霐

International Journal for Parasitology: Parasites and Wildlife 6 (2017) 202-208



Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Seasonality, richness and prevalence of intestinal parasites of three neotropical primates (*Alouatta seniculus, Ateles hybridus* and *Cebus versicolor*) in a fragmented forest in Colombia



Silvia Rondón ^{a, *}, Mario Ortiz ^a, Cielo León ^a, Nelson Galvis ^b, Andrés Link ^{b, c, d}, Camila González ^a

^a Centro de Investigaciones en Microbiología y Parasitología Tropical, CIMPAT, Departamento de Ciencias Biológicas, Universidad de los Andes, Cra. 1 N° 18^a-12, Bogotá, Colombia

^b Laboratorio de Ecología de Bosques Tropicales y Primatología, Departamento de Ciencias Biológicas, Universidad de Los Andes, Cra. 1 N° 18^a-12, Bogotá, Colombia

^c Facultad de Administración, Universidad de Los Andes, Calle 21 N° 1-20, of. SD-935, Bogotá, Colombia

^d Fundación Proyecto Primates, Cra. 11a Nº 91-55, Apartamento 202, Bogotá, Colombia

ARTICLE INFO

Article history: Received 22 April 2017 Received in revised form 14 July 2017 Accepted 20 July 2017

Keywords: Faecal smears Fragmented forest Intestinal parasites Howler monkeys Spider monkeys Capuchin monkeys

ABSTRACT

Studies on parasites infecting non-human primates are essential to better understand the potential threat to humans of zoonoses transmission, particularly under the current processes of pervasive land use change and biodiversity loss. The natural ecosystems in the Middle Magdalena river basin in Colombia have suffered a dramatic reduction and transformation into pastures and agroindustrial monocultures, threatening their biodiversity, and probably affecting the dynamics between parasites and their hosts, as well as altering the disease transmission cycles between wild populations and humans. This study evaluated seasonality, prevalence and richness of intestinal parasites in three species of neotropical primates: Cebus versicolor, Ateles hybridus and Alouatta seniculus, in a fragmented forest in the Middle Magdalena river valley, Colombia. One hundred and eighty five faecal samples were collected between 2010 and 2015. Direct faecal smears were performed with saline solution (0.85%) and iodine solution (1%), in order to identify larvae and eggs based on their morphology. A large proportion of the samples examined (72.9%) was positive for intestinal parasites; seven families of nematodes were identified: Trichuridae, Trichostrongylidae, Oxyuridae, Strongyloididae, Ancylostomatidae, Ascarididae and Gnathostomatidae, two of protozoans: Entamoebidae and Balantiididae, as well as some eggs of trematodes, cestodes and acanthocephalans. Additionally, DNA extraction and sequencing were conducted on 30 faecal samples in order to identify Giardia sp. and Blastocystis hominis, two parasite species also present in humans. Molecular testing for Giardia sp. was negative and Blastocystis hominis was identified in a single sample of Alouatta seniculus. No clear patterns were observed for prevalence of intestinal parasites according to the season; nonetheless, parasite species richness was higher during the dry season. This study builds on our current understanding of intestinal parasites infecting wild neotropical primates and provides novel information on the patterns of intestinal parasites in primate communities exposed to anthropogenic disturbance.

© 2017 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

* Corresponding author.

Several genera of protozoans and metazoans are known to infect all major clades of living non-human primates (Nunn et al., 2003). Some of these are regarded as non-pathogenic since they seem to have no detrimental effects on their hosts. However, a large number of parasites can harm their hosts, leading to physiological changes, nutritional disturbances or injuries resulting in severe weakness,

http://dx.doi.org/10.1016/j.ijppaw.2017.07.006

E-mail addresses: sy.rondon941@uniandes.edu.co (S. Rondón), mario-or@ uniandes.edu.co (M. Ortiz), cm.leon@uniandes.edu.co (C. León), nf.galvis229@ uniandes.edu.co (N. Galvis), a.link74@uniandes.edu.co (A. Link), c.gonzalez2592@ uniandes.edu.co (C. González).

^{2213-2244/© 2017} The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (http:// creativecommons.org/licenses/by-nc-nd/4.0/).

and the possibility of generating susceptibility to secondary infections that can be fatal (Toft, 1982), among other adverse physiological consequences. These processes can be exacerbated by immunosuppression, and different stress-related factors (Chapman et al., 2006; Abee et al., 2012). Likewise, deteriorated health status of animals can lead to negative impacts on their reproduction (Panayotova-Pencheva, 2013) and survival. Although intestinal parasitosis is a major cause of morbidity and mortality in New World monkeys (Guerrero et al., 2012), and some studies have been carried out in the last years (Trejo-Macías et al., 2007; Vitazkova and Wade, 2007; Chinchilla et al., 2010; Maldonado-López et al., 2014; Behie et al., 2014; Martínez-Mota et al., 2017; Rimbach et al., 2015; Solórzano-García and Pérez-Ponce de León, 2017) further research is required to better understand disease dynamics on New World primates. Currently, environmental changes and land use transformation are globally affecting the dynamics of disease transmission among wild populations of non-human primates and humans (Goldberg et al., 2008; Gottdenker et al., 2014), raising the importance and relevance of better recognizing and monitoring the current health status of wild non-human primates in their natural ecosystems.

In Colombia, the historical distribution of natural forests in the Middle Magdalena River valley has been reduced to <15% (Roncancio-Duque and Benavides Montaño, 2013); mainly due to land use changes associated with extensive cattle ranching, oil palm plantations and mining (Link et al., 2013). This pervasive process of land use change, might lead to alterations in the parasite - host dynamics since forest fragmentation favours contact between humans and non-human primates, increasing the possibility of parasite transmission (Stuart and Strier, 1995; Gillespie and Chapman, 2006; Trejo-Macías et al., 2007). Thus, it is of great importance to conduct research in this biogeographic region to better understand the processes related to parasitic infections from the perspective of the zoonotic risk of transmission to humans, and to complement previous studies such as the one conducted by Galvis et al. (unpublished data), which found higher parasite richness, prevalence and diversity in brown spiders monkeys living in small forest fragments compared to those living in a relatively undisturbed forest.

There are few studies on species richness of intestinal parasites and its effects on behaviour, health and survival of capuchin monkeys (Cebus spp.). These infections may be prevalent in primates due to their omnivorous diet, frequent contact with soil, and their large group sizes and active social behaviour (e.g., grooming) (Chinchilla et al., 2007). Previous studies on free ranging capuchin monkeys have reported several intestinal parasites, including nematodes (Strongyloides cebus, Trichostrongylidae), cestodes (Paratriotaenia oedipomidatis), acanthocephalans (Prosthernorchis elegans) and protozoans (Balantidium coli, Cryptosporidium spp) (Chinchilla et al., 2010; Guerrero et al., 2012; Parr et al., 2013). For howler monkeys (Alouatta spp.), which are considered folivores-frugivores (Arroyo-Rodríguez and Dias, 2010), several genera of parasites from different groups have been reported: protozoans (Endolimax, Entamoeba, Trichomonas, Giardia, Balantidium, Chilomastix), nematodes (Strongyloides, Trypanoxyuris, Ascaris, Ancylostoma) and trematodes (Controrchis) (Chinchilla et al., 2005; Trejo-Macías et al., 2007; Vitazkova and Wade, 2007; Solórzano-García and Pérez-Ponce de León, 2017). Regarding the genus Ateles, the diet is mainly frugivorous (González-Zamora et al., 2009). Reports of parasites infecting Ateles include nematodes (Strongyloides, Trichuris, Trypanoxyuris, Ancylostoma, Ascaris), trematodes (Controrchis) (Carrasco et al., 2008; Rimbach et al., 2015; Solórzano-García and Pérez-Ponce de León, 2017), and protozoans (Giardia, Eimeria, Entamoeba, Neobalantidium) (González Bernal, 2014; Rimbach et al., 2015).

The incidence of many parasites greatly varies according to the season, and seasonality has been suggested to affect host-pathogen interactions (Altizer et al., 2006). Inside their hosts, parasites are protected from factors such as temperature and humidity but their free-living stages may be directly affected by adverse environmental conditions. Understanding how seasonal changes affect the population dynamics of zoonotic parasites can contribute to a better understanding of their epidemiology (Burlet et al., 2011). Such studies tend to be selectively made in species with economic interest such as horses and sheep (Arece, 2007; Fusé et al., 2013), or pets like cats and dogs (Barutzki and Schaper, 2011). However, some studies have also been conducted in primates (Chapman et al., 2009; Behie et al., 2014; Duboscq et al., 2016; Martínez-Mota et al., 2017), without finding a general pattern of parasite prevalence in correlation with seasonality. Martínez-Mota et al. (2017) found lower parasite richness during rainy seasons, while Chapman et al. (2009) reported higher parasite prevalence at wet sites, and Behie et al. (2014) did not find significant differences on levels of parasitism according to seasonality. Although it is difficult to discern clear explanations for the distribution mechanisms of parasites in wild primate populations, it is critical to examine these patterns in host species that are increasingly endangered due to anthropogenic threats (MacIntosh et al., 2010).

In order to increase our knowledge on parasite prevalence in wild neotropical primates, this study aimed to assess the seasonal variation of the prevalence and richness of intestinal parasites of three sympatric primate species: *Cebus versicolor, Ateles hybridus* and *Alouatta seniculus,* living in a heavily fragmented landscape in northern South America.

2. Materials and methods

2.1. Study site

The study site is located in a forest fragment which consists of 65 ha in San Juan del Carare, Santander Department, Colombia (06°43'N, 74°09' W; 150–200 m.a.s.l) (Rimbach et al., 2013). The region has a complex mosaic of forest fragments and wetlands, within a matrix of pastures for cattle ranching and more recently agroindustrial monocultures. This area has an average temperature of 28 °C, average humidity of 80% and receives an average annual median rainfall of 3496 mm (IDEAM, 2008).

2.2. Faecal samples

Faecal samples were collected in the years 2010 (rainy season), 2011 (dry season), 2012 (dry season), 2015-1 (dry season) and 2015-2 (rainy season) (Table 1). Primates were followed from dawn to dusk and faecal samples were collected opportunistically and non-invasively from the soil immediately after defecation. Samples were

Table 1	
Temperature ($^{\circ}$ C) and	Rainfall (mms) by sampling season.

Sampling year	Month	Temperature (°C) ^a	Rainfall (mms) ^a
2010	October	28.2	502.8
	November	27.5	536.4
2011	January	28.7	180.6
	February	28.4	181.3
2012	February	29.6	58.5
	March	28.5	157.1
2015-1	January	29.0	164.8
	February		
	February	28.8	138.5
2015-2	May	29.7	205.3
	June	28.9	56.1

^a (IDEAM, 2017).

placed in Falcon tubes with 10% formalin solution until their transportation to the laboratory where direct microscopic observation was performed in order to identify parasites according to their morphology (Arnot, 1998; Ash and Orihel, 2010; Gutierrez, 2000; Lardín and Pacheco, 2015). We collected 61 samples of brown spider monkeys (*Ateles hybridus*), 38 samples of capuchin monkeys (*Cebus versicolor*) and 56 samples of red howler monkeys (*Alouatta seniculus*). Those sample sizes correspond to one sample per individual and were used for parasite prevalence and richness calculations.

In the case of 6 *A. hybridus* individuals and 6 *A. seniculus* individuals, it was possible to collect 1 to 3 additional samples from the same individual across different time periods, thereby reaching 77 samples of *A. hybridus*, 38 samples of *C. versicolor* and 70 samples of *A. seniculus*. All sampled primates were habituated, and have been individually identified by marks on their face and/or fur. Juvenile, sub-adult and adult primates were included in the sampling.

Additionally, 5 samples from *Ateles hybridus*, 15 from *Cebus versicolor* and 10 from *Alouatta seniculus* were collected during 2015-1 and placed in Falcon tubes with 96% ethanol solution. These samples were used to perform molecular analyses in order to detect *Blastocystis hominis* and *Giardia* sp. as protozoans are more difficult to detect through faecal smears.

2.3. Smears

We performed faecal smears with 0.85% saline solution and 1% iodine solution following the methods by Botero and Restrepo (2012), in order to detect intestinal parasites. This method consists of a microscope slide with a drop of saline solution placed at one end, and a drop of iodine solution at the other end. Each drop was mixed with 2 mg of stool. Each slide was examined with a microscope, systematically, using the objectives 10x, 40x and 100x. Photos of eggs and larvae were taken for later identification. For each sample, two slides were mounted in order to minimize the probability of overlooking parasites in the samples.

2.4. Molecular analyses for Blastocystis and Giardia

To perform detection of *Blastocystis hominis* and *Giardia* sp., we extracted DNA from faecal samples stored in ethanol using a QIAamp DNA Stool Kit (Qiagen), and its QIAamp DNA Stool Handbook Protocol. DNA concentration was measured for all extracts using a nanodrop device. *Blastocystis hominis* detection was performed through PCR using the primers RD5 and BhRDr (Scicluna et al., 2006) and Go Taq Master Mix, targeting a 607bp fragment. Additionally, a semi-nested PCR assay was performed for *Giardia* sp. detection, using the primers GDHeF, GDHiF and GDHiR (Read et al., 2004). Reactions were performed using Go Taq Master Mix targeting a 450–500 pb fragment.

All PCR products were visualized on agarose gel and positive samples were sequenced. Sequences were analysed using *Geneious* Software and compared with publicly available sequences using BLAST (National Center for Biotechnology Information).

2.5. Data analyses

The prevalence of parasites in each primate species was calculated as the number of infected individuals divided by the total of examined individuals (multiplied by 100). Likewise, we estimated parasite richness as the number of genera recovered from the host's faecal samples (Muehlenbein, 2005). A two-way analysis of variance (ANOVA) was performed using the RStudio Software, in order to evaluate the effect of seasonality and species on the prevalence and richness of parasites. ANOVA was followed

by a Tukey's test as a post-hoc test to elucidate differences between species.

Additionally, comparisons between social groups of the same primate species were made: SJ1 (9 individuals) and SJ2 (9 individuals) in *A. hybridus* and C0 (7 individuals), C2 (8 individuals) and C3 (8 individuals) in *A. seniculus*. Finally, seasonality of parasites was analysed in some individuals of *A. hybridus* (6 individuals) and *A. seniculus* (6 individuals), since it was possible to collect samples from the same individuals across different time periods.

3. Results

3.1. Smears

From the 185 samples analysed through faecal smears, 72.9% (n = 135) were positive for intestinal parasites. Based on their morphology we identified seven families of nematodes: *Trichuridae*, *Trichostrongylidae*, *Oxyuridae*, *Strongyloididae*, *Ancylostomatidae*, *Ascarididae* and *Gnathostomatidae*; two of protozoans: *Entamoebidae* and *Balantiididae*, as well as some eggs of trematodes, cestodes and acanthocephalans (Fig. 1).

3.2. Seasonality, prevalence and richness of parasites

Prevalence was not related to seasonality (Anova: p-value = 0.066; F-value = 3.421; Df = 1), nor were the species of primates significant predictors of prevalence (Anova: p-value = 0.117; F-value = 2.170; Df = 2); however, for the three primate species the highest prevalence values were found during the dry season (Table 2). Overall, the most prevalent parasites between 2010 and 2012 were *Strongyloididae*, *Ancylostomatidae* and *Balanti-diidae*, while in 2015 the most prevalent were *Trichuridae*, *Ascarididae*, *Strongyloididae* and *Ancylostomatidae*. Variation in parasite prevalence was observed for all groups over time, although it was not always significant. For all primate species, *Strongyloididae* and *Ancylostomatidae* in *A. seniculus*, *Balantidiidae* in *A. hybridus* and *Trichiuridae* in *C. versicolor*.

Season was related to parasite richness (Anova: p-value = 0.001; F value = 22.334; Df = 1) with the highest richness occurring during the dry season. Although parasite richness was high in the three primate species, it differed among primate species (Anova: p-value = 0.001; F value = 64.805; Df = 2), (Tukey's post-hoc test: *A. hybridus* vs. *A. seniculus* (P = 0.001); *C. versicolor* vs. *A. seniculus* (P < 0.001); *C. versicolor* vs. *A. hybridus* (P < 0.001)). Regarding seasonality, in each sampling year there were subtle variations between parasite genera circulating, except for the dry season of 2015 when *C. versicolor* had the highest richness (Table 3).

For A. seniculus, in 2011 C2 and C3 groups shared parasites belonging to two families (Strongyloididae and Ancylostomatidae). while in 2015 CO and C2 groups did not share families of parasites. On the other hand, in A. hybridus SI1 and SI2 social groups shared only two families of parasites (Strongyloididae and Balantidiidae) in 2010, and in 2011 the results were identical. Regarding the parasitological analyses at the individual level, a large variation in the presence or absence of intestinal parasites was observed across sampling periods. It may be noted that in the case of A. hybridus, Balantidiidae was found in three different individuals during three consecutive years. Meanwhile, Ancylostomatidae and Strongyloididae were present in two consecutive years, in one and two individuals each. As for A. seniculus, Ancylostomatidae was observed in two individuals during two consecutive years, while Oxyuridae and Strongyloididae were absent for three years in one individual. Generally, no clear patterns of infection among individuals and groups were observed.



Fig. 1. A. Trichuris sp., B. Oxyuridae, C. Ancylostomatidae, D. Strongyloides sp. (larva), E. Ascarididae, F. Gnathostomatidae, G. Trichostrongylidae, H-I. Trematodes, J. Entamoeba sp. (cyst), K. Acanthocephala, L. Balantidiidae.

Table 2 Prevalence (%) of intestinal parasites in each sampling year for each non-human primate species.

Parasite	Alouatta seniculus				Ateles hybridus				Cebus versicolor				
	2010 Rainy n = 7	2011 Dry n = 19	2012 Dry n = 5	2015-1 Dry n = 9	2015—2 Rainy n = 16	2010 Rainy n = 15	2011 Dry n = 18	2012 Dry n = 10	2015-1 Dry <i>n</i> = 4	2015-2 Rainy n = 14	2012 Dry n = 8	2015-1 Dry <i>n</i> = 14	2015-2 Rainy n = 16
Trichuridae	-	-	-	11.1	-	-	-	-	50	-	-	64.3	-
Oxyuridae	42.9	_	20	44.4	6.3	13.3	5.6	10	25	_	25	14.3	6.3
Trichostrongylidae	_	15.8	20	44.4	_	_	_	_	_	_	_	50	_
Strongyloididae	14.3	26.3	20	11.1	12.5	53.3	22.2	30	_	_	62.5	50	18.8
Ancylostomatidae	28.6	36.8	20	22.2	_	13.3	33.3	10	_	35.7	37.5	14.3	43.8
Ascarididae	14.3	_	_	100	_	6.7	_	_	25	7.1	12.5	35.7	6.3
Gnathostomatidae	_	_	_	_	12.5	_	_	_	_	_	_	_	_
Balantidiidae	_	_	_	_	_	26.7	77.8	30	_	_	_	_	_
Entamoebidae	_	_	-	22.2	_	_	5.6	_	_	_	_	7.1	_
Acanthocephala	_	_	_	_	_	_	_	_	_	_	12.5	14.3	37.5
Trematodes	_	_	_	11.1	_	_	_	_	_	_	_	21.4	6.3
Cestodes	14.3	5.3	20	_	-	-	5.6	-	-	-	12.5	-	6.3

Table 3

Species richness of intestinal parasites in each sampling year for each non-human primate species.

Primate species	Number of parasites	Parasite richness (%)							
	Helminths and protozoa	Total sampling	2010 Rainy	2011 Dry	2012 Dry	2015-1 Dry	2015-2 Rainy		
Alouatta seniculus Ateles hybridus Cebus versicolor	10 8 10	9/12 (75%) 8/12 (66.7%) 10/12 (83.3%)	5/12 (41.7%) 5/12 (41.7%)	4/12 (33.3%) 6/12 (50%)	5/12 (41.7%) 4/12 (33.3%) 6/12 (50%)	8/12 (66.7%) 3/12 (25%) 9/12 (75%)	3/12 (25%) 2/12 (16.7%) 7/12 (58.3%)		
	Helminths								
Alouatta seniculus Ateles hybridus Cebus versicolor	9 6 9	9/10 (90%) 6/10 (60%) 9/10 (90%)	5/10 (50%) 4/10 (40%)	4/10 (40%) 4/10 (40%)	5/10 (50%) 3/10 (30%) 6/10 (60%)	8/10 (80%) 3/10 (30%) 8/10 (80%)	3/10 (30%) 2/10 (20%) 7/10 (70%)		
	Protozoa								
Alouatta seniculus Ateles hybridus Cebus versicolor	1 2 1	1/2 (50%) 2/2 (100%) 1/2 (50%)	_ 1/2 (50%)	_ 2/2 (100%)		1/2 (50%) 1/2 (50%)			

3.3. Molecular analyses

From faecal samples stored in ethanol solution (N = 30), *Blastocystis hominis* was detected and identified by sequencing in one sample of *Alouatta seniculus*. This sequence exhibited 99% sequence identity to published sequences (Accession number: DQ232828.1). Molecular testing for *Giardia* sp. was negative for all the tested samples (N = 30).

4. Discussion

Our results provide valuable information to the study of parasites in non-human primates in Colombia, since from the perspective of zoonoses, it is essential to establish the parasite species exclusive to non-human primates, and those that could be potentially shared between non-human and humans, particularly in the current processes of land use change and biodiversity loss (Newbold et al., 2016).

Strongyloididae parasites can infect their hosts through skin penetration or by ingestion (Parr et al., 2013), and reproduces in the host's intestinal wall asexually, contributing to its high infection rates (Barutzki and Schaper, 2011). *Strongyloides* spp. are susceptible to drying and *Ancylostomatidae* survives just a few weeks, even under favourable conditions; however, these parasites are usually found in warm and humid climates (Harman, 2002), such as our sampling site. Regarding *Oxyuridae*, these parasites have a wide distribution and great tendency to spread from one individual to the other (Botero and Restrepo, 2012).

Ascarididae were present in all the samples except for the ones collected in 2011. Eggs from this group of parasites persist in the ground for long periods of time and can remain infectious for years (Newton-Fisher et al., 2006). Strait et al. (2012) state that this group of parasites is often found in the intestinal tract of non-human primates and transmission to humans is possible; therefore infected non-human primates can be considered a potential zoonotic threat. On the contrary, infection with *Gnathostomatidae* was restricted to *A. seniculus* in the last sampling period. Gnathostomiasis is likely acquired through the accidental consumption of infected snails present on the plants on which they feed.

Regarding *Balantidiidae*, they have been reported in several species of non-human primates, including New World monkeys (howler monkeys, spider monkeys and capuchin monkeys), which can be asymptomatic carriers, as the parasite is usually non-pathogenic (Strait et al., 2012). In this study it was only found in *A. hybridus* between 2010 and 2012, and we suggest that those infected individuals were asymptomatic since no signs of intestinal disorders were found. However, it is important to recognise that faecal smears are just a small part of the faeces produced by the individual, thus any evidence of intestinal disorders, such as blood, or other parasites may have been missed. Additionally, one individual of *A. hybridus* was infected with *Entamoebidae*; hence, it was the primate species most commonly infected with protozoans, although in the case of *Entamoebidae* the prevalence was very low (5.6%).

This study supports previous reports on *Trichostrongylidae*, infecting New World primates (Toft, 1982), as we observed this family in *A. seniculus* and *C. versicolor*. Meanwhile, *Trichiuridae* was found in 2010 and 2015 in all three primate species. Infection with this family is usually asymptomatic and can occur in primates of the Old and New World (Courtney, 2012).

Additionally, the three primate species were infected with cestodes. This group of parasites was absent in the 2015-1 samples, and overall the prevalence rate was low. Toft (1982) states that although these parasites can occur in large quantities, they are rarely associated with enteric lesions, so are unlikely to be detrimental to the primates' health.

Infection with trematode eggs was recorded only in 2015 in four individuals of *C. versicolor* and one individual of *A. seniculus*. The finding of a parasite that requires an intermediate host suggests that the primates ingested them while consuming fish or invertebrates (ants and crabs) (Botero and Restrepo, 2012). Invertebrates are part of the diet of *C. versicolor*, whereas in *A. seniculus* accidental consumption could have occurred because their diet is folivore-frugivorous (Arroyo-Rodríguez and Dias, 2010). On the other hand, acanthocephalans need invertebrate intermediate hosts (Barrientos, 2003), which means that the *C.versicolor* individuals that we found infected with acanthocephalans likely ingested intermediate hosts. In our study, the intermediate hosts are likely to be insects, as we found exoskeletons during the stool analysis.

We identified *Blastocystis hominis* by molecular analyses in one *A.seniculus* individual. Stensvold and Clark (2016) suggest that *B. hominis* transmission occurs through water or by fecal-oral contamination. We suggest that this infection could happen through ingestion of faeces contaminated with cysts from humans or domestic animals such as cattle, which are raised on a large scale in grassland surrounding the forest fragment of the study site. Regarding *Giardia*, Thompson (2013) indicates that it is a zoonotic parasite mainly found in wildlife as a result of environmental contamination with *Giardia* from humans or pets; this may explain the absence of this parasite in our study.

It has been suggested that habitat fragmentation leads to greater richness and prevalence of parasites (Goldberg et al., 2008). Taking into account that the study site has experienced a high level of fragmentation (Link et al., 2013), the high richness of parasites in the primate community at San Juan could be partially attributed to this factor. In future studies we recommend considering other factors that could be influencing the prevalence and richness of parasites, such as population density, social group size and fruiting phenology.

In this study, we found a relation between dry season and higher parasite richness. On the contrary, despite finding higher prevalence rates during the dry season, they are not statistically significant, and no clear patterns were found. Okoye et al. (2014) conducted a study in cats, finding higher prevalence of parasites in summer, probably due to the warm temperature that can favour eggs; in our study this factor could also explain the higher parasite prevalence and richness found in the dry season.

Diet can also affect parasite prevalence, and our results show *A. hybridus* having higher parasite prevalence than *A. seniculus*, which is mainly folivorous at this study site (Alvis Rojas, Unpublished data). This result was unexpected, since *A. hybridus* is predominantly frugivorous, having a lower probability than folivorous species of oral infection through food contamination with faeces (Vitone et al., 2004). Nonetheless, spider monkeys at San Juan consume a large proportion of leaves in their diet year round (Abondano and Link, 2012). However, primates living in fragmented and reduced habitat are often forced to feed more often on leaves than fruits (Abondano and Link, 2012). *Cebus versicolor* had the highest prevalence rate, which could be expected due to its widely varied diet and direct consumption of potential intermediate hosts. These findings support our hypothesis of higher parasite prevalence in primates with more varied diets.

Regarding social groups and individual comparisons, we expected members of the same group to share a larger proportion of parasites. However, we could not identify any patterns in this study. In the case of *A. hybridus*, it is important to consider its fission-fusion grouping patterns, which consists of large groups that could be separated into subunits of variable sizes and associations (Gebo, 2014), thus, the combination of individuals in a group can

vary. The variation in social interactions such as grooming can have profound implications for the patterns of transmission of parasites (Rimbach et al., 2015). Generally no clear patterns among individuals and social groups were observed, possibly due to specific individual characteristics. Poulin (2001) states that there may be variations in the number of parasite species harboured by individual hosts from the same population, since individual characteristics such as age, size and foraging sites influence the probability of acquiring certain species of parasites. Thus, if these characteristics change over time, the presence/absence of parasite groups in each of the individuals might also change.

This study had a small sample size, and didn't take into account variables such as group size, gender, stress factors and social connectivity; for these reasons the results should be interpreted with caution. However, this study contributes new information of intestinal parasites in wild neotropical primates and its patterns in primate communities exposed to anthropogenic disturbance.

5. Conclusions

Richness of intestinal parasites in the primate community living in the tropical rainforests of San Juan del Carare, in Colombia was higher during the dry season and had evident differences between the three diurnal primate species. However, no clear patterns were observed regarding seasonality and prevalence of intestinal parasites in the three primate species examined, either in the presence/ absence of parasites between primate social groups or individual monkeys. This study supports the hypothesis that there are differences in the richness of parasites between non-human primate species according to their diet and foraging behaviour, as well as their use of the different strata of the forest. Also, this study provides additional support for the effect of seasonality on the richness, but not the prevalence, of intestinal parasites in wild populations of primates.

Acknowledgements

This research didn't receive any specific funding from the public, commercial, or not-for-profit sectors. We thank Rufford Small Grants # 11097-2 for providing financial support, and Centro de Investigaciones en Microbiología y Parasitología Tropical (CIMPAT) at Universidad de los Andes as well as Fundación Proyecto Primates for providing all logistic support. Authors wish to thank Dr. JD Ramirez for providing *Giardia* and *Blastocystis hominis* primers. We also thank anonymous reviewers for their comments on the manuscript as well as J Rossow and B Zimmermann for English revision.

References

- Abee, C., Mansfield, K., Tardif, S., Morris, T., 2012. Nonhuman Primates in Biomedical Research: Diseases, second ed. American College of Laboratory Animal Medicine Series, USA.
- Abondano, L., Link, A., 2012. The social behavior of brown spider monkeys (Ateles hybridus) in a fragmented forest in Colombia. Int. J. Primatol. 33, 769–783. http://dx.doi.org/10.1007/s10764-012-9596-1.
- Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., Rohani, P., 2006. Seasonality and the dynamics of infectious diseases. Ecol. Lett. 9, 467–484. http:// dx.doi.org/10.1111/j.1461-0248.2005.00879.x.
- Arece, J., 2007. La epizootiología como herramienta para el control parasitario en ovinos. Pastos Forrajes 30, 35–43.
- Arnot, D., 1998. Atlas of human parasitology, 4th Edn Parasitol. Today 14, 168.
- Arroyo-Rodríguez, V., Dias, P., 2010. Effects of habitat fragmentation and disturbance on howler monkeys: a review. Am. J. Primatol. 72, 1–16. http://dx.doi.org/ 10.1002/ajp.20753.
- Ash, L., Orihel, T., 2010. Atlas de Parasitología Humana. Quinta Ed. Editorial Médica Panamericana, Buenos Aires, Argentina.
- Barrientos, Z., 2003. Zoología General, first ed. Editorial Universidad Estatal a Distancia, San José, Costa Rica.

- Barutzki, D., Schaper, R., 2011. Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010. Parasitol. Res. 109 http://dx.doi.org/10.1007/s00436-011-2402-8.
- Behie, A.M., Kutz, S., Pavelka, M.S., 2014. Cascadinf effects of climate change: do hurricane-damaged forests increase risk of exposure to parasites? Biotropica 46, 25–31.
- Botero, D., Restrepo, M., 2012. Parasitosis humanas. Quinta Ed. Corporación para Investigaciones Biológicas, Medellín.
- Burlet, P., Deplazes, P., Hegglin, D., 2011. Age, season and spatio-temporal factors affecting the prevalence of *Echinococcus multilocularis* and *Taenia taeniaeformis* in *Arvicola terrestris*. Parasit. Vectors 4, 6. http://dx.doi.org/10.1186/1756-3305-4-6.
- Carrasco, F., Tantaleán, M., Gibson, K., Williams, M., 2008. Prevalencia de helmintos intestinales de una población de monos maquisapas silvestres Ateles belzebuth chamek en el Parque Nacional de Manu, Perú. Neotrop. Helminthol. 2, 19–26.
- Chapman, C.A., Wasserman, M.D., Gillespie, T.R., Speirs, M.L., Lawes, M.J., Saj, T.L., Ziegler, T.E., 2006. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? Am. J. Phys. Anthropol. 131, 525–534. http://dx.doi.org/10.1002/ajpa.20477.
- Chapman, C.A., Speirs, M.L., Hodder, S., Rothman, J.M., 2009. Colobus monkey parasite infections in wet and dry habitats: implications for climate change. Afr. J. Ecol. 48, 555–558.
- Chinchilla, M., Guerrero, O., Gutiérrez-Espeleta, G., Sánchez, R., Valerio, I., 2007. Parásitos en monos carablanca *Cebus capucinus* (Primates: Cebidae) de Costa Rica. ScieELO 62, 170–175. http://dx.doi.org/10.4067/S0717-77122007000200011.
- Chinchilla, M., Guerrero, O., Gutiérrez-Espeleta, G., Porras, R., Ortiz, B., 2005. Parásitos intestinales en monos Congo Alouatta palliata (Primates: Cebidae) de Costa Rica. Rev. Biol. Trop. 53, 437–445.
- Chinchilla, M., Urbani, B., Valerio-Campos, I., Vanegas, J.C., 2010. Intestinal parasites in white-faced capuchin monkeys *Cebus capucinus* (Primates: Cebidae) inhabiting a protected area in the Limón province of Northeastern Costa Rica. Rev. Biol. Trop. 58, 1335–1346.
- Courtney, A., 2012. Pocket Handbook of Nonhuman Primate Clinical Medicine. CRC Press.
- Duboscq, J., Romano, V., Sueur, C., MacIntosh, A., 2016. Network centrality and seasonality interact to predict lice load in a social primate. Nature. http:// dx.doi.org/10.1038/srep22095.
- Fusé, L., Saumell, C.A., Iglesias, L., 2013. Variación estacional del parasitismo interno en equinos: fenómeno de hipobiosis de los pequeños estróngilos (Cyathostominae) en Tandil, Buenos Aires, Argentina. Rev. Med. Vet. (B. Aires) 94 (3), 62–72.
- Gebo, D., 2014. Primate Comparative Anatomy. Johns Hopkins University Press, Baltimore, Unites States of America.
- Gillespie, T.R., Chapman, C.A., 2006. Prediction of parasite infection dynamics in primate metapopulations based on attributes of forest fragmentation. Conserv. Biol. 20, 441–448. http://dx.doi.org/10.1111/j.1523-1739.2006.00290.x.
- Goldberg, T.L., Gillespie, T.R., Rwego, I.B., Estoff, E.L., Chapman, C.A., 2008. Forest fragmentation as cause of bacterial transmission among nonhuman primates, humans, and livestock, Uganda. Emerg. Infect. Dis. 14, 1375–1382. http:// dx.doi.org/10.3201/eid14.9.071196.
- González Bernal, I., 2014. Identificación de parásitos intestinales en el primate neotropical *Ateles hybridus* en un centro de paso de fauna en el municipio de Sabana de Torres, Santander. Citecsa 4, 13–18.
- González-Zamora, A., Arroyo-Rodríguez, V., Chaves, O.M., Sánchez-López, S., Stoner, K.E., Riba-Hernández, P., 2009. Diet of spider monkeys (*Ateles geoffroyi*) in Mesoamerica: current knowledge and future directions. Am. J. Primatol. 71, 8–20. http://dx.doi.org/10.1002/ajp.20625.
- Gottdenker, N.L., Streicker, D.G., Faust, C.L., Carroll, C.R., 2014. Anthropogenic land use change and infectious diseases: a review of the evidence. Ecohealth 11, 619–632. http://dx.doi.org/10.1007/s10393-014-0941-z.
- Guerrero, F., Serrano-Martínez, E., Tantaleán, M., Quispe, M., Casas, G., 2012. Identificación de parásitos gastrointestinales en primates no humanos del zoológico Parque Natural de Pucallpa, Peru. ScieELO 23, 469–476.
- Gutierrez, Y., 2000. Diagnostic Pathology of Parasitic Infections with Clinical Correlations, Second. ed. Oxford University Press, New York.
- Harman, R., 2002. Handbook of Pharmacy Healthcare: Diseases Ans Patient Advice. Pharmaceutical Press, London.
- IDEAM, 2008. Instituto de hidrologia, meteorologia y estudios ambientales [WWW Document]. http://www.ideam.gov.co/ (Accessed 12 April 2008).
- IDEAM, 2017. Instituto de hidrologia, meteorologia y estudios ambientales [WWW Document]. http://www.ideam.gov.co/ (Accessed 10 July 2017).
- Lardín, C., Pacheco, S., 2015. Helminths Handbook for Identification and Counting of Parasitic Helminth Eggs in Urban Wastewater. IWA Publihing, London.
- Link, A., de Luna, G., Burbano, J., 2013. Estado de conservación de uno de los primates más amenazados con la extinción: el mono araña cafe (*Ateles hybridus*).
 In: Primates Colombianos En Peligro de Extinción. Asociación Primatológica Colombiana, Bogotá, pp. 88–118.
- MacIntosh, A.J.J., Hernandez, A.D., Huffman, M.A., 2010. Host age, sex, and reproductive seasonality affect nematode parasitism in wild Japanese macaques. Primates 51, 353–364. http://dx.doi.org/10.1007/s10329-010-0211-9.
- Maldonado-López, S., Maldonado-López, Y., Gómez-Tagle, A., Cuevas-Reyes, P., Stoner, K., 2014. Patterns of infection by intestinal parasites in sympatric howler monkey (*Alouatta palliata*) and spider monkey (*Ateles geoffroyi*) populations in a tropical dry forest in Costa Rica. Primates 55, 383–393.
- Martínez-Mota, R., Garber, P.A., Palme, R., Gillespie, T.R., 2017. The relative effects of reproductive condition, stress, and seasonality on patterns of parasitism in wild

female black howler monkey (*Alouatta pigra*). Am. J. Primatol. http://dx.doi.org/ 10.1002/ajp.22669.

- Muehlenbein, M., 2005. Parasitological analyses of the male Chimpanzees (*Pan troglodytes schweinfurthii*) at Ngogo, Kibale National park. Uganda 179, 167–179. http://dx.doi.org/10.1002/ajp.20106.
- Newbold, T., Hudson, L.N., Arnell, A.P., Contu, S., Palma, A. De, Ferrier, S., Hill, S.L.L., Hoskins, A.J., Lysenko, I., Phillips, H.R.P., Burton, V.J., Chng, C.W.T., Emerson, S., Gao, D., Pask-Hale, G., Hutton, J., Jung, M., Sanchez-ortiz, K., Simmons, B.I., Whitmee, S., Zhang, H., 2016. Has land use pushed terrestrial biodivesity beyond the planetary boundary? A global assessment. Science (80-.) 353, 288–291.
- Newton-Fisher, N., Paterson, J., Notman, H., Reynolds, V., 2006. Primates of Western Uganda. Springer.
- Nunn, C.L., Altizer, S., Jones, K.E., Sechrest, W., 2003. Comparative tests of parasite species richness in primates. Am. Nat. 162, 597–614. http://dx.doi.org/10.1086/378721.
- Okoye, I.C., Obiezue, N.R., Okoh, F.N., Amadi, E.C., 2014. Descriptive epidemiology and seasonality of intestinal parasites of cats in Southeast Nigeria. Comp. Clin. Path 23, 999–1005. http://dx.doi.org/10.1007/s00580-013-1734-4.
- Panayotova-Pencheva, M.S., 2013. Parasites in Captive animals: a review of studies in some European Zoos. Zool. Gart. 82, 60–71. http://dx.doi.org/10.1016/ j.zoolgart.2013.04.005.
- Parr, N.A., Fedigan, L.M., Kutz, S.J., 2013. A coprological survey of parasites in whitefaced capuchins (*cebus capucinus*) from Sector Santa Rosa, ACG, Costa Rica. Folia Primatol. 84, 102–114. http://dx.doi.org/10.1159/000348287.
- Poulin, R., 2001. Interactions between species and the structure of helminth communities. Parasitology 122, S3–S11. http://dx.doi.org/10.1017/S0031182000016991.
 Read, C.M., Monis, P.T., Thompson, R.C.A., 2004. Discrimination of all genotypes of
- Read, C.M., Monis, P.T., Thompson, R.C.A., 2004. Discrimination of all genotypes of Giardia duodenalis at the glutamate dehydrogenase locus using PCR-RFLP. Infect. Genet. Evol. 4, 125–130. http://dx.doi.org/10.1016/j.meegid.2004.02.001.
 Rimbach, R., Bisanzio, D., Galvis, N., Link, A., Fiore, A. Di, Gillespie, T.R., 2015. Brown
- Rimbach, R., Bisanzio, D., Galvis, N., Link, A., Fiore, A. Di, Gillespie, T.R., 2015. Brown spider monkeys (*Ateles hybridus*): a model for differentiating the role of social networks and physical contact on parasite transmission dynamics. Phil. Trans. R. Soc. B 370. http://dx.doi.org/10.1098/rstb.2014.0110.
- Rimbach, R., Heymann, E.W., Link, A., Heistermann, M., 2013. Validation of an enzyme immunoassay for assessing adrenocortical activity and evaluation of

factors that affect levels of fecal glucocorticoid metabolites in two New World primates. Gen. Comp. Endocrinol. 191, 13–23. http://dx.doi.org/10.1016/j.ygcen.2013.05.010.

- Roncancio-Duque, N., Benavides Montaño, J.A., 2013. Parásitos intestinales en poblaciones pequeñas y aisladas de mono aullador rojo (*Alouatta seniculus*) y mono araña café (*Ateles hybridus*), Atelidae – primates en el Magdalena Medio, Colombia. Vet. Zootec, 7, 71–89.
- Scicluna, S.M., Tawari, B., Clark, C.G., 2006. DNA barcoding of Blastocystis. Protist 157, 77–85. http://dx.doi.org/10.1016/j.protis.2005.12.001.
- Solórzano-García, B., Pérez-Ponce de León, G., 2017. Helminth parasites of howler and spider monkeys in Mexico: insights into molecular diagnostic methods and their importance for zoonotic diseases and host conservation. Int. J. Parasitol. Parasites Wildl. 6, 76–84.
- Stensvold, C.R., Clark, C.G., 2016. Current status of Blastocystis: a personal view. Parasitol. Int. 65, 763–771. http://dx.doi.org/10.1016/j.parint.2016.05.015.
- Strait, K., Else, J., Eberhard, M., 2012. Parasitic diseases of nonhuman primates. In: Abee, C., Mansfield, K., Tardif, S., Morris, T. (Eds.), Nonhuman Primates InBiomedical Research. Academic Press, pp. 197–297.
- Stuart, M.D., Strier, K.B., 1995. Primates and parasites: a case for a multidisciplinary approach. Int. J. Primatol. 15, 577–593. http://dx.doi.org/10.1007/BF02735282.
- Thompson, R.C.A., 2013. Parasite zoonoses and wildlife: one health, spillover and human activity. Int. J. Parasitol. 43 (12–13), 1079–1088. http://dx.doi.org/ 10.1016/j.ijpara.2013.06.007.
- Toft, J.D., 1982. The pathoparasitology of the alimentary tract and pancreas of nonhuman primates: a review. Vet. Pathol. 7, 44–92. http://dx.doi.org/10.1177/ 030098588201907s06.
- Trejo-Macías, G., Estrada, A., Mosqueda, M.A., 2007. Survey of helminth parasites in populations of *Alouatta palliata mexicana* and *A.pigra* in Continuous and in fragmented habitat in Southern Mexico. Int. J. Primatol. 28, 931–945.
- Vitazkova, S., Wade, S., 2007. Effects of Ecology on the gastrointestinal parasites of Alouatta pigra. Int. J. Primatol. 28, 1327–1343.
- Vitone, N.D., Altizer, S., Nunn, C.L., 2004. Body size, diet and sociality influence the species richness of parasitic worms in anthropoid primates. Evol. Ecol. Res. 6, 183–199.