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ORIGINAL PAPER

Characterization of *Blastocystis* species infection in humans and mantled howler monkeys, *Alouatta palliata aequatorialis*, living in close proximity to one another

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Abstract This study characterizes *Blastocystis* species infections in humans and mantled howler monkeys, *Alouatta palliata aequatorialis*, living in close proximity to one another in northwestern Ecuador. *Blastocystis* species were identified from 58 of 96 (60.4 %) mantled howler monkey fecal samples, and 44 of 55 human fecal samples (81.5 %) by polymerase chain reaction. Using single-stranded conformation polymorphism, we were able to efficiently separate and sequence subtypes (STs) within mixed samples without the need for cloning. *Blastocystis* ST1, ST2, and ST3 were found in people, and two individuals were infected with more than one subtype. All monkey samples were ST8. The lack of shared subtypes between humans and monkeys suggests that no *Blastocystis* transmission occurs between these species in spite of close proximity in some instances. Based on analysis of demographic data from a questionnaire given to human participants, individuals who boiled their water before consumption were significantly less likely to be infected with *Blastocystis* (44.4 %) compared to those who did not (93.8 %) (p=0.002). No other risk factors were significant, although hunters, females, individuals living in large families, and those living closer to forested habitat tended to have a higher proportion of *Blastocystis* infections.

Keywords Gastrointestinal parasites · Epidemiology · Blastocystis infection · *Alouatta palliata aequatorialis* · Humans · Transmission · Zoonoses

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Introduction

Transmission of parasites between humans and wildlife poses a potential health risk to both groups (Polly 2005; Thompson 2013). The spread of emerging pathogens in either human or wildlife populations places each at risk of new infections or from expansion of previously rare pathogens (Muriuki et al. 1998; Daszak et al. 2000; Pedersen et al. 2005; Pedersen and Davies 2010). In the tropics, some 40 % of infectious diseases have been linked to nonhuman primate origins, likely due to the close evolutionary relatedness of humans to nonhuman primates and their often close contact with each other (Wolfe et al. 2007; Pedersen and Davies 2010). Coupled with this idea is the fact that the tropics are where some of the greatest ecological disturbances are occurring, creating a hotbed for future zoonoses (Morse 1995; Patz et al. 2000; Daszak et al. 2001; Chapman et al. 2005; Gillespie et al. 2005; Laurance et al. 2006; Mosandl et al. 2008; Püttker et al. 2008).

To investigate the likelihood of parasitic exchange between humans and wildlife, we chose the mantled howler monkey, *Alouatta palliata aequatorialis*, as our study species. This primate species lives in close proximity to people and often lives in degraded habitat or areas near agricultural fields which might be conducive to parasite transmission (i.e., crop raiding), and other gastrointestinal parasite studies have been conducted with *Alouatta* species (Gilbert 1994; Stuart et al. 1998; Cruz et al. 2000; Phillips et al. 2004; Stoner and Gonzalez 2006; Eckert et al. 2006; Vitazkova and Wade 2006; Trejo-Macías et al. 2007; Cristobal-Azkarate et al. 2010). Here we focused on *Blastocystis* species, namely because it is one of the most commonly encountered parasite species from our previous morphological study on howlers (Helenbrook 2014; Helenbrook et al. 2015). *Blastocystis* species represent a complex of genetic groups subdivided into subtypes (STs) representing phylogenetic lineages (Stensvold

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et al. 2007). The occurrence of 17 distinct subtypes in people and other wild and domestic animals has been summarized previously (Noel et al. 2005; Stensvold et al. 2007; Stensvold et al. 2009; Parkar et al. 2010; Alfellani et al. 2013a). For convenience, we will refer to this collection of subtypes heretofore as "*Blastocystis*"—as is the convention.

There are few reports identifying *Blastocystis* in wild howler monkeys (Phillips et al. 2004; Stoner and Gonzalez 2006; Milozzi et al. 2012; Ramirez et al. 2014), and this may simply be due to lack of testing and reporting. However, a study of *Blastocystis* subtypes in numerous animal hosts found in Colombia found that two sampled howler monkeys were ST4-positive (Ramirez et al. 2014). In captive howlers, ST8 has been reported from captive *Alouatta caraya* (Alfellani et al. 2013b). In other primates, including humans, a variety of subtypes have been found. For example, in Old World primates and in humans, several authors have reported ST1, ST2, ST3, and ST4 (Abe 2004; Noel et al. 2005; Scicluna et al. 2006; Yoshikawa et al. 2009; Parkar et al. 2010; Petrasova et al. 2011; Stensvold et al. 2012; Alfellani et al. 2013c). *Blastocystis* ST5 is widely found in apes (including humans), livestock, and several species of Old World monkeys (Noel et al. 2005; Parkar et al. 2010; Yan et al. 2007; Stensvold et al. 2009; Alfellani et al. 2013a, b, c). Subtype 6 has been reported in domestic animals and people (Scicluna et al. 2006; Parkar et al. 2010; Petrasova et al. 2011); subtype 7 has been found in primarily in birds, although it has also been reported in humans (Noel et al. 2005; Parkar et al. 2010; Alfellani et al. 2013b); subtype 8 has been found in several captive primate species, their caregivers, marsupials, and in a pheasant species (Stensvold et al. 2009; Petrasova et al. 2011; Alfellani et al. 2013b; Ramirez et al. 2014); subtype 9 has been found in humans, but not in nonhuman primates (Yoshikawa et al. 2004; Noel et al. 2005; Petrasova et al. 2011; Stensvold et al. 2012); and subtypes 10, 13, and 15 have all been found in numerous Old World primates (Alfellani et al. 2013b).

Subtyping can be conducted directly from DNA extracted from feces and is typically conducted using conventional PCR and sequencing of the small subunit ribosomal DNA (Scicluna et al. 2006; Whipps et al. 2010). However, when more than one subtype is present in a host, expensive and time-consuming cloning techniques—coupled with sequencing—are normally required to identify subtypes. A potentially faster and less expensive technique known as single-strand conformation polymorphism (SSCP) has previously been used to detect *Blastocystis* subtypes in humans (Menounos et al. 2008). The advantage of SSCP is that mixed subtypes can be separated on a gel and these fragments can then be sequenced by conventional methods.

The aim of this study was to examine the subtype composition of *Blastocystis* in humans and monkeys living in close proximity, using the SSCP methodology where mixed infections are encountered. For humans, questionnaire data will be used to determine whether there are particular attributes of people that make them more likely to harbor *Blastocystis* and a specific subtype. If interspecies transmission was happening or there was a common source of infection, then we would expect to find identical subtypes in both monkeys and people. Conversely, the occurrence of distinct subtypes in humans and monkeys would suggest limited transmission and lack of a common source of infection. We would also expect that if direct or indirect transmission (environmental sources or reservoir host transmission) was occurring, then people who are in closest contact with nonhuman primates—in this case researchers and hunters—would be the most likely to have the same *Blastocystis* subtypes found in howler monkeys.

Methods

Fecal sample collections were conducted in Ecuador at the Bilsa Biological Station (0° 21′ N, 79° 44′ W) for howler monkeys (N=96) and from people (N=54) living in the local reserve or in adjacent communities from June to August 2011 (Fig. 1). This field site has both primary and secondary forest, human population expansion into a buffer zone surrounding primate populations, and areas where humans and monkeys live as little as 5 m away from each other. Fecal samples were collected using sterile tongue depressors and a subsample placed in RNAlater (Qiagen Inc., Valencia, CA) for subsequent *Blastocystis* PCR analysis. Location of each sample was recorded using global positioning system (GPS) and howler monkey group demographics noted when feasible. Contamination was minimized by wearing disposable gloves. Howler monkey field methods have previously been described in further detail (Helenbrook 2014; Helenbrook et al. 2015).

Fecal samples were collected from individuals living at the field station and in two communities outside the reserve at Dogola and La Yecita. Community meetings were advertised by word of mouth and information regarding the experiment was conveyed to possible participants. The research team would work through a questionnaire with each participant, asking questions about their age, size of family, occupation, interaction with animals (both domestic and wild, including nonhuman primates), method for sterilizing water, and whether they hunted (Helenbrook 2014). Information was provided on how to collect a fecal sample so as to minimize contamination using sterile surgical gloves. All samples were delivered to a central location within 3 h of collections to be preserved. We also visited each participant's house in order to gather GPS data and assess distance to nearest forested area.

Genomic DNA was extracted from approximately 200 mg of feces using the QIAamp DNA Stool Mini Kit

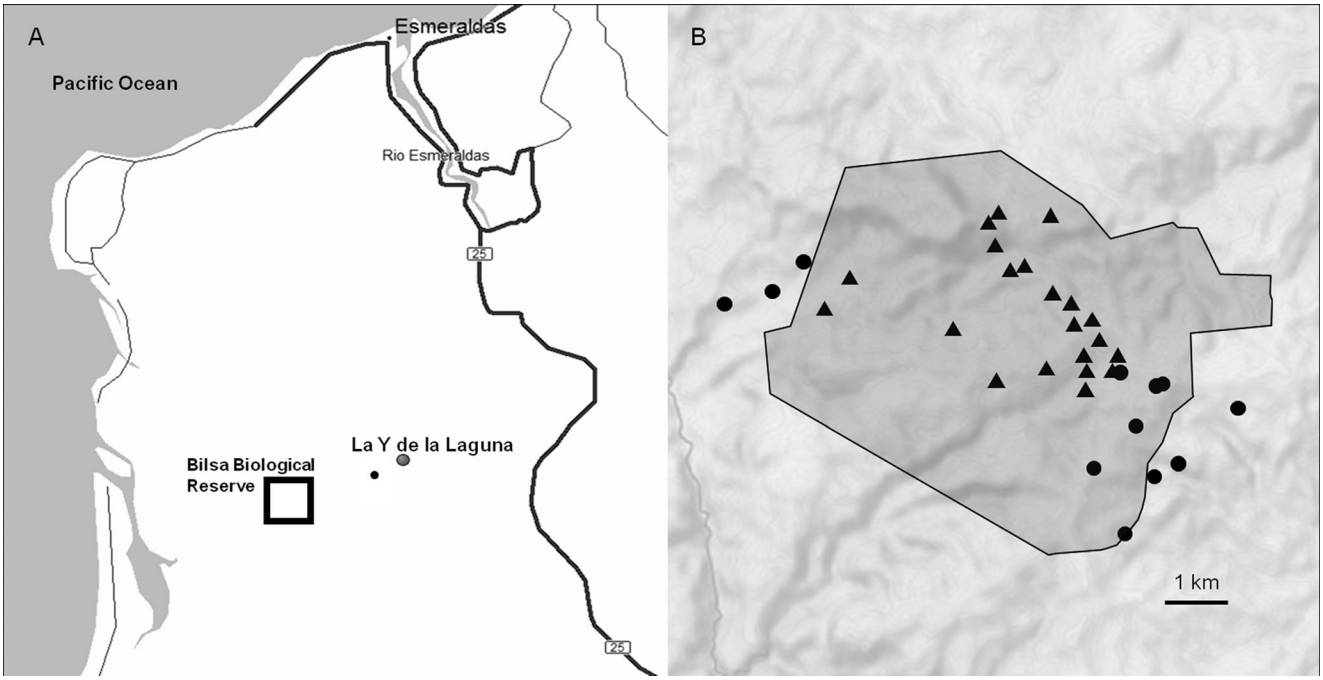

Fig. 1 **a** Field site location in the Tumbes-Choco-Magdalena bioregion of northwestern Ecuador. **b** Ninety-six mantled howler monkey samples were collected within the Bilsa reserve (*triangles*), along with 54 human samples from two surrounding communities and individuals working at the field research station (*circles*)

following the manufacturer's instructions. Partial *Blastocystis* species small subunit ribosomal DNA (SSU rDNA) was sequenced using previously published PCR-based primer sets BLF and BLR (Menounos et al. 2008), BH1F (Whipps et al. 2010) and BHRDr (Scicluna et al. 2006), and bl1400ForC and bl1710RevC (Stensvold et al. 2006). DNA was amplified by PCR in 25-μL reaction volumes in Quick-Load® Taq 2× Master Mix (New England Biolabs, Ipswich, MA), 0.25 μM of each primer, and 3 μL of template DNA using an C1000 Thermal Cycler (Bio-Rad) for 40 cycles at 95 °C for 30 s, 53 °C for 60 s, and 68 °C for 60 s, preceded by an initial denaturation at 95 °C for 3 min, and followed by a final extension at 68 °C for 7 min. Product amplification was initially evaluated by agarose gel electrophoresis. Samples that did not amplify were run two more times under the same PCR regime, and if no amplification was observed, they were categorized as negative. Positive samples were then run using SSCP to determine if multiple subtypes were present. For SSCP, 15 μL of each PCR product was mixed with 28 μL of loading buffer (10 mM NaOH, 95 % formamide, 0.05 % bromophenol blue, and 0.05 % xylene cyanol). Samples were denatured at 95 °C for 10 min and then snap-cooled by placing on ice for 5 min. Thirty-five microliters of sample was subjected to electrophoresis on a Bio-Rad Protean II xi Cell apparatus on a 0.4-mm-thick 12 % polyacrylamide gel in 1× TBE buffer at 300 V for 6 h at 6–8 °C. A thermostatically controlled refrigerated circulator was used to maintain a constant temperature throughout the buffer chamber. Polyacrylamide gels were post-stained with 10 μg/mL ethidium bromide, and distinct amplified fragments were sampled with a sterile pipette tip, which was transferred to a new PCR reaction for re-amplification. Amplified products were purified with E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Inc. Norcross, Georgia). Purified PCR samples were sequenced using forward primers BH1F, BLF, or bl1400ForC (depending on original PCR primers used) in reactions using the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1, on the ABI3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were uploaded to BioEdit (Hall 1999) and edited to remove base-calling errors, and subtype was determined based on highest sequence similarity from BLAST results in GenBank and checked against the phylogenetic framework of Whipps et al. (2010). Amplified nucleotide sequences obtained from both howler monkeys and people were deposited in GenBank (Table 1).

Data were described using mean and standard deviation for continuous variables and proportions for categorical variables. Mann-Whitney *U* test was used to analyze univariate association of each potential risk factor between those who harbored *Blastocystis* versus those that did not. We compared binomial dichotomized data using Fisher's exact test. Comparison of infected and uninfected individuals was calculated using Kruskal-Wallis nonparametric analysis of variance across three or more categorical responses. All effects were considered significant at the level of $p<0.05$ using STATISTICA 10 for Windows (StatSoft, Inc., Tulsa, USA).

Table 1 *Blastocystis* species small subunit ribosomal DNA sequence types in howler monkeys and humans. Unique sequences within a subtype were assigned an alphabetic designator (A, B, or C) and the number (*N*) of each unique type is listed

Host and subtype	Accession number	*N*
Howler monkey host		
Subtype 8		
ST8-A	KM374608	29
ST8-B	KM374609	1
ST8-C	KM374610	1
Howler total		31[b]
Humans host		
Subtype 1		
ST1-A	KM374611	18
ST1-B	KM374612	1
ST1-C	KM374613	1
ST1-D	KF848584 (96 %)[a]	1
Subtype 2		
ST2-A	KF848606 (100 %)[a]	2
ST2-B	KM374619	4
ST2-C	KM374614	2
ST2-D	KM374615	1
ST2-E	KM374616	1
Subtype 3		
ST3-A	KM374617	11
ST3-B	KM374618	1
Human total		43[c]

[a] Accession numbers for highest sequence match are included for ST1-D and ST2-A because the sequences are less than 200 bp

[b] DNA sequences generated solely from primer sets BLF/BLR (~260 bp) and BH1F and BHRDr (~600 bp). Sixteen howler monkey samples were amplified using bl1400ForC and bl1710RevC, yielding subtype 8; however, these samples are not included in this table because of a lack of overlap between sequences

[c] Similarly, only human sequences amplified with BLF/BLR and BH1F and BHRDr are included. Two other samples could only be amplified using bl1400ForC and bl1710RevC

Results

Fifty-eight out of 96 (60.4 %) howler monkey fecal samples tested positive for *Blastocystis* by PCR of SSU rDNA sequence. Eighteen out of 19 groups (94.7 %) harbored at least one positive individual. Fourteen out of 19 groups (73.7 %) over half of the individuals (≥50 %) were positive. Eleven of the 58 howler monkey samples were consistently positive for *Blastocystis* using two different primer sets; however, sufficient SSU ribosomal sequence could not be obtained to determine subtype, likely due to either low-quality DNA or insufficient DNA to visualize using SSCP. Of those that were sequenced, BLAST results revealed that all 47 samples were ST8. Sixteen of these samples could only be amplified using primer set bl1400ForC/bl1710RevC and were not included in Table 1 as the sequence was not congruent with those amplified using BLF/BLR and BH1F/BHRDr. Three unique sequences that varied by no more than 8 nucleotides could be categorized as ST8.

Forty-four out of 54 (81.5 %) human samples were positive, of which 1 was positive for *Blastocystis* but could not be subtyped likely due to low-quality DNA. Human samples contained ST1, ST2, and ST3 (Table 1 and Fig. 2). Two human samples harbored mixed subtypes: ST1 and ST3 ($N=1$), and ST1 and ST2 ($N=2$). Subtypes 1, 2, and 3 were further categorized into unique sequence types that varied by as little as a single base pair in some cases (Table 1).

Attributes and potential risk factors of people living in two communities surrounding the biological reserve were compared with the presence of *Blastocystis* species and *Blastocystis* subtypes (Table 2). The presence of *Blastocystis* was significantly higher in those who did not boil their water ($p=0.002$). Ninety-four percent (93.8 %) of people who did not boil their water had *Blastocystis*, while only 44.4 % of those who did had a detectable infection. Comparisons among all other risk factors (i.e., age, sex, family size, anti-parasitic treatment in last year, or monkeys within 1 km of home) were not statistically significant; however, several trends were observed. All self-described hunters ($N=6$) were infected, while 80.5 % of those who did not were infected with *Blastocystis*. Individuals who were infected tended to be younger (mean=26.5) versus those who were uninfected (mean=31.3; $p=0.68$). A greater proportion of females (mean=89.7 %) were infected compared to males (mean=26.5 %; $p=0.09$). Smaller family size was associated with reduced prevalence (mean=6.7 vs 8.1; $p=0.35$), and those who were infected with *Blastocystis* reported living much closer to rainforests (mean=420.1 m) than those who were not infected (mean=797.5 m; $p=0.38$).

Discussion

We found no evidence of *Blastocystis* transmission between howler monkeys and people living in close proximity to one another. Specifically, ST8 was the only *Blastocystis* subtype found in monkeys, whereas ST1, ST2, and ST3 were detected in people living in the surrounding community. Two people were found with mixed *Blastocystis* subtypes, yet none harbored ST8. A lack of identical *Blastocystis* subtypes in sampled howler monkeys and people living in close proximity suggests that zoonotic transmission of this parasite is not occurring between humans and howler monkeys. It also suggests that there is no common source of infection, or perhaps that there are differences in host specificity in these particular

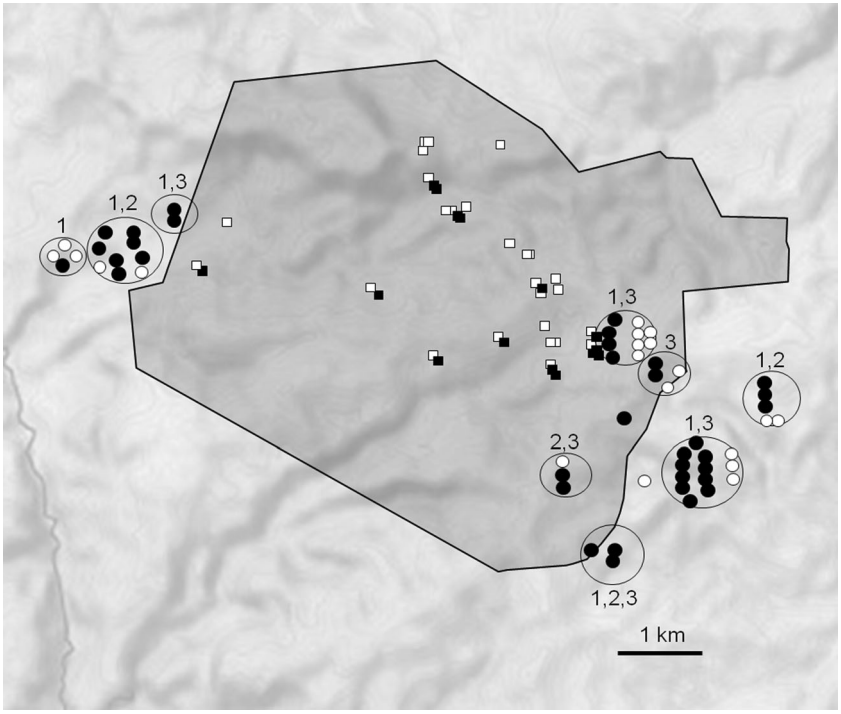

Fig. 2 Fifty-eight out of 96 monkey samples from 19 groups contained *Blastocystis* ST8 (*black squares*). Those samples which tested negative for *Blastocystis* are reported as *white squares*. Forty-four out of 55 humans were infected (*dark circles*) with either ST1, ST2, ST3, or a combination of two subtypes (N=3). Small *white circles* are individuals which tested negative for *Blastocystis*. *Larger circles* surrounding human samples represent individual households

subtypes. Subtype 8 has primarily been reported in nonhuman primates, although, in some cases, it has been found in zookeepers working with captive primates (Stensvold et al. 2009). The presence of ST8 in sampled wild howler monkeys suggests that captive howlers with ST8 could be harboring a wild form of *Blastocystis*, as opposed to them acquiring the ST8 infection from handlers.

The primary mode of gastrointestinal zoonotic transmission occurs through contaminated food or water (Slifko et al. 2000; Ithoi et al. 2011; Leelayoova et al. 2008). In *Blastocystis*, production of thick-walled cysts are believed to provide protection in an external environment once transmitted via a fecal-oral route (Garcia 1999). In the case of wild howler monkeys, they have rarely been seen drinking from river water. Instead, drinking water is obtained from arboreal cisterns or standing bodies of water (Glander 1978), suggesting little opportunity for humans to transmit *Blastocystis* to howler monkeys via rivers. The only other real opportunity for transmission from people to howlers would be if monkeys came to the ground and happened upon human fecal material. Howlers are known to traverse on the ground, especially in disturbed habitats (Pozo-Montuy and Serio-Silva 2007).

Conversely, the main source of drinking water in people living near these sampled howler monkeys is rivers (Table 2). Because howler monkeys are routinely found living near rivers and in close proximity to people, this raises the possibility that howler fecal matter could wash off leaves and rooftops into rivers where people retrieve their drinking water. In this scenario, people could conceivably be infected with howler monkey ST8 if they drank contaminated water, yet this was not observed. Those who did not boil their water were much more likely to harbor *Blastocystis*, which suggests that the river is a potential source of *Blastocystis*. However, without testing the river water directly, there is no way to know if ST8 is present.

In 78 % of reported cases, this drinking water is left untreated before consumption. By boiling water, the threat of ingesting these infectious stages would likely be reduced, as has been recorded in other areas throughout the world (Taamasri et al. 2000; Leelayoova et al. 2008; Abdulsalam et al. 2012). We did find that individuals who boiled their water were significantly less likely to have *Blastocystis* (p=0.002) than those that did not boil water before consuming. Ninety-three percent of those who reportedly did not boil their water before consumption were infected with *Blastocystis*, while only 44 % of those who did were positive. Based on multivariate logistic regression analysis, those who did not boil their water were 3.5 times more likely to be infected (p=0.02). The benefits of simple water treatment are likely not limited to *Blastocystis*, but also other gastrointestinal macroparasites, protozoa, bacteria, and viruses (Levy et al. 2008).

Other risk factors may be associated with the presence of *Blastocystis*; however, several variables were answered uniformly or there simply were not enough responses in a particular category. For example, all but four participants lived within relative close proximity (<1 km) to howler

Table 2 Potential risk factors associated with overall prevalence of *Blastocystis* and subtypes based on questionnaire topics

Characteristics	*N* (%)	*Blastocystis N* (%)[a]	ST1 *N* (%)	ST2 *N* (%)	ST3 *N* (%)	Mixed *N* (%)
Age						
0–15	17 (31.5)	14 (82.4)	7 (50.0)	2 (14.3)	3 (21.4)	1 (7.1)
16–30	13 (24.1)	13 (100.0)	7 (53.8)	4 (30.8)	3 (23.1)	1 (7.7)
31–45	11 (20.4)	7 (63.6)	2 (28.6)	2 (28.6)	3 (42.9)	0 (0)
46–60	9 (16.7)	6 (66.7)	3 (42.9)	1 (14.3)	2 (28.6)	0 (0)
60+	1 (1.9)	1 (100.0)	1 (100.0)	0 (0)	0 (0)	0 (0)
Unreported	4 (7.3)	3 (75.0)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0)
Gender						
Female	29 (53.7)	26 (89.7)	13 (50.0)	5 (19.2)	6 (23.1)	1 (3.8)
Male	25 (46.3)	17 (68.0)	7 (41.2)	5 (29.4)	6 (35.3)	1 (5.9)
Unreported	1 (1.8)	1 (100.0)	1 (100.0)	–	–	–
Family size						
1–3	13 (24.1)	10 (76.9)	3 (30.0)	2 (20.0)	4 (40.0)	0 (0)
4–6	9 (16.7)	6 (66.7)	1 (16.7)	3 (50.0)	2 (33.3)	0 (0)
7–10	19 (35.2)	14 (73.7)	9 (64.3)	4 (28.6)	2 (14.3)	1 (7.1)
11–13	13 (24.1)	13 (100.0)	7 (53.8)	1 (7.7)	4 (30.8)	1 (7.7)
Unreported	1 (1.8)	1 (100.0)	1 (100.0)	–	–	–
Treated with antiparasitic drugs in last year						
Yes	19 (35.2)	13 (68.4)	4 (30.8)	6 (42.9)	3 (21.4)	0 (0)
No	27 (50.0)	24 (88.9)	12 (50.0)	3 (12.5)	7 (29.2)	1 (4.2)
Unreported	9 (16.4)	7 (77.8)	5 (71.4)	1 (14.3)	2 (28.6)	1 (14.3)
Boil water						
Yes	9 (16.7)	4 (44.4)*	2 (50.0)	0 (0)	2 (50.0)	0 (0)
No	32 (59.3)	30 (93.8)*	13 (43.3)	7 (23.3)	9 (30.0)	2 (6.7)
Unreported	14 (25.5)	10 (71.4)*	6 (60.0)	3 (30.0)	1 (10.0)	0 (0)
Method of water treatment						
No treatment (river)	34 (63.0)	30 (88.2)	13 (43.3)	7 (23.3)	9 (30.0)	2 (6.7)
Boil	7 (13.0)	4 (57.1)	2 (50.0)	0 (0)	2 (50.0)	0 (0)
Filtered	–	–	–	–	–	–
Rain water	–	–	–	–	–	–
Multiple	–	–	–	–	–	–
Unreported	14 (25.5)	10 (71.4)	6 (60.0)	3 (30.0)	1 (10.0)	0 (0.0)
Monkeys within 1 km of house						
Yes	45 (83.3)	37 (82.2)	16 (43.2)	7 (18.9)	12 (32.4)	1 (2.7)
No	4 (7.4)	3 (75.0)	3 (100.0)	1 (33.3)	0 (0)	1 (33.3)
Unreported	6 (11.0)	4 (67.0)	2 (50.0)	2 (50.0)	0 (0)	0 (0)
Monkeys within 1 k of farm						
Yes	37 (68.5)	30 (81.0)	15 (50.0)	5 (16.1)	10 (33.3)	2 (7.0)
No	8 (14.8)	7 (87.5)	3 (42.9)	3 (42.9)	0 (0)	0 (0)
No farm	7 (13.0)	5 (71.4)	1 (20.0)	2 (40.0)	2 (40.0)	0 (0)
Unreported	3 (5.0)	2 (66.7)	2 (100.0)	0 (0)	0 (0)	0 (0)
Hunt wildlife						
Yes	6 (11.1)	6 (100)	1 (16.7)	1 (16.7)	4 (66.7)	0 (0)
No	41 (75.9)	33 (80.5)	18 (54.5)	7 (21.2)	7 (21.2)	2 (6.1)
Unreported	8 (14.5)	5 (62.5)	2 (40.0)	2 (40.0)	1 (20.0)	0 (0)
How often do you hunt?						
Never	41 (75.9)	33 (80.5)	18 (54.5)	7 (21.2)	7 (21.2)	2 (6.1)
Everyday	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2–3 times per week	3 (5.6)	3 (100.0)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0)

Table 2 (continued)

Characteristics	*N* (%)	*Blastocystis N* (%)[a]	ST1 *N* (%)	ST2 *N* (%)	ST3 *N* (%)	Mixed *N* (%)
1 time per week	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1 time per month	1 (1.9)	1 (100.0)	0 (0)	0 (0)	1 (100.0)	0 (0)
Unreported	10 (16.7)	7 (77.8)	2 (28.6)	2 (28.6)	3 (42.9)	0 (0)
Hunt or eat monkey?						
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No	45 (83.3)	37 (82.2)	17 (45.9)	8 (21.6)	11 (29.7)	2 (5.4)
Unreported	10 (16.7)	7 (70.0)	4 (40.0)	2 (20.0)	1 (10.0)	0 (0)

Categories ST1–Mixed represent number and percentage of total *Blastocystis*-positive infections

*$p<0.05$

[a] Includes positive PCR-based samples which did not provide sufficient quality sequence to determine subtype

monkeys based on survey results. All participants also had some contact with domesticated animals and nobody reportedly eats monkeys, precluding us from testing the affect of proximity to wildlife on presence or absence of *Blastocystis*. Several studies have found similar *Blastocystis* subtypes in people and their domestic animals, which makes this a worthy avenue of exploration (Lee et al. 2012). In another example, only six people reportedly hunted which is likely an underestimate based on personal communications with local community members. Nevertheless, all reported hunters were positive for *Blastocystis* while 80.5 % of nonhunters were infected.

The effect of *Blastocystis* infection on human and wildlife health is rather contentious and still remains poorly understood (Boorom et al. 2008). Infection with *Blastocystis* species does not necessarily indicate that an individual will indeed have gastrointestinal disease, although *Blastocystis* infections have been associated with various gastrointestinal symptoms as well as headache, fatigue, and depression (Boorom et al. 2008; Denoeud et al. 2011). The inconsistent gastrointestinal response to infection may be a product of antibiotic resistance, host immune response and overall health, variability in subtype infection, or any combination of these factors (Stensvold et al. 2007; Whipps et al. 2010).

Only one other study has described *Blastocystis* subtypes in wild South American primates, in which two sampled howler monkeys were positive for ST4 (Ramirez et al. 2014). Other subtypes, including ST1, ST2, ST3, ST4, ST5, and ST8, have previously been reported in Old World primates and captive howler monkeys (Abe 2004; Noel et al. 2005; Yoshikawa et al. 2009; Petrasova et al. 2011; Alfellani et al. 2013b); however, with only two Colombian howler monkey samples reported by Ramirez et al. (2014), we are unable to ascertain whether our results are common and applicable to other howler populations or species. Regarding the differences we observed from our sequences categorized as either ST1, 2, 3, or 8 (Table 1), these could represent distinct parasites, but just as likely this subtle variation within a subtype could represent sequence differences in the ribosomal DNA which is represented in the genome by multiple copies (Denoeud et al. 2011; Poirier et al. 2014). Regardless, there were clear breaks between subtypes found in humans and monkeys beyond these minor differences, whatever their source.

We anticipate that the use of SSCP to uniquely identify multiple subtypes within a single sample may play a particularly useful role in understanding the interactions of particular *Blastocystis* subtypes throughout domestic animals, wildlife, and people. Our methods also provide an inexpensive and relatively quick way to diagnose mixed subtypes in individual samples obtained from either people or nonhuman primates. Although we did not find direct evidence of zoonotic transmission, this does not preclude the possibility that people living in close contact with domestic animals and wildlife could still be at risk of obtaining *Blastocystis* under certain conditions, as evidenced from shared subtypes of infected primate zookeepers reported in other studies.

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