

Do habituation, host traits and seasonality have an impact on protist and helminth infections of wild western lowland gorillas?

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Abstract Increased anthropogenic activity can result in parasite exchanges and/or general changes in parasite communities, imposing a health risk to great apes. We studied protist and helminth parasites of wild western lowland gorilla groups in different levels of habituation, alongside humans inhabiting Dzanga-Sangha Protected Areas in the Central African Republic. Faeces were collected yearly during November and December from 2007 to 2010 and monthly from November 2010 to October 2011. Protist and helminth infections were compared among gorilla groups habituated, under habituation and unhabituated, and the effect of host traits and seasonality was evaluated. Zoonotic potential of parasites found in humans was assessed. No significant differences in clinically important parasites among the groups in different stages of habituation were found, except for *Entamoeba* spp. However, humans were infected with four taxa which may overlap with taxa found in gorillas. Females were less infected

with spirurids, and adults had higher intensities of infection of *Mammomonogamus* sp. We found seasonal differences in the prevalence of several parasite taxa, but most importantly, the intensity of infection of unidentified strongylids was higher in the dry season. This study highlights that habituation may not necessarily pose a greater risk of protist and helminth infections in gorilla groups.

Keywords Western lowland gorilla · Parasite · Habituation · Human impact

Introduction

Habituation is a process whereby animals become acclimated to human presence over time until they accept human observers as part of their environment (Tutin and Fernandez

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1991; Williamson 1988). The consequent proximity to the animals is highly beneficial to both tourism and research (Higham and Shelton 2011). However, the process may be highly stressful for the wildlife concerned (Blom et al. 2004; Cipolletta 2003; Doran-Sheehy et al. 2009; Shutt et al. 2014). These chronic stressors could have a significant impact on animal immunity, increasing their susceptibility to diseases (Hofer and East 1994; Hudson 1992; Meder 1994; Woodford et al. 2002). Although wild animals cope with unpredictable stressors in their environments (Cyr and Romero 2009), the unpredictable chronic stressors generated by habituation are expected to decrease resistance to infections by immunosuppression (Boonstra 2012; Burchfield 1979). Moreover, close contact between habituated animals and humans increases the risk of human pathogen spillover.

Viral respiratory infections represent the most prominent example of diseases affecting wild great apes that can result from direct or close contact with humans. Due to the resultant high morbidity and mortality among chimpanzees and gorillas, communicable viruses are of major conservation concern (Homsy 1999; Nishida et al. 2003; Woodford et al. 2002). Gastrointestinal parasites are also commonly listed as a threat to primate conservation efforts (Chapman et al. 2005; Woodford et al. 2002). Growing molecular evidence supports that human-gorilla interactions may result in transmission of parasites between humans and non-human primates (e.g., Graczyk et al. 2002; Hasegawa et al. 2014; Nizeyi et al. 1999; Sak et al. 2013, 2014). Several studies have also reported a higher prevalence and species richness of gastrointestinal parasites in non-human primates living in habitats disturbed by humans, suggesting a negative impact of humans on great ape health (Gillespie and Chapman 2006, 2007; Kowalewski et al. 2011; Mbora et al. 2009; Sá et al. 2013).

To shed more light on possible effects of human-gorilla interactions on gorilla parasite communities, using coproscopic methods we studied protist and helminth parasites of wild western lowland gorillas *Gorilla gorilla gorilla* (Savage and Wyman 1847) in the Dzanga-Sangha Protected Areas, Central African Republic, one of the first sites to have habituated lowland gorillas for both ecotourism and research (Cipolletta 2004). As increased anthropogenic impacts on non-human primate populations may result in general changes in primate parasite communities over time (Nunn and Altizer 2006), we compared the communities of protists and helminths among gorilla groups at different stages of the habituation process. We also took into consideration individual gorilla traits and seasonality, which were previously shown to have an impact on the prevalence of parasites and on the infection intensities of selected helminths (Benavides et al. 2012; Masi et al. 2012). By using coproscopic methods, we are not able to evaluate the epidemiology and zoonotic transmission of parasites in the human-ape interface, but we highlighted the parasites occurring in both humans in contact

with gorillas, with a special focus on those with zoonotic potential, which require to be more deeply analysed in follow-up molecular studies using genotyping and subtyping tools.

Material and methods

Study site

The study was conducted in Dzanga-Sangha Protected Areas (DSPA) in the Central African Republic (CAR). Activities in DSPA are directed by the DSPA administration under the collaborative management of the CAR Government and World Wildlife Fund. In 1997, the DSPA launched the Primate Habituation Programme (PHP) with the specific aim of habituating western lowland gorillas for tourism and research (Blom et al. 2004). The study gorilla groups inhabit semi-deciduous forest interspersed by natural clearings, locally called 'bais'. The climate is characterized by marked seasonal variation (Cipolletta 2004), with dry (November–March) and wet (April–October) months (Shutt et al. 2014). Human population density is low, estimated at one person per square kilometre, with the greatest concentration (60% of people) located in the village of Bayanga (Blom et al. 2004; Remis and Robinson 2012). Sampling was carried out around two permanent PHP research camps: Bai Hokou (2° 50' N, 16° 28' E) and Mongambe (2° 55' N, 16° 23' E).

Gorilla groups

We studied several groups of western lowland gorillas (*Gorilla gorilla gorilla*) at different habituation stages (i) fully habituated (Makumba, Mayele), (ii) under habituation (Mata, Wonga) and (iii) unhabituated. The Makumba group, ranging in the surroundings of Bai Hokou research camp, has been followed since 2000 and opened to tourists in September 2004; the Mayele group, ranging near Mongambe research camp, has been followed since 2005 and was opened to tourists at the end of 2009. Both groups are followed daily by habituation teams and intermittently visited by tourists and film crews. Habituation of the Mata group at Bai Hokou and Wonga in Mongambe started in 2008; the group was not fully habituated at the time of sample collection. Samples were also collected opportunistically from 14 other unhabituated groups and 6 lonely males.

Sample collection

We collected samples in two periods: long-term investigation (yearly) during November and December from 2007 to 2010 (but no samples in 2008) from all groups (however, no samples from unhabituated groups and groups under habituation

were collected in 2007) and 1-year intensive investigation (monthly) from November 2010 to October 2011, which individually identified samples from habituated groups (Makumba, Mayele) used for statistics and the Mata group and unhabituated groups for coproscopy. We collected 2 g of fresh faeces and fixed it in 4% formaldehyde immediately. For Makumba (during both sampling periods) and Mayele (intensive investigation) groups, we collected samples immediately after defecation from identified group members during focal follows. For unhabituated groups and groups under habituation and Mayele (long-term investigation), we collected fresh faecal samples from night nests early in the morning (within 3 h from the time we suspected the gorillas had left the nests) and each sample corresponding to a different but unidentified individual. We implemented modified Harada-Mori faecal cultures (Hasegawa 2009) to develop infectious L3 larvae from fresh faeces to enable identification of strongylid nematodes. We fixed developed L3 larvae into 4% formaldehyde for morphological examination. We collected samples non-invasively, adhering to site regulations regarding proximity and other behavioural rules.

Human samples were obtained from PHP employees and other inhabitants of nearby villages during regular health monitoring in November/December from 2007 to 2010. PHP employees reside in the Park during rotating work shifts. Human sample collection was approved by an ethics committee (Anthropology Department Research Ethics and Data Protection Committee, University of Durham, UK), and prior informed consent was obtained from all volunteers.

Coproscopic analyses

We examined all of the 593 samples by coproscopy. Before parasitological analyses, we homogenized each faecal sample, strained them through a sieve and centrifuged. We took the weight of the sediment in order to later count the eggs and prefixed the sediment up to 10 ml with 4% formaldehyde. Initial coproscopic examination was carried out using Sheather's flotation with modified sugar solution (s. g. 1.33) (Sheather 1923), followed by centrifuge sedimentation (Zajac and Conboy 2012), and microscopy (Olympus BX41, Olympus CX40). We also quantified strongylid nematode eggs (classified into unidentified strongylid eggs and *Mammomonogamus* sp. eggs, the latter being the only strongylid nematode distinguishable by egg morphology) in samples collected during intensive investigation, directly from the sediment. We took 2 ml of faecal suspension, centrifuged and examined whole sediment. We counted all the strongylid eggs present in the sediment and then calculated the number of eggs per gram of sediment according to the following formula: $n = N/(m)$, where n = number of eggs/g of sediment, N = number of eggs in examined amount of sediment and m = weight of examined sediment. For detection of small

adult/larvae nematodes, we used a 'gauze-washing' method (Hasegawa 2009). We examined the larvae to genus level from coprocultures based on their morphology (Little 1981).

Data analyses and statistics

We reported individual parasite presence/absence and individual parasite richness, i.e. the number of unique parasite taxa recovered per individual. All of the effects were tested using penalized quasi-likelihood generalized linear mixed models (glmmPQL) using a binomial or Poisson distribution (Bolker 2008). This was implemented using the glmmPQL package in R (R Development Core Team 2015). We did not run the models with binomial distribution for parasite taxa where only few samples were positive (*Strongyloides* sp., *Bertiella* sp.). For *Entamoeba* spp., prevalence in unhabituated groups was 0% and thus the glmmPQL model was run only with samples from under habituation and habituated gorillas; additionally, the prevalence of *Entamoeba* spp. among groups in different stages of habituation was compared separately using 2*5 Fisher exact test.

Long-term investigation (2007–2010) We tested the effect of habituation by including 'habituation stage' (habituated, under habituation, unhabituated) and year of sampling (2007, 2009, 2010) as fixed effects on (i) protist and helminth taxa occurrence in gorillas and (ii) parasite richness. We also included 'group identity' (Mayele, Makumba, Mata, Wonga and unhabituated groups) as a random effect to account for differences in social groups that are not necessarily attributed to the habituation process. Since the Makumba habituated group had more than one sample collected, we randomly chose one sample from each individual per year. We included all nest samples from a single day for gorillas under habituation (Mata, Wonga groups) and the Mayele group in each year. The unhabituated gorilla groups were only sampled once, and thus all samples were used. No samples from unhabituated groups and groups under habituation were collected in 2007.

One-year intensive investigation (2010/2011) We analysed the effect of host traits and seasonality in datasets of identified individuals from Makumba and Mayele groups on (i) protist and helminth taxa occurrence in gorillas, (ii) infection intensity of unidentified strongylids and *Mammomonogamus* sp. and (iii) parasite richness. For the Makumba group, we used two or three samples per individual per month. For the Mayele group, we randomly chose two identified samples from the silverback and identified samples from at least one female and juvenile per month. We tested the effect of 'group identity' (fixed effect, categories: Makumba, Mayele), 'sex' (fixed effect, categories: male, female), 'age' (fixed effect, categories: adult, subadult, juvenile) and 'season' (fixed effect,

categories: dry, wet), including also ‘individual identity’ as a random effect.

Results

In total, we examined 593 faecal gorilla samples and 58 human stool samples using coproscopy. For statistical analyses, we used 480 samples, 182 samples for long-term investigation (2007–2010) and 298 samples for 1-year intensive investigation (2010/2011). Detected parasites were divided into 15 categories (eight protists and seven helminths), with four taxa found in both humans and gorillas (Fig. 1, for overall sample prevalence, see Table 1; for prevalence in different groups, years, host traits and seasons in gorillas, see Supplement Tables 1, 2, 5, 6, 7, 8). L3 infectious larvae developed in coprocultures from gorilla and human faeces belonged to genera *Necator* and *Oesophagostomum*. Furthermore, we detected larvae of *Probstmayria* sp. by using the gauze-washing method (Fig. 1, Table 1).

During the long-term investigation (2007–2010), we found no effect of habituation stage on prevalence of detected parasites or parasite richness with the exception of *Entamoeba* spp. (Table 2). Differences between the years were detected in *Troglodytella/Gorillophilus* spp., *Prototapirella gorillae*, *Entamoeba* spp., *Mammomonogamus* sp. and spirurids (Table 2; Supplements: Table 4a, b, e, g, h).

During the 1-year intensive investigation (2010/2011), we found significant differences between gorilla groups in the prevalence of *Entamoeba* spp. and spirurids (Table 3; Supplement Table 9e, h). A significant effect of sex was detected only on the prevalence of spirurids, whilst a significant effect of age was detected only on the intensity of infection of *Mammomonogamus* spp. (Table 3, Supplement Tables 10 h and 12b). Significant differences between seasons were found in prevalence of unidentified entodiniomorphids, spirurids and intensities of infection of unidentified strongylids (Table 3, Supplement Tables 10c, h and 12a). For non-significant results of other taxa, see Tables 3, 4, 9, 10, and 12 in the Supplement.

Discussion

To evaluate possible effects of human-gorilla interactions, host traits and seasonality on protist and helminth parasites, we studied wild western lowland gorillas at different habituation stages over 5 years. With a few exceptions, the spectrum of protist and helminth parasites detected in all studied wild western lowland gorilla groups at different habituation stages can be considered as typical of this host species and corresponds well with results of previous studies on gorillas at the same site (Freeman et al. 2004; Masi et al. 2012) and

elsewhere such as Mondika, Republic of Congo (Lilly et al. 2002) and Lope, Gabon (Landsoud-Soukate et al. 1995).

The effect of habituation on gorilla parasites

Protist and helminth parasites are commonly discussed as a health risk linked to great ape habituation (Chapman et al. 2005; Woodford et al. 2002). Although there were differences in the level of human-ape contact among the studied groups, we only found significant differences in a few parasite taxa across the gorilla groups. Importantly, none of these organisms are considered seriously pathogenic for gorillas.

Most important are the differences in *Entamoeba* spp. During the long-term investigation (2007–2010), we found a significantly higher *Entamoeba* spp. prevalence in the habituated groups in comparison to the group under habituation and unhabituated and also a higher prevalence of *Entamoeba* spp. in 2010 compared to 2009. During 1-year intensive investigation (2010/2011), we found higher prevalence of *Entamoeba* spp. in Makumba, longer habituated group, than in Mayele. Comparative data are rare; Chapman et al. (2006) found *Entamoeba coli* four times more prevalent in red colobus (*Piliocolobus tephrosceles*) living in forest edge habitat where greater levels of contact between humans and wildlife occur, than in those living within the forest. This evidence can indicate a negative impact of close contact with humans in the habituated group, even more on the Makumba group which has been habituated for the longest period of time and over time of habituation. However, pathogenicity of individual species of the genus *Entamoeba* differs significantly, from harmless mutualist species to pathogenic species such as *Entamoeba histolytica*. As it is impossible to distinguish most of the *Entamoeba* species by light microscopy (Jirků-Pomajbíková et al. 2016), our results must be interpreted with extreme caution.

We found significant changes in commensal entodiniomorphid ciliate prevalence (*Troglodytella/Gorillophilus* spp., *Prototapirella gorillae*) over time. However, the long-term persistence of individuals negative for entodiniomorphid ciliates in entodiniomorphid ciliate-positive groups is unlikely and with repeated sampling prevalence may reach up to 100% (Modrý et al. 2009). Observed changes in prevalence of ciliates may thus more likely represent their natural fluctuation, which depends on the diverse ecological and host characteristics (Abe and Iriki 1989; Bakuza and Nkwengulila 2009; Williams and Coleman 1992). We also found changes in spirurid prevalence over time and differences in spirurid prevalence between Makumba and Mayele groups during the 1-year intensive investigation, which may reflect the insect intermediate host availability, rather than the impact

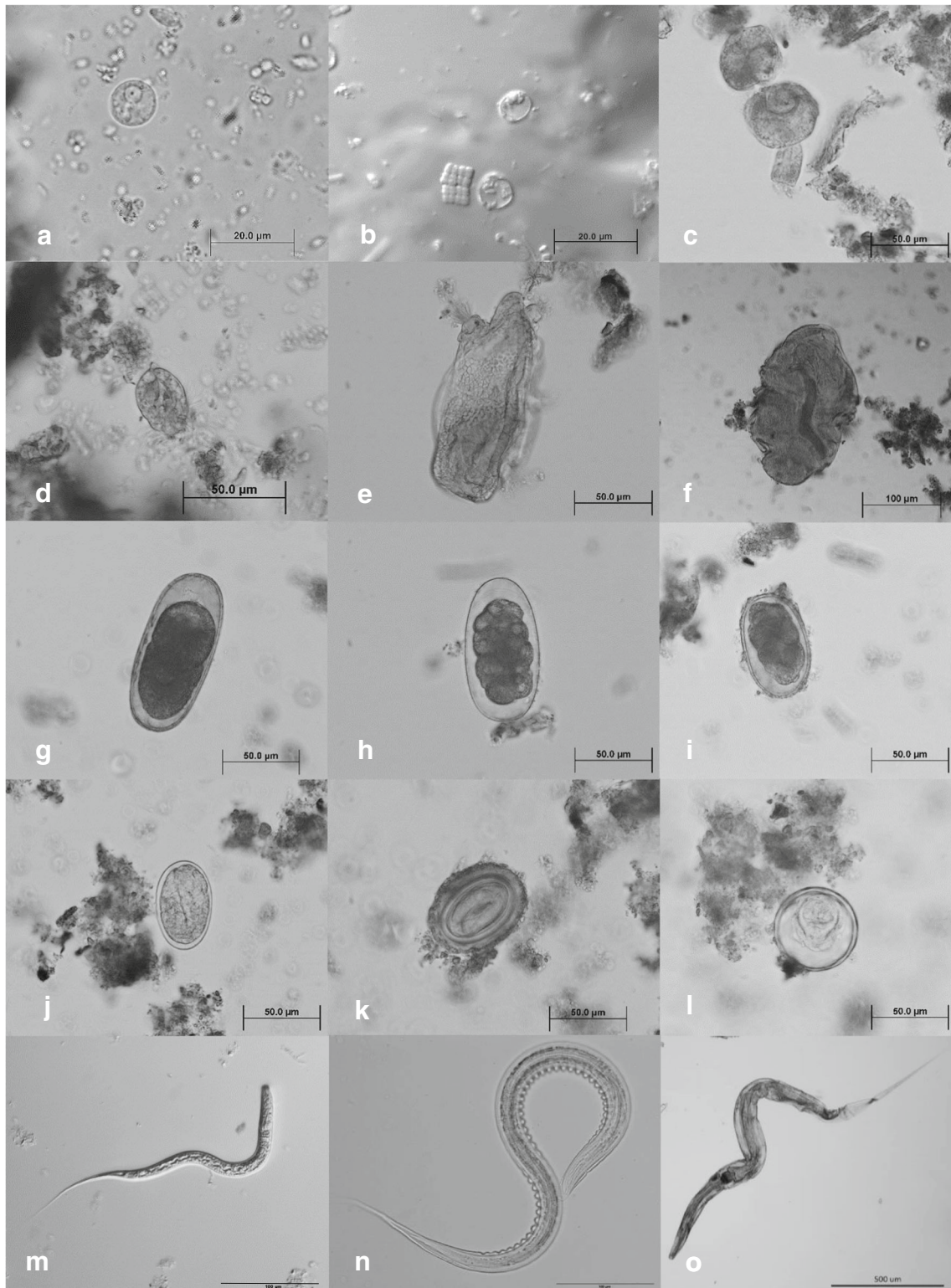


Fig. 1 Parasites found in western lowland gorillas in DSPA. **a** *Entamoeba* sp. **b** *Blastocystis* sp. **c** Unidentified entodiniomorphid ciliate. **d** Unidentified entodiniomorphid ciliate. **e** *Prototapirella gorillae*. **f** *Troglodytella/Gorillophilus* sp. **g** *Mammomonogamus* sp. **h**

Unidentified strongylid egg. **i** Unidentified strongylid egg. **j** *Strongyloides* sp. **k** Spirurid egg. **l** *Bertiella* sp. **m** *Oesophagostomum* sp. **n** *Necator* sp. **o** *Probstmayria* sp. Scale bar used: 20 μm for **a, b**; 50 μm for **c, d, e, g, h, i, j, k, l**; 100 μm for **f, m, n**; 500 μm for **o**

of habituation. Last, *Mammomonogamus* sp. was least prevalent in 2010 during long-term investigation; however, interpretation

of this result is difficult due to the lack of information about the parasite's transmission route, life cycle and pathogenicity.

Table 1 Parasites found in western lowland gorillas and humans in DSPA and parasite total sample prevalence. Highlighted parasites found in both gorillas and humans

	Parasite	Gorillas	Humans
Protists	<i>Blastocystis</i> sp.	16%	62%
	<i>Entamoeba</i> spp.	35%	24%
	<i>Endolimax nana</i>	–	7%
	<i>Iodamoeba bütschlii</i>	–	3%
	<i>Chilomastix mesnili</i>	–	5%
	<i>Giardia intestinalis</i>	–	5%
	<i>Prototapirella gorillae</i>	75%	–
	<i>Troglodytella/Gorillophilus</i> spp.	71%	–
	Unidentified entodiniomorphids	56%	–
Helminths	<i>Strongyloides</i> spp.	3%	12%
	Unidentified strongylids	96%	47%
	<i>Necator</i> spp. ^a		
	<i>Oesophagostomum</i> spp. ^a		
	<i>Mammomonogamus</i> sp.	64%	–
	Spirurids	17%	–
	<i>Ascaris lumbricoides</i>	–	55%
	<i>Trichuris trichiura</i>	–	66%
	<i>Bertiella</i> sp.	< 1%	–
	<i>Probstmayria</i> sp. ^b		–

^a Species detected by studying of morphology of L3 larvae developed in coprocultures

^b Detected by gauze-washing method

Parasites with zoonotic potential found in humans

DSPA humans were infected with nine taxa, comparable with other studies on humans from humid tropical areas (Ashford et al. 1990; Lilly et al. 2002; Wumba et al. 2010). Four taxa identified in humans may overlap with those found in gorillas (*Entamoeba*, *Blastocystis*, *Necator/Oesophagostomum*, *Strongyloides*). Hasegawa et al. (2014) evaluated the zoonotic potential of *Necator* spp., studying DNA sequences originating from L3 infectious larvae from infected DSPA humans, gorillas and chimpanzees. Evidence suggests that *Necator*

hookworms are shared by humans and great apes cohabiting the same tropical forest ecosystem (Kalousová et al. 2016), which is likely greatly facilitated by percutaneous migration of infective L3 larvae. Alternatively, although transmission of both *Strongyloides fuelleborni* and *S. stercoralis* between humans and non-human primates has been suggested (Hasegawa et al. 2010), recent results of Hasegawa et al. (2016) employing molecular methods showed that only humans carry *S. stercoralis* and that *S. fuelleborni* in humans in DSPA differ from those in great apes. Both *Blastocystis* sp. and *Entamoeba* spp. are considered as zoonotic parasites (Parkar et al. 2010; Feng et al. 2011). Some are highly host specific, whilst other subtypes display moderate or low host specificity (Noel et al. 2005; Stensvold et al. 2009). Studies utilizing molecular tools are needed to clarify the epidemiology of these taxa in DSPA.

Although *Ascaris lumbricoides* and *Trichuris* spp. were found in the sampled humans in high prevalence, we did not detect them in the studied gorillas. Both helminths were found in very low prevalence in other gorilla populations (Kalema-Zikusoka et al. 2005; Lilly et al. 2002; Makouloutou et al. 2014; Sleeman et al. 2000). In these cases, the presence of these parasites in gorilla groups may indicate direct transmission from humans. Interestingly, both taxa are commonly diagnosed in captive animals (University of Veterinary and Pharmaceutical Sciences Brno, unpublished data) which might result from cross-transmission from other mammals in the epidemiologically complex zoo environment as recorded e.g. for *Ascaris* in captive chimpanzees (Nejsum et al. 2006). The virtual absence of these ‘typically human’ parasites in DSPA gorillas may indicate a low rate of transmission.

The effect of host traits on parasites of habituated gorillas

The impact of sex and age on parasites of primates has been well documented (e.g. Miller 1960; Muller-Graf et al. 1996). Individual factors were shown as more important for parasite infections than other extrinsic factors such as temperature or the home range size (Benavides et al. 2012). We found a

Table 2 The effect of habituation on parasites in gorilla groups in DSPA, long-term investigation 2007–2010

Effect of group	<i>Entamoeba</i> spp. more prevalent in habituated groups than in groups under habituation and unhabituated (Fisher 2*5 test: $p = 0.013$)
Effect of year	<i>Troglodytella/Gorillophilus</i> spp. more prevalent in 2009 than 2007 (glmmPQL: $t = -2.152$, $p = 0.033$)
	<i>Prototapirella gorilla</i> more prevalent in 2010 than 2007 and 2009 (glmmPQL: $t = 3.223$, $p = 0.001$; glmmPQL: $t = 3.135$, $p = 0.002$)
	<i>Entamoeba</i> spp. more prevalent in 2010 than in 2009 (glmmPQL: $t = 2.195$, $p = 0.030$)
	<i>Mammomonogamus</i> sp. less prevalent in 2010 than in 2007 and 2009 (glmmPQL: $t = -3.125$, $p = 0.002$; glmmPQL: $t = -3.534$, $p = 0.001$)
	Spirurids more prevalent in 2010 than in 2009 (glmmPQL: $t = 1.977$, $p = 0.050$)

Table 3 The effect of group, host traits and season on parasites in habituated groups in DSPA, 1-year intensive investigation 2010/2011

Effect of group	Spirurids more prevalent in Mayele than in Makumba (glmmPQL: $t = 2.163$, $p = 0.045$) <i>Entamoeba</i> spp. more prevalent in Makumba than in Mayele group (glmmPQL: $t = -2.225$, $p = 0.040$)
Effect of sex	Spirurids less prevalent in males than in females (glmmPQL: $t = -4.392$, $p << 0.001$)
Effect of age	Intensity of infection of <i>Mammomonogamus</i> sp. lower in both juveniles and subadults than in adults (glmmPQL: $t = -2.158$, $p = 0.046$; glmmPQL: $t = -2.555$, $p = 0.021$)
Effect of season	Unidentified entodiniomorphids lower in wet season than in dry (glmmPQL: $t = -2.993$, $p = 0.003$) Spirurids more prevalent in wet season than in dry (glmmPQL: $t = 3.267$, $p = 0.001$) Intensity of infection of unidentified strongylids lower in wet season than in dry (glmmPQL: $t = -2.576$, $p = 0.011$)

significantly higher infection intensity of *Mammomonogamus* sp. in adult gorillas compared to subadults and juveniles. The life cycle of *Mammomonogamus* sp. is unknown, which makes our results difficult to interpret. A similar trend was detected in cattle in Mindanao, Philippines, which could reflect an increase in infection prevalence of this species with age (Van Aken et al. 1996), possibly as a result of increased exposure over time with no development of adaptive immunity (Benavides et al. 2012).

Sex-related differences were found only in spirurids, where male gorillas were less frequently infected than females. This could potentially be explained by sex differences in diet, as spirurid infectious larvae develop in insects and, e.g. in Mondika, Republic of Congo, gorilla females spent more time feeding on insects (including termites and ants) compared to males (Doran-Sheehy et al. 2009). Also, Pettifer (1984) explains higher prevalence of the spirurid *Physaloptera caucasica* in females of chacma baboons (*Papio ursinus*) by sex differences in the consumption of the arthropod intermediate hosts.

Effect of seasonality on parasites of gorillas

We found that infection intensity of unidentified strongylids was higher during the dry season compared to the rainy season. The same trend was observed by Masi et al. (2012) at the same locality. Seasonal differences in strongylid nematodes are commonly observed in non-human primates (e.g. Huffman et al. 1997; Krief et al. 2005; MacIntosh et al. 2010; Masi et al. 2012; Rothman et al. 2008, 2009; Trejo-Macias and Estrada 2012); however, the reported trend is usually opposite to our findings. In humans, studies in West Africa showed that environmental populations of strongylid L3 larvae are highest during the rainy season (Udonsi et al. 1980); however, the highest faecal egg counts in host faeces were lagged at 2–7 months after the rainy season (Knight and

Merret 1981; Nwosu 1981). This phenomenon reflects worm maturation in the host's intestine and supports our findings.

We also found a higher prevalence of spirurids in the wet season than in the dry season, possibly caused by the seasonal availability to access the insects consumed by gorillas. In Ugalla, Tanzania, Kalousová et al. (2014) observed higher prevalence of spirurids in chimpanzees during the dry season, when the Ugalla chimpanzees were observed to consume larvae of Curculionidae, a possible intermediate host. Differences in prevalence of unidentified entodiniomorphids between the seasons can be caused by their natural fluctuation (Abe and Iriki 1989).

Conclusions

During monitoring of parasites in groups of western lowland gorilla in DSPA, we found no significant effect of habituation on protist and helminth infections. Only prevalence of *Entamoeba* spp. significantly differed among gorilla groups in different levels of habituation; however, the pathogenicity of species within the genus *Entamoeba* is variable and thus the results must be interpreted with caution. Compared to bacterial and viral infections, which may cause serious outbreaks in habituated ape groups (Leendertz et al. 2006), the pathogenicity of gastrointestinal protists is much lower or absent. In the case of helminth infections, the adverse effects usually result from a high abundance/infection intensity, depending on the infectious dose and the complexity of the life cycles of particular taxa. Following the best practice guidelines for tourism and habituation and for health monitoring and disease control in great ape populations developed and recommended by the IUCN (Gilardi et al. 2015; Macfie and Williamson 2010), strictly followed in DSPA, should help mitigate disease transmission risk from human-originating helminthiasis caused by *Ascaris* and *Trichuris*. Undoubtedly, continuous monitoring

of potentially zoonotic pathogens in situations of close contact at the human-ape interface is highly beneficial. Gorilla females were generally less infected than males, and adults had higher intensities of infection; however, in both cases the parasites have an intermediate host in their life cycle or the life cycle is mostly unknown, therefore to make a conclusion is complicated. The found seasonal differences in strongylid nematode infections correspond with other studies.

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Compliance with ethical standards Human sample collection was approved by an ethics committee (Anthropology Department Research Ethics and Data Protection Committee, University of Durham, UK), and prior informed consent was obtained from all volunteers.

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