans, aside from researchers (9). This suggests that close proximity to humans could be important for *E. histolytica* infection in chimpanzees. Lower *E. histolytica* infection rates in humans could

also be a result of improved hygiene and sanitation in the region due to the implementation of interventions such as the Jane Goodall Institute's TACARE program (*31*) and protocols intended to improve sanitation in staff quarters in Gombe (*17*).

The frequency of *E. histolytica* was expected to be higher in the Mitumba chimpanzee community due to the natural border it shares with Mwamgongo village. However, surprisingly, *E. histolytica* frequency did not differ between the two chimpanzee communities under investigation. Similarly, presence of *E. histolytica* did not differ when comparing humans residing in the Kasekela and Mitumba camps. Although, this may be an artifact of the low statistical power due to the small human population size inside the park.

Water is a possible source for transmission of *E. histolytica*, as the cysts of this parasite are very resistant and can survive for several months in water and the environment (*32*). In Gombe, humans drink from the same stream as NHPs. They use the same lake for bathing and washing clothes and cooking utensils that baboons and chimpanzees drink from (*33*). Despite playing and drinking from Lake Tanganyika (**Figure 2**) more often than chimpanzees (*33*), baboons had a significantly lower frequency (6.3%; $p = 0.0369$) of infection than chimpanzees. Therefore, future studies using comprehensive water sampling to investigate *E. histolytica* contamination in water sources used by humans and NHPs in the GGE would be beneficial to the understanding of *E. histolytica* transmission in this system.

Persons experiencing gastrointestinal symptoms, which included diarrhea and stomach cramping, were more likely to be infected with *E. histolytica* than those who were not experiencing gastrointestinal symptoms. This suggests that individuals may

have been suffering from symptomatic *E. histolytica*, which highlights the importance of controlling *E. histolytica* infection in this region.

The epidemiology of *Entamoeba* spp. is complicated, as pathogenic and non-pathogenic species are morphologically similar. The recent separations of *E. histolytica, Entamoeba dispar*, and *Entamoeba nuttalli* further complicate the matter. In 1993, *E. histolytica* was re-described as separated from the non-pathogenic *E. dispar*, which was first introduced by Brumpt in 1925 (*34*). The name *E. nuttalli* was revived in 2007 for another pathogenic *Entamoeba* spp. strain that was similar to *E. histolytica*, but phylogenetically between *E. histolytica* and *E. dispar* (*35*). This study targeted the SSU-rRNA gene of *E. histolytica*, which is distinct from *E. nuttalli* (*37*), thus the infection rates we report for *E. histolytica* represent only *E. histolytica;* however, our approach does not rule out the presence of *E. nuttalli* in the GGE and a subset of the *Entamoeba* spp. positive specimens may represent *E. nuttalli.* Most studies to date have only identified *E. nuttalli* in NHPs (*35*,*36*); however, *E. nuttalli* has been detected in humans (*37*). Our findings highlight the potential for zoonotic transmission of *Entamoeba* spp. and stress the need for further studies to determine cross-species transmission of both *E. nuttalli* and *E. histolytica* at the human–wild primate interface.

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6. Tables

Table 1. List of primers used in PCR for detection of *Entamoeba* spp. and *E. histolytica* in humans and non-human primates in and around Gombe National Park, Tanzania.

Primer name	**Primer characterization**	**Primer sequence (5'-3')**	**Reference**
Eg-SS-F1	*Entamoeba* spp. forward	TGTGATTAAAACGCTCGTAGTTGAA	Foo et al. (2012)
Eh-SS-F1	*E. histolytica* forward conserved	GAAGCATTGTTTCTAGATCTGA	Foo et al. (2012)
Eg-SS-CR1	*Entamoeba* common reverse	CTCGTTCGTTACCGGAATTAACC	Foo et al. (2012)

Table 2. Detection of *Entamoeba* spp. and *E. histolytica* in three primate species sampled in and around Gombe National Park, Tanzania.

Host	Positive for *Entamoeba* spp.	Positive for *E. histolytica*	Percent *E. histolytica* of *Entamoeba* spp.
Humans	**60.3% (161/267)**	**9.4% (25/267)**	**15.5% (25/161)**
Mwamgongo Village	65.8% (100/152)	9.9% (15/152)	15.0% (15/100)
Kasekela	51.3% (39/76)	10.5% (8/76)	20.5% (8/39)
Mitumba	56.4% (22/39)	5.1% (2/39)	9.1% (2/22)
Chimpanzees	**65.6% (158/241)**	**16.2% (39/241)**	**24.7% (39/158)**
Kasekela	71.2% (121/170)	17.6% (30/170)	24.8% (30/121)
Mitumba	52.1% (37/71)	12.7% (9/71)	24.3% (9/37)
Baboons			
All groups	**88.6% (70/79)**	**6.3% (5/79)**	**7.1% (5/70)**

Table 3. Infection rate of *E. histolytica* detected by location in and around Gombe National Park, Tanzania.

Host	Positive/Total	Infection Rate (95% CI)
Humans		
Mwamgongo Village	14/96	0.15 (0.09-0.25)
Kasekela	7/61	0.11 (0.05-0.24)
Mitumba	2/33	0.06 (0.02-0.24)
Chimpanzees		
Kasekela	21/58	0.36 (0.24-0.56)
Mitumba	8/27	0.30 (0.15-0.59)
Baboons		
All groups	5/46	0.11 (0.05-0.26)

Table 4. Risk factors for *E. histolytica* infection in humans living in and around Gombe National Park, Tanzania.

			95% CI		
Variable	**n**	**Adjusted OR**	**Lower**	**Upper**	**p**
Sex (female vs. male)[a]	182	0.8701	0.3536	2.1552	0.7619
Season (dry vs. wet)[b]	182	1.7688	0.7841	3.9902	0.1695
Age (≤7 years)[c]	188	1.1043	0.3559	3.4262	0.8636
Location (Mitumba vs Mwamgongo)[b]	124	0.4841	0.1028	2.2794	0.3588
Location (Kasekela vs Mwamgongo)[c]	156	0.9120	0.3213	2.5884	0.8626
Location (Mitumba vs Kasekela)[b]	92	0.4143	0.0917	1.8718	0.2521
Mwamgongo vs park resident[a]	182	1.3026	0.5203	3.2609	0.5723
Work in agricultural fields or forest[b]	182	0.9238	0.3229	2.6429	0.8826
Water not boiled before consumption[b]	182	1.0272	0.3755	2.8101	0.9583
Experienced gastrointestinal symptoms[b]	182	2.2723	1.0318	5.0043	0.0416*
Used commercial or traditional medicine[b]	182	0.2752	0.0230	3.2966	0.3085
Consumption of water from open water source[b]	182	1.0724	0.3791	3.0337	0.8951
Infected with another enteric pathogen[b,d]	182	0.5461	0.1936	1.5403	0.2528

*significant at α= 0.05
[a]controlling for age
[b]controlling for age and sex
[c]controlling for sex
[d]*Cryptosporidum hominis,* ETEC-ST, ETEC-LT*, Salmonella, or Shigella*

Table 5. Risk factors for *E. histolytica* infection in chimpanzees in Gombe National Park, Tanzania.

			95% CI		
Variable	**n**	**OR**	**Lower**	**Upper**	**p**
Sex (female vs. male)	83	1.2153	0.5659	2.6096	0.6171
Season (dry vs. wet)	83	1.0194	0.4802	2.1641	0.9600
Age (≤10 years)	83	0.6474	0.2664	1.5731	0.3371
Location (Kasekela vs Mitumba	83	1.4845	0.6189	3.5604	0.3761
Observed to have diarrhea	83	0.6687	0.3165	1.4131	0.2918
Infected with another enteric pathogen[a]	83	0.5677	0.2435	1.3236	0.1899

[a]*Cryptosporidum hominis,* ETEC-ST, ETEC-LT, *Salmonella, or Shigella*

Table 6. Risk factors for *E. histolytica* infection in baboons in Gombe National Park, Tanzania.

			95% CI		
Variable	**n**	**OR**	**Lower**	**Upper**	**p**
Sex (female vs. male)	47	2.1704	0.2459	19.1560	0.4855
Season (dry vs. wet)	47	0.2436	0.0268	2.2130	0.2097
Age (≤10 years)	47	0.1771	0.0201	1.5597	0.1189

7. Figures

Figure 1. Life cycle of *Entamoeba histolytica* (*12*).

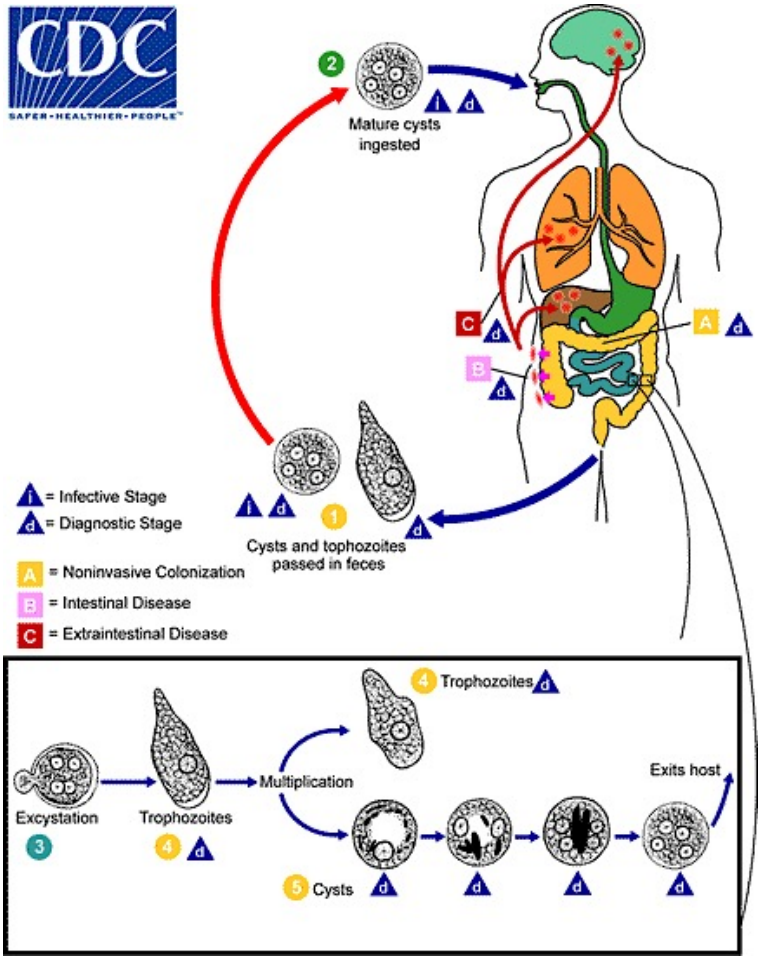

Figure 2. Chimpanzee communities located in Gombe National Park, Tanzania.