Parasitology

cambridge.org/par

Entamoeba histolytica infection in humans, chimpanzees and baboons in the Greater Gombe Ecosystem, Tanzania

Jessica R. Deere[1,*], Michele B. Parsons[2,3], Elizabeth V. Lonsdorf[4], Iddi Lipende[5], Shadrack Kamenya[5], D. Anthony Collins[5], Dominic A. Travis[6] and Thomas R. Gillespie[1,2]

[1]Department of Environmental Health, Rollins School of Public Health, Emory University, 1518 Clifton Road NE, Atlanta, GA 30322, USA; [2]Department of Environmental Sciences and Program in Population Biology, Ecology, and Evolutionary Biology, Emory University, Suite E510, 400 Dowman Drive, Atlanta, GA 30322, USA; [3]Division of Global Health, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, Georgia 30329, USA; [4]Department of Psychology, Franklin and Marshall College, 415 Harrisburg Ave, Lancaster, PA 17603, USA; [5]The Jane Goodall Institute, P.O. Box 1182, Kigoma, Tanzania and [6]College of Veterinary Medicine, University of Minnesota, 1988 Fitch Avenue, St. Paul, MN 55108, USA

Research Article

*Present address: College of Veterinary Medicine, University of Minnesota, 1988 Fitch Avenue, St. Paul, MN 55108, USA

Cite this article: Deere JR, Parsons MB, Lonsdorf EV, Lipende I, Kamenya S, Collins DA, Travis DA, Gillespie TR (2019). *Entamoeba histolytica* infection in humans, chimpanzees and baboons in the Greater Gombe Ecosystem, Tanzania. *Parasitology* **146**, 1116–1122. https://doi.org/10.1017/S0031182018001397

Received: 22 March 2018
Revised: 13 July 2018
Accepted: 20 July 2018
First published online: 30 August 2018

Key words:
Amebiasis; diarrhoea; primates; protozoa; wildlife disease

Author for correspondence:
Thomas R. Gillespie, E-mail: thomas.gillespie@emory.edu

Abstract

Entamoeba histolytica is an enteric parasite that infects approximately 50 million people worldwide. Although *E. histolytica* is a zoonotic parasite that has the potential to infect non-human primates, such transmission is poorly understood. Consequently, this study examined whether *E. histolytica* is present among humans, chimpanzees and baboons living in the Greater Gombe Ecosystem (GGE), Tanzania. The primary aims were to determine patterns of *E. histolytica* infection in a system with human-nonhuman primate overlap and to test associations between infection status and potential risk factors of disease. *Entamoeba* spp. occurred in 60.3% of human, 65.6% of chimpanzee and 88.6% of baboon samples. *Entamoeba histolytica* occurred in 12.1% of human, 34.1% of chimpanzee and 10.9% of baboon samples. Human *E. histolytica* infection was associated with gastrointestinal symptoms. This was the first study to confirm the presence of *E. histolytica* in the GGE. The high sample prevalence of *E. histolytica* in three sympatric primates suggests that zoonotic transmission is possible and stresses the need for further phylogenetic studies. Interventions targeting better sanitation and hygiene practices for humans living in the GGE can help prevent *E. histolytica* infection in humans, while also protecting the endangered chimpanzees and other primates in this region.

Introduction

Entamoeba histolytica is an enteric protozoan parasite that causes the disease amebiasis, the second leading cause of death from intestinal parasitic disease worldwide, following cryptosporidiosis (Ali, 2015). The exact extent of morbidity and mortality is currently a point of contention; however, estimates suggest that it infects approximately 50 million people worldwide, killing approximately 40 000–100 000 people annually (Inam *et al.*, 2016). In fact, in 2010, amebiasis was responsible for 55 500 deaths worldwide (Lozano *et al.*, 2012). Infection occurs through fecal-oral transmission, wherein the mature cyst of *E. histolytica* from fecal-contaminated food or water is ingested. *Entamoeba histolytica* infection is particularly problematic in developing nations due to less capacity for sanitation and hygiene practices (Ashbolt, 2004).

There are more than 20 species of *Entamoeba*, with varying pathogenic potential and host specificity (Nozaki and Bhattacharya, 2015). Unfortunately, many of these species of *Entamoeba* are morphologically indistinguishable from *E. histolytica*, including the potentially-pathogenic but rare *Entamoeba nuttalli* (Levecke *et al.*, 2015), as well as several nonpathogenic species of *Entamoeba*, including *Entamoeba dispar*, *Entamoeba moshkovskii* and *Entamoeba bangladeshi* (Nozaki and Bhattacharya, 2015; Jirku-Pomajbíková *et al.*, 2016). Consequently, molecular diagnostics are required to differentiate these species. Prevention and control of amebiasis is further complicated by the zoonotic potential of *E. histolytica*, which is known to infect both human and captive nonhuman primates (NHPs) (Rivera *et al.*, 2010). *Entamoeba histolytica* is of more urgent global health concern as a neglected tropical disease (Ximénez *et al.*, 2011). Importantly, although *E. nuttalli* is genetically similar to *E. histolytica* (Tachibana *et al.*, 2007), it cannot be detected by polymer chain reaction (PCR) based on the gene targeted in the current study (ribosomal small subunit) (Levecke *et al.*, 2015). Despite the clinical significance of *E. histolytica*, few studies have been conducted on the risks zoonotic transmission pose to public health (Thompson and Smith, 2011).

Understanding the risk of zoonotic disease transmission is crucial to both human and animal health, especially in systems characterized by high rates of human-animal overlap

CAMBRIDGE UNIVERSITY PRESS

(Pedersen *et al.*, 2005; Bowden and Drake, 2013). Anthropogenic habitat change causes humans and NHPs to come into closer and more frequent contact, which leads to an increase in the risk of zoonotic disease transmission (Gillespie *et al.*, 2008; Nunn and Gillespie, 2016). Gombe National Park, Tanzania is home to seven species of NHPs, including endangered eastern chimpanzees (*Pan troglodytes schweinfurthii*) and olive baboons (*Papio anubis*) (Gillespie *et al.*, 2010). Many primates are habituated to human presence and live in close proximity to humans, thus human–primate interaction – through means such as primate crop-raiding, shared water sources and human presence in primate habitat – is common (Parsons *et al.*, 2015). Additionally, habituation for research and tourism provides opportunities for primates to be exposed to new diseases (Pusey *et al.*, 2008; Gilardi *et al.*, 2015). As *Entamoeba* spp. have been identified in chimpanzees and baboons, there is a need to investigate whether it is possible for pathogenic species of *Entamoeba* to transfer to humans in the Greater Gombe Ecosystem (GGE) (Gillespie *et al.*, 2010; Howells *et al.*, 2011).

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 (Pusey *et al.*, 2008). Since the establishment of the national park following Jane Goodall's research on chimpanzees in 1960 (Goodall, 1986), the woody vegetation and forest cover have increased inside the park (Pusey *et al.*, 2008). However, rapid human population growth and the dependence of the Tanzanian economy on agriculture, has resulted in deforestation for conversion to farmland in the GGE (Pusey *et al.*, 2008). Furthermore, desertification and soil degradation from droughts have caused natural resources to decline, leading to fragmented landscapes that increase human–wildlife contact (Parsons *et al.*, 2014).

The wild chimpanzee population in Gombe National Park has been studied continuously for over 50 years and bolsters the national economy of Tanzania through tourism (Pusey *et al.*, 2008). There are three chimpanzee communities: Mitumba, Kasekela and Kalande (Pusey *et al.*, 2007). This study focused on the two habituated communities of chimpanzees that experience different degrees of human encroachment (Pusey *et al.*, 2007). Mitumba, the smaller northern community, is located in proximity to Mwamgongo, a village of approximately 5000 humans and their livestock. Whereas, Kasekela, the larger central chimpanzee community, is located in the less disturbed forest (Parsons *et al.*, 2015). Researchers, tourists, park management staff and local field assistants are the only humans allowed to reside inside the park, but the park border is not fenced; therefore, local villagers and their animals have access to the park (Parsons *et al.*, 2015). 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 (Pusey *et al.*, 2007). Therefore, the GGE provides a unique setting to study disease transmission among a dense human population and the NHPs they encounter, both directly and indirectly.

Entamoeba histolytica has been identified microscopically in Gombe (Gillespie *et al.*, 2010) 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 amebiasis (Wilson *et al.*, 2012), infection with this intestinal parasite in humans may cause diarrhoea, haemorrhagic dysentery, liver abscesses and death (Ali *et al.*, 2008). Invasive amebiasis has also been observed in NHPs through necropsy and studies have shown that *E. histolytica* infection in NHPs mimics human infection (Haq *et al.*, 1985; Verweij *et al.*, 2003). Clinical complications of *E. histolytica* infection for both humans and NHPs may cause detrimental impacts on the human population as well as the endangered chimpanzee population.

The present study aimed to (1) quantify the presence of *E. histolytica* in a system with human-NHP overlap, and (2) test associations between infection status in humans, chimpanzees and baboons and potential risk factors of enteric disease. We predicted that these primate species would have similar sample prevalence of *E. histolytica* given that they frequently come into contact, potentially acting as reservoirs for one another. Specifically, we hypothesized that the sample prevalence of *E. histolytica* would be higher in the Mitumba chimpanzee community, than in Kasekela, because of the natural border it shares with Mwamgongo. To investigate the potential of zoonotic transmission of *E. histolytica* in this system, we examined patterns of infection with the parasite and assessed risk factors for infection among humans, chimpanzees and baboons in the GGE, Tanzania.

Materials and methods

Study site and sample collection

This study was conducted in the GGE, 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 (estimated population size ~5000) and residents of Gombe (~100) comprised the human subject pool. Human subjects were chosen by methods described in complementary projects (Parsons *et al.*, 2014, 2015). Residents of the park consist of Tanzania National Park staff (TANAPA), Jane Goodall Institute (JGI) researchers and members of their families who reside at the park (Mitumba) or JGI (Kasekela). As part of a routine observational monitoring program (Lonsdorf *et al.*, 2018), chimpanzees were sampled in Mitumba (71 samples from 27 individuals) and Kasekela (170 samples from 58 individuals) communities at quarterly intervals. Baboons (79 samples from 46 individuals) 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 topmost 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 labelled with a unique identification number and date of collection. Information such as observer name, location and the 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, USA.

DNA extraction and molecular detection

Nucleic acid was extracted from 587 human, chimpanzee and baboon (267, 241 and 79, respectively) fecal samples 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.* (1999). DNA extracts were subsequently tested using a polymerase chain reaction (PCR) assay adapted from Foo *et al.* (2012). First, a segment (~748 bp) of the *Entamoeba* spp. small subunit ribosomal

https://doi.org/10.1017/S0031182018001397 Published online by Cambridge University Press

ribonucleic acid (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). Appropriate positive and negative controls were used for all analyses. 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 from Foo *et al.* (2012). PCR amplification cycles were performed in an Eppendorf Mastercycler pro (Hamburg, Germany) thermal cycler. PCR amplification cycles for *Entamoeba* spp. and *E. histolytica,* independently, were optimized from those described (Foo *et al.*, 2012). For *Entamoeba* spp., thermal cycler settings were 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55.8 °C for 30 s and 72 °C for 30 s; 72 °C for 10 min, and 4 °C ∞. For *E. histolytica*., thermal cycler settings were 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 52.1 °C for 30 s and 72 °C for 30 s; 72 °C for 10 min and 4 °C ∞. The amplified PCR products were resolved by 1.0% agarose gel electrophoresis stained with Invitrogen™ UltraPure™ Ethidium Bromide (Fisher Scientific, Pittsburgh, PA) and run for 30 min at 80 v. DNA bands were visualized under UV illumination and photographed using a Molecular Imager Gel Doc™ XR System (BIO-RAD, Hercules, CA).

Human risk factor survey and NHP health-monitoring program

A cross-sectional survey was administered by trained local field assistants in the national language (Swahili) to minimize response bias. Human subjects were selected by methods described in complementary projects (Parsons *et al.*, 2014, 2015) and enrollment was facilitated by a health officer. Each human subject was surveyed on topics related to acquiring enteric diseases. Data were first recorded on paper forms and then entered into spreadsheets in Microsoft Excel (Redmond, WA).

Chimpanzee and baboon behavioural and observational data were provided by the Gombe Ecosystem Health Program (Lonsdorf *et al.*, 2018). Continuous behavioural and observational data, such as primate behaviour and fecal condition, were non-invasively collected. The long-term behavioural record of these primates allows for the inclusion of demographic data such as age and sex.

Statistical analyses and control for sample bias

PCR results were manually recorded and entered into Microsoft Excel (Redmond, WA). Chimpanzee and baboon individuals can have uneven sampling because of the degree of habituation, distance of travel after defecation, or other factors, thus we calculated sample prevalence using the number of individuals instead of the number of samples (Gillespie *et al.*, 2010). To control for sample bias, we calculated sample prevalence 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*. If any single individual sample was confirmed positive for *E. histolytica*, the subject was considered positive for the collection period.

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 estimating 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.

Results

In total, 587 fecal samples were screened for *Entamoeba* spp., including 267 human, 241 chimpanzee and 79 baboon specimens (Table 1); representing approximately 2% of Mwamgongo, 94% of park residents, 100% of Mitumba chimpanzee community, 89% of Kasekela chimpanzee community and 23% of all baboons. Overall, *Entamoeba* spp. (including *E. histolytica*) were detected by PCR from 389 (66.3%) fecal samples and *E. histolytica* was detected by PCR from 69 (11.8%) fecal samples. Fecal samples represent all samples collected, which include paired fecal samples collected during the dry and wet seasons.

More than half of the samples collected from each primate species were positive for *Entamoeba* spp. (Table 1). Approximately 60% of human samples screened were positive for *Entamoeba* spp. One-hundred of the 152 Mwamgongo samples (65.8%) and 61 of the 115 park resident samples (53%) were positive for *Entamoeba* spp. For all chimpanzees sampled, 65.6% of samples were positive for *Entamoeba* spp., 71.2% of Kasekela chimpanzees and 52.1% of Mitumba chimpanzees. Almost 90% of baboon samples were positive for *Entamoeba* spp.

Sample prevalence, representing individual infection, was calculated for individuals confirmed positive for *E. histolytica* (Table 2). The sample prevalence of *E. histolytica* was higher in chimpanzees (34.1%) than humans (12.1%) or baboons (10.9%). Of the 23 detections of *E. histolytica* in humans, 14 (60.9%) resided in Mwamgongo and nine (39.1%) lived inside of the park (seven in Kasekela camp and two in Mitumba camp). Humans living in Kasekela camp had more positive individuals (7/61) than Mitumba camp (2/33). Similarly, Kasekela chimpanzees had more positive individuals (21/58) compared with Mitumba chimpanzees (8/27).

Chimpanzees had a significantly higher sample prevalence among individuals positive for *E. histolytica* than either humans and baboons (Fisher's exact test $P = 0.0326$ and $P = 0.0369$, respectively). No significant difference in sample prevalence of positive individuals was 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 sample prevalence observed between humans living inside and outside 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. Logistic regression models were specified to include possible confounding variables within the survey results. After checking for confounding effects through comparisons of unadjusted and adjusted ORs among all the variables, age and sex were controlled for in the models which had more than a 10% difference between unadjusted and adjusted ORs. Therefore, adjusted ORs are reported where applicable (Table 3). 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; $P = 0.0416$; Table 3). When reviewing survey data for *E. histolytica* positive persons, 7/23 reported cramping; four reported having diarrhea; one sought treatment at the village clinic (Metronidazole); and three had a watery or bloody stool. Seven of the 23 individuals positive for *E. histolytica* lived in a household with at least one other *E.*

https://doi.org/10.1017/S0031182018001397 Published online by Cambridge University Press

Table 1. Detection of *Entamoeba* spp. and *E. histolytica* in three primate species (total samples) in and around Gombe National Park, Tanzania

Host[a]	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	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)

[a]Some individuals have repeat sampling due to collection during both wet and dry seasons.

Table 2. Sample prevalence of *E. histolytica* detected by species and location in and around Gombe National Park, Tanzania

Host	Positive/Total	Sample prevalence (95% CI)
Humans		
Mwamgongo	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)

histolytica positive person. Chimpanzee and baboon demographic factors such as age and sex were not risk factors for *E. histolytica* infection in this sample (Tables 4 and 5). Diarrhea was also not a reliable predictor of *E. histolytica* infection in chimpanzees (Table 4). No significant association with the season was observed in humans, chimpanzees, or baboons (Tables 3–5). Human samples showed a smaller amount of infected samples during the wet season (36%); whereas, chimpanzee and baboon samples contained a higher amount of infection during the wet season (72% and 80%, respectively).

Discussion

This was the first study to molecularly confirm the presence of *E. histolytica* in the GGE. We sampled humans, chimpanzees and baboons, resulting in 587 samples overall. *E. histolytica* was found in all communities sampled. High sample prevalence identified among all species could indicate significance, as death from infectious diseases is the leading cause of mortality for Gombe chimpanzees (Lonsdorf *et al.*, 2018). However, chimpanzees had significantly higher sample prevalence than both humans and baboons, yet diarrhoea was not a risk factor for *E. histolytica* presence in chimpanzees. Therefore, chimpanzees with *E. histolytica* may not be symptomatic; however, our methods do not confirm current infection, so we cannot confirm this. Furthermore, we did not have baboon fecal consistency data. Further investigations into whether the NHPs of the GGE are symptomatic is necessary. Interestingly, Rivera *et al.* (2010) confirmed *E. histolytica* in NHPs, but also noted that they did not exhibit typical symptoms of amebiasis. NHPs could be more resistant against infection from *E. histolytica* than humans.

Our methodology was 2-fold: first screen all samples for *Entamoeba* spp., then identify *E. histolytica* in the *Entamoeba* spp. positive samples. This is important because pathogenic and non-pathogenic species are morphologically similar. The recent separation of *E. histolytica, E. dispar* and *E. nuttalli* adds to the complexity of the epidemiology of *Entamoeba* spp. In 1993, *E. histolytica* was re-described as separated from the non-pathogenic *E. dispar*, which was first introduced by Brumpt in 1925 (Diamond and Clark, 1993). The name *E. nuttalli* was revived in 2007 for another pathogenic *Entamoeba* spp. strain that is similar to *E. histolytica*, but phylogenetically between *E. histolytica* and *E. dispar* (Tachibana *et al.*, 2007). Most studies to date have only identified *E. nuttalli* in NHPs (Tachibana *et al.*, 2007, 2013; Levecke *et al.*, 2010; Rivera *et al.*, 2010); however, it has been detected in human zoo caretakers who were in contact with *E. nuttalli* positive NHPs (Levecke *et al.*, 2015), suggesting that transmission of *E. nuttalli* from NHPs to humans might also be possible.

While we recognize that the absence of DNA sequencing is a limitation of this study, we targeted the SSU-rRNA gene of *E. histolytica*, which is distinct from *E. nuttalli* (Levecke *et al.*, 2010, 2015). Thus, the reported sample prevalence for *E. histolytica* represent only *E. histolytica;* however, our approach does not rule out the presence of *E. nuttalli* in the GGE. A subset of the *Entamoeba* spp. positive specimens may represent *E. nuttalli*.

Entamoeba spp. were detected in more than 60% of all samples collected in the GGE, further indicating the importance of understanding the distribution of *Entamoeba* in this system. Sample prevalence ranging from 6 to 15% in humans and 30–36% in chimpanzees suggest there is a risk for zoonotic transmission. The sample prevalence of *E. histolytica* was expected to be higher in the Mitumba chimpanzee community due to the natural border it shares with Mwamgongo, assuming greater human-NHP contact. However, surprisingly, *E. histolytica* sample prevalence did not differ significantly between the two chimpanzee communities.

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 (Hemmati *et al.*, 2015). In Gombe, humans drink from the same stream as NHPs and use the same lake for bathing and washing clothes and utensils that baboons consume (Wallis and Lee, 1999). Despite contacting Lake Tanganyika more often than chimpanzees (Murray *et al.*, 2000), baboons had a significantly lower sample prevalence than chimpanzees. Therefore, future studies using comprehensive water sampling

https://doi.org/10.1017/S0031182018001397 Published online by Cambridge University Press

Table 3. Risk factors for *E. histolytica* infection in humans living in and around Gombe National Park, Tanzania

Variable	*n*	Adjusted OR	95% CI		*P*
			Lower	Upper	
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

*Significant at $\alpha = 0.05$.
[a]Controlling for age.
[b]Controlling for age and sex.
[c]Controlling for sex.

Table 4. Risk factors for *E. histolytica* infection in chimpanzees in Gombe National Park, Tanzania

Variable	*n*	OR	95% CI		*P*
			Lower	Upper	
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

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

Variable	*n*	OR	95% CI		*P*
			Lower	Upper	
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

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.

Another potential factor involved with the transmission of *E. histolytica* is seasonality. Although we did not find a significant association between infection status and season among the three primate species, there is often an association between diarrheal disease and season (Haque *et al.*, 2006). Studies have shown that *E. histolytica* infection in humans does have marked seasonality, with peaks during the wet season (Mukherjee *et al.*, 2010). However, other studies have also found no relationship between *E. histolytica* infection and season (Haque *et al.*, 2006; Mukherjee *et al.*, 2010). In this study, humans had higher sample prevalence during the dry season; in contrast, chimpanzees and baboons had higher sample prevalence during the wet season. Therefore, a more in depth investigation into the seasonal relationship of *E. histolytica* infection could increase knowledge of the transmission dynamics of *E. histolytica* in this system.

While a recent study detected no *E. histolytica* in a group of chimpanzees living in the Issa Valley in Tanzania (Jirku-Pomajbíková *et al.*, 2016), approximately 100 km east of the Gombe chimpanzee population, the Gombe chimpanzees showed a significantly higher sample prevalence 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 (Jirku-Pomajbíková *et al.*, 2016). This suggests that close proximity to humans could be an important factor in potential *E. histolytica* infection in chimpanzees and vice versa. Chimpanzees could also be at higher risk because they consume red colobus monkeys (*Colobus badius tephrosceles*) and bushpigs (*Potamochoerus larvatus*) (Gilby, 2006). *Entamoeba histolytica* has been found in colobus monkeys (Gillespie *et al.*, 2005) and in non-primate mammals, such as dogs (Alam *et al.*, 2015); therefore, transmission could occur through consumption of cysts from infected animals. Reports show that the Issa Valley chimpanzees consume blue duiker (*Philantomba monticola*) meat, but not red colobus monkeys or bushpigs, even though they are both present in the habitat (Ramirez-Amaya *et al.*, 2015). This further supports the possibility that the Gombe chimpanzees become infected by ingesting cysts from the colonic content of their prey. Additional investigations into the presence of *E. histolytica* in other wildlife and domestic animals in the GGE could shed further light into the transmission of *E. histolytica* in this system. Moreover, lower *E. histolytica* sample prevalence in humans could be a result of improved hygiene and sanitation in the region due to the implementation of interventions such as the JGI's TACARE program (Mavanza and Grossman, 2007) and protocols intended to improve sanitation in staff quarters in Gombe (Gillespie *et al.*, 2010).

https://doi.org/10.1017/S0031182018001397 Published online by Cambridge University Press

Persons experiencing gastrointestinal symptoms, which included diarrhoea 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 emphasizes the importance of controlling *E. histolytica* infection in this region. The role of NHPs in *E. histolytica* transmission has not yet been elucidated. Our findings highlight the potential for zoonotic transmission of *Entamoeba* spp. and stress the need for further phylogenetic studies to determine cross-species transmission of both *E. nuttalli* and *E. histolytica* at the human–NHP interface. Interventions targeting better sanitation and hygiene practices for humans living in and around Gombe National Park can help prevent *E. histolytica* infection in humans, while also protecting the endangered chimpanzees and other primates in this region.

Acknowledgments. We are grateful to Deus Mjungu, Juma Baranyikwa and the field assistants of the Jane Goodall Institute's Gombe Stream Research Centre for assisting in the collection of chimpanzee and baboon demographic and health data and nonhuman primate fecal specimens. We thank the Kigoma district health officers for assistance with human survey and sampling and the Tanzanian Commission for Science and Technology, Tanzania National Parks and Tanzania Wildlife Research Institute for permission to conduct the research. We thank J. Bodager, K. Cross, M. Hensley, S. Kuthyar, L. Rautman and D. Ryu for laboratory assistance.

Financial support. This work was supported by the Morris Animal Foundation (MAF D09ZO-041 and MAF D09ZO-634), the Emory University Global Health Institute, the Arcus Foundation, the Leo S. Guthman Fund, and the National Institutes of Health (R01 AI58715).

Conflict of interest. None.

Ethical standards. 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 with deferral from CDC Institutional Review Board, 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 NHPs 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 primates.

References

Alam MA, Maqbool A, Nazir MM, Lateef M, Khan MS and Lindsay DS (2015) Entamoeba infections in different populations of dogs in an endemic area of Lahore, Pakistan. *Veterinary Parasitology* **207**, 216–219.

Ali IKM (2015) Intestinal Amebae. *Clinics in Laboratory Medicine* **35**, 393–422.

Ali IKM, Clark CG and Petri WA (2008) Molecular epidemiology of amebiasis. *Infection, Genetics and Evolution* **8**, 698–707.

Ashbolt NJ (2004) Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology* **198**, 229–238.

Bowden SE and Drake JM (2013) Ecology of multi-host pathogens of animals. *Nature Education Knowledge* **4**, 5.

da Silva A, Bornay-Llinares F, Moura I, Slemenda S, Tuttle J and Pieniazek N (1999) Fast and reliable extraction of protozoan parasite DNA from fecal specimens. *Molecular Diagnosis* **4**, 57–64.

Diamond LS and Clark CG (1993) A Redescription of Entamoeba Histolytica Schaudinn, 1903 (Emended Walker, 1911) separating it from Entamoeba Dispar Brumpt, 1925. *Journal of Eukaryotic Microbiology* **40**, 340–344.

Foo PC, Chan YY, See Too WC, Tan ZN, Wong WK, Lalitha P and Lim BH (2012) Development of a thermostabilized, one-step, nested, tetraplex PCR assay for simultaneous identification and differentiation of Entamoeba species, *Entamoeba histolytica* and Entamoeba dispar from stool samples. *Journal of Medical Microbiology* **61**, 1219–1225.

Gilardi KV, Gillespie TR, Leendertz FH, Macfie EJ, Travis DA, Whittier CA and Williamson EA (2015) *Best Practice Guidelines for Health Monitoring and Disease Control in Great Ape Populations*. IUCN SSC Primate Specialist Group, Gland, Switzerland.

Gilby IC (2006) Meat sharing among the Gombe chimpanzees: harassment and reciprocal exchange. *Animal Behaviour* **71**, 953–963.

Gillespie TR, Greiner EC and Chapman CA (2005) Gastrointestinal parasites of the Colobus monkeys of Uganda. *Journal of Parasitology* **91**, 569–573.

Gillespie TR, Nunn CL and Leendertz FH (2008) Integrative approaches to the study of primate infectious disease: implications for biodiversity conservation and global health. *American Journal of Physical Anthropology Suppl* **47**, 53–69.

Gillespie TR, Lonsdorf EV, Canfield EP, Meyer DJ, Nadler Y, Raphael J, Pusey AE, Pond J, Pauley J, Mlengeya T and Travis DA (2010) Demographic and ecological effects on patterns of parasitism in eastern chimpanzees (Pan troglodytes schweinfurthii) in Gombe National Park, Tanzania. *American Journal of Physical Anthropology* **143**, 534–544.

Goodall J (1986) *The Chimpanzees of Gombe: Patterns of Behavior*. Boston: Belknap Press of the Harvard University Press.

Haq A, Sharma A, Ahmad S, Khan HM and Khan N (1985) Experimental infection of rhesus monkeys with *Entamoeba histolytica* mimics human infection. *Laboratory Animal Science* **35**, 481–484.

Haque R, Mondal D, Duggal P, Roy S, Farr BM, Sack RB, Kabir M and Petri Jr. WA (2006) *Entamoeba histolytica* infection in children and protection from subsequent amebiasis *Entamoeba histolytica* infection in children and protection from subsequent amebiasis. *Society* **74**, 904–909.

Hemmati A, Hooshmand E and Hosseini MJ (2015) Identification of *Entamoeba histolytica* by molecular method in surface water of Rasht City, Iran. *Iranian Journal of Public Health* **44**, 238–243.

Howells ME, Pruetz J and Gillespie TR (2011) Patterns of gastro-intestinal parasites and commensals as an index of population and ecosystem health: the case of sympatric western chimpanzees (Pan troglodytes verus) and guinea baboons (Papio hamadryas papio) at Fongoli, Senegal. *American Journal of Primatology* **73**, 173–179.

Inam A, Mittal S, Rajala MS, Avecilla F and Azam A (2016) Synthesis and biological evaluation of 4-(2-(dimethylamino)ethoxy)benzohydrazide derivatives as inhibitors of *Entamoeba histolyica*. *European Journal of Medicinal Chemistry* **124**, 445–455.

Jirku-Pomajbíková K, Čepička I, Kalousová B, Jirku M, Stewart F, Levecke B, Modrý D, Piel AK and Petrželková KJ (2016) Molecular identification of Entamoeba species in savanna woodland chimpanzees (Pan troglodytes schweinfurthii). *Parasitology* **143**, 741–748.

Levecke B, Dreesen L, Dorny P, Verweij JJ, Vercammen F, Casaert S, Vercruysse J and Geldhof P (2010) Molecular identification of *Entamoeba* spp. in captive nonhuman primates. *Journal of Clinical Microbiology* **48**, 2988–2990.

Levecke B, Dorny P, Vercammen F, Visser LG, Van Esbroeck M, Vercruysse J and Verweij JJ (2015) Transmission of *Entamoeba nuttalli* and *Trichuris trichiura* from Nonhuman primates to humans. *Emerging Infectious Diseases* **21**, 1871–1872.

Lonsdorf EV, Gillespie TR, Wolf TM, Lipende I, Raphael J, Bakuza J, Murray CM, Wilson ML, Kamenya S, Mjungu D, Collins DA, Gilby IC, Stanton MA, Terio KA, Barbian HJ, Li Y, Ramirez M, Krupnick A, Seidl E, Goodall J, Hahn BH, Pusey AE and Travis DA (2018) Socioecological correlates of clinical signs in two communities of wild chimpanzees (Pan troglodytes) at Gombe National Park, Tanzania. *American Journal of Primatology*, **80**, e22562.

Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Abdulhak A, Bin Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, De Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L,

https://doi.org/10.1017/S0031182018001397 Published online by Cambridge University Press

Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FGR, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Memish ZA, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KMV, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld RC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh P, Yip P, Zabetian A, Zheng Z, Lopez AD, Murray CJL (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet* **380**, 2095–2128. doi: 10.1016/S0140-6736(12)61728-0.

Mavanza M and Grossman AA (2007) Conservation and family planning in Tanzania: the TACARE experience. *Population and Environment* **28**, 267–273.

Mukherjee AK, Das K, Bhattacharya MK, Nozaki T and Ganguly S (2010) Trend of *Entamoeba histolytica* infestation in Kolkata. *Gut Pathogens* **2**, 2009–2010.

Murray S, Stem C, Boudreau B and Goodall J (2000) Intestinal parasites of baboons (Papio Cynocephalus Anubis) and Chimpanzees (Pan Troglodytes) in Gombe National Park Intestinal Parasites of Baboons (Papio Cynocephalus Anubis) and Chimpanzees (Pan Troglodytes) in Gombe. *Journal of Zoo and Wildlife Medicine* **31**, 176–178.

Nozaki T and Bhattacharya A (eds) (2015) *Amebiasis: Biology and Pathogenesis of Entamoeba*. Japan: Springer.

Nunn CL and Gillespie TR (2016) Pathogens and primate conservation. In: Wich S and Marshall A (eds). *Primate Conservation: An Introduction*. Oxford, UK: Oxford University Press, pp. 157–174.

Parsons MB, Gillespie TR, Lonsdorf EV, Travis D, Lipende I, Gilagiza B, Kamenya S, Pintea L and Vazquez-Prokopec GM (2014) Global positioning system data-loggers: a tool to quantify fine-scale movement of domestic animals to evaluate potential for zoonotic transmission to an endangered wildlife population. *PLoS ONE* **9**, 1–7.

Parsons MB, Travis D, Lonsdorf EV, Lipende I, Roellig DMA, Kamenya S, Zhang H, Xiao L and Gillespie TR (2015) Epidemiology and molecular characterization of *Cryptosporidium* spp. in humans, wild primates, and domesticated animals in the Greater Gombe Ecosystem, Tanzania. *PLoS Neglected Tropical Diseases* **9**, 1–14.

Pedersen AB, Altizer S, Poss M, Cunningham AA and Nunn CL (2005) Patterns of host specificity and transmission among parasites of wild primates. *International Journal for Parasitology* **35**, 647–657.

Pusey AE, Pintea L, Wilson ML, Kamenya S and Goodall J (2007) The contribution of long-term research at Gombe National Park to chimpanzee conservation. *Conservation Biology* **21**, 623–634.

Pusey AE, Wilson ML and Anthony Collins D (2008) Human impacts, disease risk, and population dynamics in the chimpanzees of Gombe National Park, Tanzania. *American Journal of Primatology* **70**, 738–744.

Ramirez-Amaya S, Stewart FA and Piel AK (2015) Savanna chimpanzees (Pan troglodytes schweinfurthii) consume and Share Blue Duiker (*Philantomba monticola*) meat in the Issa Valley, Ugalla, Western Tanzania. *Pan Africa News* **22**, 17–21.

Rivera WL, Yason JADL and Adao DEV (2010) *Entamoeba histolytica* and *E. dispar* infections in captive macaques (*Macaca fascicularis*) in the Philippines. *Primates* **51**, 69–74.

Tachibana H, Yanagi T, Pandey K, Cheng XJ, Kobayashi S, Sherchand JB and Kanbara H (2007) An *Entamoeba* sp. Strain isolated from rhesus monkey is virulent but genetically different from *Entamoeba histolytica*. *Molecular and Biochemical Parasitology* **153**, 107–114.

Tachibana H, Yanagi T, Lama C, Pandey K, Feng M, Kobayashi S and Sherchand JB (2013) Prevalence of *Entamoeba nuttalli* infection in wild rhesus macaques in Nepal and characterization of the parasite isolates. *Parasitology International* **62**, 230–235.

Thompson RCA and Smith A (2011) Zoonotic enteric protozoa. *Veterinary Parasitology* **182**, 70–78.

Verweij JJ, Vermeer J, Brienen EAT, Blotkamp C, Laeijendecker D, van Lieshout L and Polderman AM (2003) *Entamoeba histolytica* infections in captive primates. *Parasitology Research* **90**, 100–103.

Wallis J and Lee DR (1999) Primate conservation: the prevention of disease transmission. *Space Science Reviews* **96**, 317–330.

Wilson IW, Weedall GD and Hall N (2012) Host-Parasite interactions in *Entamoeba histolytica* and *Entamoeba dispar*: what have we learned from their genomes? *Parasite Immunology* **34**, 90–99.

Ximénez C, Morán P, Rojas L, Valadez A, Gómez A, Ramiro M, Cerritos R, González E, Hernández E and Oswaldo P (2011) Novelties on amoebiasis: a neglected tropical disease. *Journal of Global Infectious Diseases* **3**, 166–174.

https://doi.org/10.1017/S0031182018001397 Published online by Cambridge University Press