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GASTROINTESTINAL PARASITES OF ECUADORIAN MANTLED HOWLER MONKEYS (ALOUATTA PALLIATA AEOQUATORIALIS) BASED ON FECAL ANALYSIS

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ABSTRACT: An analysis of gastrointestinal parasites of Ecuadorian mantled howler monkeys, Alouatta palliata aequatorialis, was conducted based on examination of fecal smears, floatations, and sedimentations. At least 1 type of parasite was detected in 97% of the 96 fecal samples screened across 19 howler monkey groups using these techniques. Samples averaged 3.6 parasite species per individual (±1.4 SD). Parasites included species representing genera of 2 apicomplexans: Cyclospora sp. (18% of individual samples) and Isospora sp. (3%); 6 other protozoa: Balantidium sp. (9%), Blastocystis sp. (60%), Chilomastix sp. (4%), Dientamoeba sp. (3%), Entamoeba species (56%), Iodamoeba sp. (5%); 4 nematodes: Enterobius sp. (3%), Capillaria sp. (78%), Strongyloides sp. (88%) which included 2 morphotypes, Trypanoxyuris sp. (12%); and the platyhelminth Controrchis sp. (15%). A statistically significant positive correlation was found between group size and each of 3 different estimators of parasite species richness adjusted for sampling effort (ICE: $r^2 = 0.24, P = 0.05$; Chao2: $r^2 = 0.25, P = 0.05$, and Jackknife: $r^2 = 0.31, P = 0.03$). Two significant associations between co-infecting parasites were identified. Based on the prevalence data, individuals infected with Balantidium sp. were more likely to also be infected with Isospora sp. ($\chi^2 = 6.02, P = 0.01$), while individuals harboring Chilomastix sp. were less likely to have Capillaria sp. present ($\chi^2 = 4.03, P = 0.04$).

In the last decade, an increasing number of studies have focused on parasitism in wild primate populations, emanating mainly from an interest in zoonotic pathogen transmission (Muruiki et al., 1998; Pedersen et al., 2005; Howells et al., 2011), conserving host species (Wallis and Lee, 1999; Gillespie et al., 2005; Smith et al., 2008), and ecosystem health (Marcogliese, 2005). Many of the parasitic diseases in primates are zoonotic (Pedersen, 2005; Wolfe et al., 2007), and concern is growing that anthropogenic disturbances are changing the dynamics of these ecological systems (Morse, 1995; Patz et al., 2000; Daszak et al., 2001; Chapman et al., 2005; Puttker et al., 2008). A first step in understanding this relationship is examining the parasite community of a primate that is intricately associated with humans, such as howler monkeys (Alouatta spp.), and is often detrimentally affected by their actions.

The genus Alouatta consists of 6 species and 22 subspecies spanning most of Central and South America (Crockett, 1998). Five subspecies of Alouatta palliata are recognized, Alouatta palliata palliata found in Honduras, Nicaragua, Costa Rica, Panama, and El Salvador; Alouatta palliata mexicana found in southern Mexico and Guatemala; Alouatta palliata aequatorialis found in Panama, W. Colombia, W. Ecuador and N.W. Peru; and Alouatta palliata trabeata and Alouatta palliata coibensis found solely in Panama (Crockett, 1998; IUCN, 2012). Alouatta spp. are among the largest New World primates, living in groups averaging 14 individuals and ranging from 4-40 individuals per group (Rowe, 1996; IUCN, 2012). Howlers are arboreal, spending the vast majority of their time in the forest canopy, seasonal and non-seasonal forests, and in mangroves and swamps, providing key ecological services such as seed dispersal (Anzures-Dadda et al., 2011). They are largely folivorous, though it has also been shown that upwards of 40% of their diet is fruit during favorable seasons (Estrada et al., 1999; Pinto et al., 2003; Vitazkova, 2009). An indiscriminate diet is likely one of the main reasons that they are so adaptable to changing ecological landscapes, whether natural or anthropogenic.

The gastrointestinal parasite communities of several howler monkey species (Alouatta spp.) have been studied throughout Central and South America including A. palliata (Stuart et al., 1998; Trejo-Macias et al., 2007), Alouatta pigra (Eckert et al., 2006; Stoner and Gonzalez Di Pierro, 2006; Vitazkova and Wade, 2006; Trejo-Macias et al., 2007; Cristobal-Azkarate et al., 2010), Alouatta caraya (Stiles et al., 1929; Cruz et al., 2000), and Alouatta seniculus (Gilbert, 1994; Phillips et al., 2004). These howler monkey studies have found an array of gastrointestinal parasites including protozoans, nematodes, and platyhelminths (Pope, 1966; Stuart et al., 1990; Amato et al., 2002; Godoy et al., 2004; Phillips et al., 2004; Eckert et al., 2006; Vitazkova and Wade, 2007; Cristobal-Azkarate et al., 2010; Valdespino et al., 2010). A complete literature review, including non-gastrointestinal parasites, can be found in Stuart et al. (1998) and Vitazkova (2009).

Gastrointestinal parasite communities of mantled howler monkeys (A. p. aequatorialis) from Ecuador have yet to be described; therefore, the main objective of this study was to determine what parasites are found in this threatened howler monkey subspecies. These data will also be used in our long-term research project designed to study the effects of habitat disturbance (i.e., human proximity and basal area) on parasite communities in the mantled howler monkey and to assess zoonotic transmission of gastrointestinal parasites. We anticipate that a broad array of protozoans, nematodes, and platyhelminths will be recovered based on findings from other related howler monkey studies throughout Central and South America.

We also examined the relationship between group size and gastrointestinal parasite species richness because several studies have documented increased parasite species richness in larger groups (Freeland, 1979; Nunn et al., 2003). Individual parasitism is expected to increase with larger social groups due to higher densities and increased contact rate (Cote and Poulin, 1995; Arneberg et al., 1998; Nunn et al., 2003). Unfortunately, the effect may or may not be real, as some of these studies fail to account for sampling effort—larger groups are expected to have more parasites simply because more individuals are sampled. Modeling by Wilson et al. (2003) suggests that per capita risk of infection

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from directly transmitted parasites is actually decreased in larger groups, and Mbora et al. (2009) found smaller groups had higher parasite prevalence and species richness, though the results were not significant. Presumably, maintaining groups could be advantageous against the spread of parasitism if clustering of individuals reduced the likelihood of an infected individual entering a new group (Black, 1966; Freeland, 1976, 1979; Wilson et al., 2003). Conversely, larger group limitations include increased intra-group parasite transmission along with higher localized host density, increased stress levels, and larger travel requirements in search of food (Wrangham, 1980; Cote and Poulin, 1995; Sterck et al., 1997; Nunn and Dokey, 2006; Borries et al., 2008; Majolo et al., 2008). If gastrointestinal parasites infecting howler monkeys are transmitted directly or via fecal-oral route, then larger groups are expected to have more parasite species per individual.

Lastly, gastrointestinal parasite species have previously been shown to interact with each other both experimentally and in the wild (Poulin, 2001; Nacher, 2011). Two main relationships have been previously reported: competitive interactions where 1 parasite species outcompetes another, causing an inhibitory effect (Poulin, 2001); and where infection of a host by 1 parasite species increases host susceptibility to secondary infection (Murphy et al., 2013). We will investigate this phenomenon using fecal analysis to evaluate whether certain parasites co-occur or some occur in the absence of others.

**MATERIALS AND METHODS**

The Bilsa Biological Station, a private reserve of 3,300 hectares in northeastern Ecuador (35 km west of Quinindé, 0°21’N, 79°44’W), is located in a pre-montane tropical forest along the Pacific coast at an altitude of 350–576 m (Fig. 1A, B). This area is part of the Tumbes-Choco-Magdalena bioregion and is currently under threat due to localized host density, increased stress levels, and larger travel requirements in search of food (Wrangham, 1980; Cote and Poulin, 1995; Sterck et al., 1997; Nunn and Dokey, 2006; Borries et al., 2008; Majolo et al., 2008). If gastrointestinal parasites infecting howler monkeys are transmitted directly or via fecal-oral route, then larger groups are expected to have more parasite species per individual.

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Jackknife (Chao and Lee, 1992), and Incidence-based Coverage Estimator (ICE) (Gotelli and Colwell, 2001, 2011; Walther and Martin, 2001). We investigated 3 adjusted species richness estimators to ensure that the observed results were robust to choice of adjustment. Groups with 2 or fewer individuals were not included in analyses because estimates of adjusted species richness would be unreliable based on so few individuals. Estimated species richness adjusted for sampling effort was then modeled as a function of group size to assess the relationship between species richness and group size. Estimated species richness adjusted for sampling effort is a continuous variable, so ordinary least squares regression was used to quantify the association between estimated species richness and group size.

Attribute agreement analysis coupled with Cohen’s kappa was used to evaluate the ability of the 3 methods employed to detect parasites. No gold-standard for presence of each parasite species is available, so each method was compared to the final assessment, which was a summation of fecal smears, flotations, and sedimentation results. In the case of Blastocystis sp., we used PCR-based results as a gold standard. Co-infection was assessed using the chi-square ($\chi^2$) test of independence (with the Yates adjustment) for pairs of parasite species. Unless otherwise specified, all statistical analyses were conducted using STATISTICA 10 for Windows (StatSoft, Inc., Tulsa, Oklahoma).

RESULTS

Almost all samples (97%) contained at least 1 type of gastrointestinal parasite. Parasite species richness per fecal sample ranged from 0 to 7 species. Of the 96 fecal samples, 23 had 5–7 parasite species, 68 had 2–4 parasite species, 3 had a single parasite, and 2 had no parasites detected. Samples averaged 3.6 parasite species per individual ($\pm$1.4 SD). Thirteen parasites encompassing 13 genera were identified, representing 2 apicomplexans, 6 other protozoa, 4 nematodes, and 1 platyhelminth (Fig. 2; Table I). At least 1 apicomplexan was found in 20% of all samples, other protozoans were present in 88% of samples, and nematodes were detected in 95% of the samples.

Average length and range of parasites are listed in Table I. Three parasite groups require additional explanation. First, Strongyloides spp. egg length ranged from 14.4 μm to 71.3 μm and, when we plotted a frequency distribution of the lengths, this produced a bimodal curve with peaks at 36 μm and at 52.8 μm. Over half (52%) of identified eggs fell below 48 μm in length, a
reported minimum for *Strongyloides* species in howlers. The 2 apparent morphotypes observed were considered a single entity for the purposes of analysis here. Secondly, 58% of samples contained *Entamoeba* spp. Several morphotypes existed with cysts that averaged 12.8 μm in length and ranged from 9.2–16.3 μm. The number of nuclei within each cyst could not reliably be determined; thus, all findings were categorized as *Entamoeba* spp. Lastly, PCR-based confirmation was conducted for *Blastocystis* spp. Seven percent (7%) of fecal smears were positive, along with 1% of flotations and 4% of sedimentations, while 60% were positive using PCR detection.

Recovery efficiency was dependent on fecal extraction method (Table I). For example, *Strongyloides* spp. were found in 88% of samples based on all methods combined, but only 58% of smears, 3% of flotations, and 73% of fecal sedimentations were positive. Similarly, a *Capillaria* sp. was present in 78% of samples yet only 64% of smears, 56% of flotations, and 38% of sedimentations were positive. Thirteen parasite species were recovered from fecal smears compared to 9 species from flotations and 10 from sedimentations. The highest parasite prevalence for 6 species was found using smears, while only 1 species had the highest prevalence from flotations and 3 species from sedimentations.

There is clearly a benefit to using multiple extraction methods as each is suited to recovering different parasite species (Table I).

Considering the 19 howler groups with at least 2 individuals, parasite species richness varied from 3 to 11 parasite species and averaged 3.5 species per group (±0.6 SD; Fig. 3). Sixteen groups (84%) harbored 5 or more distinct parasite species and 3 groups (16%) had 2-4 species of parasite. Eleven groups (58%) exhibited at least 1 apicomplexan. Nematode and protozoan species were found in all other groups. Parasite species richness increased with group size, regardless of the method used to adjust for sampling effort (Fig. 4): Jackknife \( F_{1,14} = 6.2, r^2 = 0.31, P = 0.03 \); Chao2 \( F_{1,14} = 4.7, r^2 = 0.25, P = 0.05 \); and ICE \( F_{1,14} = 4.5, r^2 = 0.24, P = 0.05 \).

Co-infection status was evaluated for all parasite pair combinations. Two significant associations were found. Of those individuals infected with *Balantidium* sp., 22% were also infected with *Isospora* sp., which is significantly greater than expected (3%) under the hypothesis of no association between the 2 species (\( \chi^2 = 6.02, P = 0.01 \)). A negative association was found between *Chilomastix* sp. and *Capillaria* sp. Only 25% of the individuals infected with *Chilomastix* sp. were also infected with *Capillaria* sp., far fewer than the expected *Capillaria* sp. prevalence of 78%.
under the null hypothesis of no association between the 2 species ($\chi^2 = 4.03, P = 0.04$).

**Discussion**

Parasite species from 13 genera were found in 19 groups of Ecuadorian mantled howler monkeys. These 13 genera represent a higher number than observed in previous howler monkey studies. The higher species richness observed for these howler groups relative to previous studies could be due to several factors including differences in sampling effort and technique, extraction methodology, environmental conditions (Stuart et al., 1990, 1998; Stoner, 1996; Eckert et al., 2006; Cristobal-Azkarate et al., 2010), anthropogenic disturbance (Daszak et al., 2001; Chapman et al., 2005; Puttker et al., 2008), host densities, and parasite attributes such as intermittent shedding of parasites by hosts in various seasons, differences in host immunity (Stear et al., 1995), initial infection dose (Christensen et al., 1995), and parasite fecundity (Dineen et al., 1965). Our study may simply have recovered more parasites because we used 3 different methods to retrieve parasites from feces (plus PCR-based detection for *Blastocystis* spp.) in order to maximize the likelihood of recovering various types of parasites, whereas the other studies limited retrieval and detection to 1 or 2 extraction methods. We are not suggesting that this is the only explanation for increased species richness. However, looking only at our fecal flotation results, 9 species were recovered as opposed to 13 species recovered when all 3 extraction methods were combined. Sedimentation methods resulted in the recovery of 10 parasite species. Fecal smears did result in the detection of all 13 species and also had the highest number of positive matches (when compared to a combination of all methods) for 7 parasite species (Table II). Yet, only 2 other howler monkey studies have

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**Table I.** Percent parasite prevalence (P) with 95% confidence intervals (CI), mean intensity (MI) estimated as number of eggs per gram of feces using three fecal extraction techniques, and mean and range of length of eggs or cysts.

<table>
<thead>
<tr>
<th>Parasite group</th>
<th>Parasite species</th>
<th>Smear</th>
<th>Flotation</th>
<th>Sedimentation</th>
<th>Total sampled (n = 96)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P %</td>
<td>CI MI</td>
<td>P % CI MI</td>
<td>P % CI MI</td>
</tr>
<tr>
<td>Apicomplexa</td>
<td><em>Cyclospora</em> sp.</td>
<td>8.3</td>
<td>4–16 7.0</td>
<td>3.1 1–9 134.0</td>
<td>9.4 5–17 13.4</td>
</tr>
<tr>
<td></td>
<td><em>Isospora</em> sp.</td>
<td>1.0</td>
<td>0–6 1.0</td>
<td>1.0 0–6 1.0</td>
<td>1.0 0–6 1.0</td>
</tr>
<tr>
<td>Other Protozoa</td>
<td><em>Balantidium</em> sp.</td>
<td>8.3</td>
<td>4–16 2.4</td>
<td>1.0 0–6 1.0</td>
<td>1.0 0–5 1.0</td>
</tr>
<tr>
<td></td>
<td><em>Blastocystis</em> sp.</td>
<td>7.3</td>
<td>3–15 1.1</td>
<td>1.0 0–6 1.0</td>
<td>1.0 0–6 1.0</td>
</tr>
<tr>
<td></td>
<td><em>Chilomastix</em> sp.</td>
<td>3.1</td>
<td>1–9 3.0</td>
<td>0.0 0–5 0.0</td>
<td>0.0 0–5 0.0</td>
</tr>
<tr>
<td></td>
<td><em>Dientamoeba</em> sp.</td>
<td>3.1</td>
<td>1–9 1.0</td>
<td>0.0 0–5 0.0</td>
<td>0.0 0–5 0.0</td>
</tr>
<tr>
<td></td>
<td><em>Entamoeba</em> sp.</td>
<td>55.0</td>
<td>45–65 6.8</td>
<td>2.0 0–5 1.0</td>
<td>1.0 3–13 1.3</td>
</tr>
<tr>
<td></td>
<td><em>Iodamoeba</em> sp.</td>
<td>5.2</td>
<td>2–12 1.0</td>
<td>0.0 0–5 0.0</td>
<td>0.0 0–5 0.0</td>
</tr>
<tr>
<td>Nematoda</td>
<td><em>Enterobius</em> sp.</td>
<td>1.0</td>
<td>0–6 1.0</td>
<td>0.0 0–5 0.0</td>
<td>0.0 0–5 0.0</td>
</tr>
<tr>
<td></td>
<td><em>Capillaria</em> sp.</td>
<td>63.5</td>
<td>53–72 7.3</td>
<td>56.3 46–66 17.2</td>
<td>37.5 28–48 3.2</td>
</tr>
<tr>
<td></td>
<td><em>Strongyloides</em> sp.</td>
<td>58.3</td>
<td>48–68 3.8</td>
<td>3.1 1–9 1.0</td>
<td>1.0 72.9 63–81 4.4</td>
</tr>
<tr>
<td></td>
<td><em>Trypanoxyuris</em> sp.</td>
<td>6.3</td>
<td>3–13 1.8</td>
<td>1.0 0–6 7.0</td>
<td>1.0 3–13 6.0</td>
</tr>
<tr>
<td>Platyhelminth</td>
<td><em>Controrchis</em> sp.</td>
<td>3.1</td>
<td>1–9 1.0</td>
<td>0.0 0–5 0.0</td>
<td>0.0 0–5 0.0</td>
</tr>
</tbody>
</table>

* Blasto* cystis confirmation using PCR-based detection.

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**Table II.** Percent agreement and kappa statistics for parasite detection using 3 extraction methods (fecal flotation, sedimentation, and smear). Aggregated data from all methods were used as the gold-standard. N = 96 fecal samples assessed by all 3 methods. Kappa values >0.81 are considered near perfect agreement, 0.41–0.60 corresponds to moderate agreement, and <0.20 is considered poor.

<table>
<thead>
<tr>
<th>Genera</th>
<th>Flotation</th>
<th>Sedimentation</th>
<th>Smear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agreement %</td>
<td>Kappa</td>
<td>Agreement %</td>
</tr>
<tr>
<td><em>Cyclospora</em></td>
<td>85</td>
<td>0.26</td>
<td>92</td>
</tr>
<tr>
<td><em>Isospora</em></td>
<td>98</td>
<td>0.49</td>
<td>98</td>
</tr>
<tr>
<td><em>Balantidium</em></td>
<td>92</td>
<td>0.18</td>
<td>91</td>
</tr>
<tr>
<td><em>Blastocystis</em>†</td>
<td>40</td>
<td>−0.02</td>
<td>39</td>
</tr>
<tr>
<td><em>Chilomastix</em></td>
<td>96</td>
<td>0.00</td>
<td>97</td>
</tr>
<tr>
<td><em>Dientamoeba</em></td>
<td>97</td>
<td>0.00</td>
<td>97</td>
</tr>
<tr>
<td><em>Entamoeba</em></td>
<td>44</td>
<td>0.03</td>
<td>49</td>
</tr>
<tr>
<td><em>Iodamoeba</em></td>
<td>90</td>
<td>0.15</td>
<td>95</td>
</tr>
<tr>
<td><em>Enterobius</em></td>
<td>97</td>
<td>0.00</td>
<td>99</td>
</tr>
<tr>
<td><em>Capillaria</em></td>
<td>77</td>
<td>0.51</td>
<td>58</td>
</tr>
<tr>
<td><em>Strongyloides</em></td>
<td>18</td>
<td>0.02</td>
<td>88*</td>
</tr>
<tr>
<td><em>Trypanoxyuris</em></td>
<td>95</td>
<td>0.00</td>
<td>95</td>
</tr>
<tr>
<td><em>Controrchis</em></td>
<td>95</td>
<td>0.75</td>
<td>88</td>
</tr>
</tbody>
</table>

* Significantly higher prevalence than other unmarked methods based on 95% confidence interval.
† For Blastocystis sp., the gold-standard was PCR data.
utilized this method (Eckert et al., 2006; Stoner and Gonzalez Di Pierro, 2006).

Group size

Group size was associated with parasite species richness; larger groups harbored a greater number of parasite species (Fig. 4). This finding is consistent with the hypothesis proposed by Freeland (1979) and summarized by Altizer et al. (2003) in which the number of intestinal protozoan species is a function of group size. In larger groups, individuals are hypothetically more likely to be infected either from increased environmental contamination, from an increased exposure to immigrants (Freeland, 1976), or from expanded traveling needed to secure food resources (Chapman et al., 2008). Conversely, other studies have found no association of parasitism with group size (Chapman et al., 2008) or a negative association (Snailth et al., 2008). The positive relationship found between group size and parasite richness is likely a product of several contributing factors closely associated with the number of individuals within a group (Cote and Poulin, 1995; Altizer et al., 2003) including group density, individual stress levels, food availability, and subsequent altered ranging behaviors that could bring individuals into contact with fecal-contaminated areas. This could mean that fluctuations in certain environmental factors, such as food sources, would result in a change in stress levels, group density, and number of individuals (Chapman et al., 2006).

Parasite interactions

There was 1 case where a host was more likely to harbor a parasite species in the presence of another—individuals positive for *Isospora* sp. were much more likely to be found in individuals with *Balantidium* sp. There was also only 1 case where 2 parasites showed a negative association. Individuals infected with *Chilomastix* sp. were much less likely to be infected with *Capillaria* sp. There is little evidence in the literature to suggest that presence of a *Chilomastix* sp. actually inhibits or influences the presence of *Capillaria* sp. In fact, *Chilomastix mesnili* is found in primates of all sorts and is often associated with other parasitic infections (Cox, 2001; Ekanayake et al., 2006; Chapman et al., 2011). In either case, it is not possible to definitively identify what type of interaction is occurring simply based on patterns of co-infection; however, there is certainly evidence elsewhere to suggest that competitive interactions between parasites occurs (Petney and Andrews, 1998; Pedersen and Fenton, 2007; Graham, 2008) as well as parasite-induced immunosuppression—where the presence of 1 parasite species benefits another (Cox, 2001). Poulin (2001) summarizes possible causes of gastrointestinal parasite relationships, including the possibility that certain hosts might be more susceptible to parasitism than are other individuals.

Parasite species identification

We may have encountered 2 distinct *Strongyloides* morphotypes in our study, as the eggs we measured ranged in length from 14 μm to 71.3 μm, and the distribution of egg length was bimodal with modes at 36 μm and at 52.8 μm. Because of significant overlap, we could not confidently separate large and small eggs for the purposes of our analysis here. Multiple *Strongyloides* species have been found in the same red colobus monkey population, though it was not reported whether both were in a single individual (Gillespie and Chapman, 2008). The howler parasite literature typically only lists genus because these are difficult to identify to species level from eggs, and there are more than 50 described *Strongyloides* species throughout the world (Speare, 1989). It is possible that some of the recovered eggs we found that were longer than 48 μm are attributable to *Strongyloides stercoralis*, and this species does have a distribution throughout humans in the Neotropics (Olsen et al., 2009). However, 52% of identified *Strongyloides* eggs were less than 48 μm long, which puts them outside the previous size estimates for any species reported from howlers. Besides egg length, there were...
no other discernible morphological differences between the morphotypes (Fig. 2A, B).

All but 3 parasite genera (Cyclospora sp., Capillaria sp., and Balantidium sp.) have been previously reported in other howler monkey species (Gilbert, 1994; Stoner 1996; Eckert et al., 2006; Stoner and Gonzalez-Di Pierro, 2006; Vitazkova and Wade, 2006; Trejo-Macias et al., 2007; Cristobal-Azkarate et al., 2010; Kowalzik et al., 2010). In most cases we were not able to identify to a species level based simply on morphology, yet we can speculate based on other factors. Entamoeba coli could have been responsible for some howler infections, as cyst morphology was consistent with other reports (Table I). It has also been reported in humans living in the same province as our field study (Gatti et al., 2002). Nonetheless, we cannot rule out the presence of other Entamoeba spp.

Trypanoxyuris sp. eggs averaged 42.5 μm in length, similar to previous findings which ranged from 31.2–49.3 μm in A. palliata in Mexico (Table I; Cristobal-Azkarate et al., 2010). Trypanoxyuris minutus has been reported in nearly every other Central and South American country, which leads us to believe that this is T. minutus (Thatcher and Porter, 1968; Gilbert, 1994; Stuart et al., 1998; Cristobal-Azkarate et al., 2010). This parasite is relatively important because it has previously been reported as the cause of death of a howler in Brazil (Amato et al., 2002).

We observed eggs of a Capillaria species in 78% of our samples with eggs averaging 48.8 μm long. Over 250 Capillaria spp. have been described in vertebrates, though only eggs from Capillaria hepatica and Capillaria brochieri have been reported from primates (Brack et al., 1994; Graczyk et al., 1999). Capillaria hepatica eggs were reported to be from 50 μm (Brack et al., 1994) to 54.3 ± 0.5 μm (Graczyk et al., 1999). Capillaria brochieri eggs in chimpanzees were documented from 45–55 μm, which would encompass our observations (Justine, 1987). However, Capillaria species have only been found in A. caraya but not in A. palliata (Godoy et al., 2004).

Balantidium coli is the only known ciliated protozoan to infect humans and has been found in Ecuadorian human populations (Chiriboga Urquizo et al., 1985). However, no cases of B. coli have been reported in howler monkeys, though Balantidium species have been reported in A. caraya (Stiles et al., 1929). Trophozoites averaged 44.5 μm (30.1–107.3 μm), which is within the normal variable size limit of 30–300 μm in length (Fig. 1). Cyclospora species have been previously reported from Ethiopian and Kenyan primates (Eberhard et al., 1999, 2001). However, Cyclospora in South American non-human primates has not been reported. Human infections of Cyclospora cayetanensis have been reported in South American countries including Peru (Ortega et al., 1994) and Venezuela (Chacin-Bonilla et al., 2001), which suggests that this parasite might be found in Ecuador. Chilomastix sp. was also identified to the genus level. Only 1 other howler study (A. caraya) reported this parasite, a study in Brazil (Stiles et al., 1929).

Kowalzik et al. (2010) previously hypothesized that black howler monkeys (A. pigra) who eat the leaves, fruit, and stems of the ceiboa tree, Cecropia peltata, are likely to become infected with Controrchis sp. through the ingestion of infected ants. Our study found only 15% of samples positive for Controrchis sp. compared to 80–89% prevalence in black howler monkeys (Vitazkova and Wade, 2006; Kowalzik et al., 2010). Aside from the difference in host species, there is the possibility that forest structure differences between the studies might play a role. The cecropia tree is a pioneer species and thus we would expect Controrchis sp. to be more prevalent in disturbed forest. Only 20% of the Bilsa Biological Reserve is considered secondary forest (Ortega-Andrade et al., 2010), while Kowalzik et al. (2010) describe their site as a “hurricane-damaged” forest and Vitazkova and Wade (2006) sampled monkeys in secondary growth forest, cattle pastures, and plantations. If indeed these Controrchis species were limited to degraded environments, then this might very well explain the reduced prevalence in our study. Vitazkova and Wade (2006) identified the parasite from black howlers as Controrchis biliophilus. Based on descriptions and size estimates of eggs (41 μm–50 μm) from other studies (Stuart et al. 1990), the eggs we observed are consistent with Co. biliophilus.

Pathogenicity

Although we took a conservative approach to identify species only to genus, some of the species in this study are from genera where most of the members are considered asymptomatic. Others may be associated with host impacts ranging from mild signs of infection to death (Abbott and Majeed, 1984; Foreyt, 2001; Chapman et al., 2005; Trejo-Macias et al., 2007). In the case of Blastocystis sp., Iodamoeba sp., and En. coli there is little evidence to suggest pathogenicity (Toft, 1982; Chapman et al., 2005; Coyle et al., 2011). Alternatively, Strongyloides spp. (Abbott and Majeed, 1984; Chapman et al., 2005), Capillaria spp. (Abbott and Majeed, 1984), Balantidium sp. (Kuntz, 1982; Toft, 1982), Enterobius sp. (Toft, 1982; Chapman et al., 2005), and Chilomastix sp. (Chapman et al., 2005) have been shown to be pathogenic in other primates. Entamoeba spp. (Kuntz, 1982) could be either asymptomatic (En. coli) or cause amoebiasis (Entamoeba histolytica), but is limited in this case by our inability to identify these parasites to a species level (Chapman et al., 2005). Similarly, Isospora sp. may or may not lead to coccidiosis in non-human primates (Lindsay et al., 1997; Duszynski et al., 1999). Information on the effect of these parasite species in A. palliata is limited, especially as it relates to multiple infections; however, based on evidence found in other organisms there is the possibility that host health and fitness could be affected (Murray et al., 1998; Chapman et al., 2007; Cristobal-Azkarate, 2010). Multiple infections in combination or single infections coupled with reduced host immune response could influence pathogenicity, leading to illness or in some circumstances death (Amato et al., 2002).

In conclusion, our study found several parasites which may have relevance to primate and human health: Balantidium sp., Isospora sp., Enterobius sp., and Strongyloides sp. We also found that group size was positively correlated with gastrointestinal parasitism—a finding which builds on previous primate studies by accounting for sampling effort. Describing gastrointestinal parasites infecting non-human primate species, and understanding factors that impact parasite communities, is important to both human health and primate conservation. Future natural and man-made changes to the environment have been predicted to increase spill-over events from wildlife populations to people and vice versa (Daszak et al., 2000). The data presented here provide the foundation for future studies on howler monkey parasites in Ecuador.
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