Fecal glucocorticoids and gastrointestinal parasite infections in wild western lowland gorillas (*Gorilla gorilla gorilla*) involved in ecotourism

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ABSTRACT

Wildlife ecotourism can offer a source of revenue which benefits local development and conservation simultaneously. However, habituation of wildlife for ecotourism can cause long-term elevation of glucocorticoid hormones, which may suppress immune function and increase an animal’s vulnerability to disease. We have previously shown that western lowland gorillas (*Gorilla gorilla gorilla*) undergoing habituation in Dzanga-Sangha Protected Areas, Central African Republic, have higher fecal glucocorticoid metabolite (FGCM) levels than both habituated and unhabituated gorillas. Here, we tested the relationship between FGCM levels and strongylid infections in the same gorillas. If high FGCM levels suppress the immune system, we predicted that FGCM levels will be positively associated with strongylid egg counts and that gorillas undergoing habituation will have the highest strongylid egg counts, relative to both habituated and unhabituated gorillas. We collected fecal samples over 12 months in two habituated gorilla groups, one group undergoing habituation and completely unhabituated gorillas. We established FGCM levels and fecal egg counts of *Necator*/*Oesophagostomum* spp. and *Mammomonogamus* sp. Controlling for seasonal variation and age-sex category in strongylid infections we found no significant relationship between FGCMs and *Nectator/Oesophagostomum* spp. or *Mammomonogamus* sp. egg counts in a within group comparison in either a habituated group or a group undergoing habituation. However, across groups, egg counts of *Nectator/Oesophagostomum* spp. were lowest in unhabituated animals and highest in the group undergoing habituation, matching the differences in FGCM levels among these gorilla groups. Our findings partially support the hypothesis that elevated glucocorticoids reduce a host’s ability to control the extent of parasitic infections, and show the importance of non-invasive monitoring of endocrine

function and parasite infection in individuals exposed to human pressure including habituation process and ecotourism.

Key Words: Primate, conservation, endocrine, parasites, immunity

This work was supported by a NERC/ESRC interdisciplinary PhD studentship and grants from the Primate Society of Great Britain, the International Primatological Society, the Biosocial Society UK, and Rufford Small Grants for Nature Conservation. This publication is an outcome of the HPI-lab/Laboratory for Infectious Diseases Common to Human and Non-Human Primates. Klara Petrželková and Barbora Pafčo was supported by institutional support of Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic (RVO:68081766) and during writing by the Czech Science Foundation Grant (18–24345S).

Highlights:

- Habituation and ecotourism may have negative impacts on endangered animals including chronically elevated glucocorticoid levels
- We found no significant relationship between glucocorticoid metabolites and parasite infection in gorillas under human pressure
- Both glucocorticoid metabolites and strongylid egg counts were lowest in unhabituated animals and highest in a group undergoing habituation

1. Introduction

Ecotourism is promoted as a tool to conserve endangered species and habitats as it can bring development benefits to local people (Williamson and Macfie, 2010). Wildlife must often be habituated to human presence before ecotourism activities can commence. Habituation refers to an animals' waning fear response following repeated stimulation (in this case the arrival of humans in their environment) without reinforcement (Williamson and Feistner, 2001). Human disturbance can lead to chronic overproduction of metabolic hormones controlled by the hypothalamic-pituitary-adrenal axis, such as glucocorticoids (Walker et al., 2006; Behie et al., 2010; Shutt et al., 2014; French et al., 2017). This can disrupt the production of cytokines and lymphocytes (Cyr and Romero, 2009; McEwen, 1998; Wingfield and Sapolsky, 2003) and contribute to immunosuppression (Råberg et al., 1998; Sapolsky, 1998; Sapolsky et al., 2000) and thus to the host's ability to control parasitic infections, resulting in higher parasite fecundity and higher parasite egg outputs (Else, 2005; Moreau and Chauvin, 2010; Periago and Bethony, 2012; Quinnell et al., 2004). While an acute rise in glucocorticoids can be part of an adaptive physiological response to a stressor (which habituation to human presence likely presents for wild-living animals, e.g. Barja et al., 2007; Chen et al., 2020; Palme et al., 2019; Shutt et al., 2012), chronically elevated glucocorticoid output is linked to pathology, reduced fitness and increased mortality (Boonstra, 2013; Cyr and Romero, 2007; McEwen, 1998; Pride, 2005; Sapolsky et al., 2000; Selye, 1955; Wingfield and Romero, 2010). Animals subject to habituation may therefore have increased susceptibility to diseases (Hofer and East, 1998; Hudson et al., 1992; Meder, 1994; Woodford et al., 2002).

Several studies have tested the hypothesis that increased glucocorticoid output is associated with parasite infections in non-human primates, with various results. Some studies have found a positive relationship between fecal glucocorticoid metabolite output and the number of different gastrointestinal parasite taxa (parasite richness) or parasite prevalence (male chimpanzees, *Pan troglodytes:* Muehlenbein, 2006; mandrills, *Mandrillus sphinx:* Setchell et al., 2010; red-fronted lemurs, *Eulemur fulvus rufus:* Clough et al., 2010), while other studies found no such relationship (red colobus, *Piliocolobus tephrosceles:* Chapman et al., 2007; white-handed gibbons, *Hylobates lar:* Gillespie et al., 2013, red-capped mangabeys, *Cercocebus torquatus:* Friant et al., 2016). However, parasite richness can be a problematic measure as most parasites cannot be identified to the species level by coproscopic methods (Modrý et al., 2018), sequential individual samples are needed to accurately diagnose parasite infections (Muehlenbein, 2005; Setchell et al., 2010), and richness measures include organisms with no or positive implications for host health. Intensity of infection may be a more relevant measure of parasite infection, but studies comparing fecal glucocorticoid hormones and intensity of parasite infection also found inconsistent outcomes (positive association: red colobus, *Piliocolobus tephrosceles*: Chapman et al., 2007; red-fronted lemurs, *Eulemur fulvus rufus*: Clough et al., 2010; gray-cheeked mangabeys, *Lophocebus albigena:* Arlet et al., 2015*;* guenons, *Cercopithecus mitis:* Foerster et al., 2015; Barbary macaque, *Macaca sylvanus*: Müller-Klein et al., 2019; no or negative association: savanna baboons, *Papio cynocephalus*: Habig et al., 2019).

Habituation and ecotourism are novel contexts to investigate the relationship between physiology and pathogens (Muehlenbein, 2006), particularly in light of general concerns that ecotourism may potentially affect health status of the individuals involved negatively. To test this relationship, and inform conservation strategies, we carried out a multidisciplinary study focusing on the impact of habituation, research and ecotourism on the physiology of western lowland gorillas (*Gorilla gorilla gorilla*) in Dzanga-Sangha Protected Areas (DSPA), Central African Republic. Habituation of western lowland gorillas takes 4–8 years and in the early stages of habituation gorillas typically show behavioral indications of an acute stress response (Blom et al., 2004). In line with this, we have previously shown that a group of gorillas undergoing habituation for ecotourism had significantly higher fecal glucocorticoid metabolite (FGCM) levels than a well-habituated group, and both these groups had higher FGCMs than gorillas that were not habituated or followed by humans (Shutt et al., 2014). Despite these significant differences in HPA-axis activity related to the habituation process, the gastrointestinal bacteriome composition in the gorilla groups were almost unaffected, i.e. higher FGCM levels were associated with only minor changes in bacteriome composition (Vlčková et al., 2018).

The DSPA gorillas are infected by a wide spectrum of gastrointestinal parasites (Pafčo et al., 2017). There are no significant differences in clinically important parasites among groups at different stages of habituation, except for *Entamoeba* spp., which are significantly more prevalent in habituated groups than in the group under habituation and unhabituated gorillas (Pafčo et al., 2017). However, the pathogenicity of species within the genus *Entamoeba* is variable and these results do not necessarily suggest that the habituated gorillas experience higher levels of pathogenic infections. Nevertheless, gorillas at all habituation levels are infected by strongylid nematodes, which are considered pathogenic (Collet et al., 1986; Brooker and Hotez, 2004; Terio et al., 2011).

In this study, we explore the relationship between FGCM levels, and the intensities of strongylid infections (expressed as fecal egg counts). If increased glucocorticoid levels compromise the immune system, and therefore the host's ability to regulate the level of parasite infections, then we predicted that: (1) Within groups, FGCM levels will be positively associated with strongylid egg counts and (2) Across groups, strongylid egg counts will be lowest in the groups with the lowest FGCM levels (completely unhabituated gorillas), and highest in the group with the highest FGCM levels (the group undergoing habituation).

2. MATERIALS AND METHODS

2.1 Study site and subjects

We conducted our study around the Primate Habituation Programme research camps Bai Hokou (33 N 663109, 316187 UTM) and Mongambe (33 N 654357, 322606 UTM) in DSPA, Central African Republic. For a more detailed description of the study site see Blom (2001). Gorilla habituation aimed at developing ecotourism and research activities began in 1997. We studied three groups of western lowland gorillas (*Gorilla gorilla gorilla*) at different habituation stages, and unhabitated gorillas (Table 1). Groups are named after their dominant silverback male. Makumba group (fully habituated) ranges in the surroundings of Bai Hokou research camp, has been followed since 2000 and visited by tourists since September 2004. Mayele group (fully habituated, but more recently) ranges near Mongambe research camp, has been followed since 2005 and visited by tourists since the end of 2009. Both groups are followed daily by project teams and visited intermittently by tourists and film crews. Habituation of Mata group (under habituation) at Bai Hokou started in 2008 and the group was still under habituation during the study. We collected samples opportunistically from several groups of unhabituated gorillas. For more details about the groups see Shutt et al. (2014).

2.2 Fecal sample collection

We worked with local BaAka trackers to collect gorilla fecal samples non-invasively from November 2010 to November 2011. We collected 411 samples (Table 1). Fecal collection methods differed among the groups. The habituated status of Makumba and Mayele groups allowed collection of the samples within 30 min (usually within 5 min) of defecation from identified individuals of known age and sex. For the group under habituation and unhabituated groups we collected samples <6 h old from night nests. Experienced trackers assigned feces from those groups to the silverback or animals of all other age-sex category based on fecal bolus size, nest size and position in relation to other individuals, and the presence of silver hairs (Remis, 1997; Shutt et al., 2014; Tutin et al., 1995). Genetic studies have shown that although dung size estimation is an unreliable means for assessing age and sex class, assignment of dung to the silverback is reliable in combination with other clues like silver hairs (Bradley et al., 2008; McNeilage et al., 2001, 2006).

To test Prediction 1, we obtained 257 samples for Makumba group and 74 samples for Mata group. We attempted to sample each individual in Makumba group three times per month, but only achieved two samples per individual in some months. For Mata group we took two samples per month for the silverback and two for other individuals representing other age/sex-groups. To test Prediction 2, we collected an additional 50 samples from Mayele group and 30 samples from completely unhabituated gorillas.

For hormone analysis we weighed approximately 0.5 g of feces from each sample using a portable balance and homogenized it well before placing it in 4 ml of 90% ethanol in water.

We detected no effects of urine contamination on FGCMs (Shutt et al., 2012). Nevertheless, we took all samples from the center of the fecal bolus where it should not have been affected by urine. We followed validated methods to avoid variation in our FGCM measurements resulting from sampling, extraction and storage (Shutt, et al. 2012). This meant we extracted all samples within 24 h and stored dried fecal metabolite extracts in the field before shipping them to the German Primate Centre´s endocrinology laboratory for FGCM analysis. We collected samples for parasitological analyses from the same fecal bolus as for hormone sampling. We took approximately 2 g of feces and fixed it with 4% formaldehyde in 25 ml vials. We stored samples at ambient temperature before shipping them to the Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, for parasitological analyses. We adhered to the research protocols defined by the Administration of DSPA, and Durham University Life Sciences Ethical Review Process Committee approved the study.

Table 1. Number of individuals and samples collected for all groups

Age-sex category	MAKUMBA fully habituated		MAYELE fully habituated		MATA undergoing habituation		UNHABITUATED unhabituated	
	Individuals	Samples	Individuals	Samples	Individuals*	Samples	Individuals	Samples
Silverback male	1	35	1	17	1	24	unknown	14
Sub-adult male	1	25	0		0		unknown	
Adult female	2	57	4		4		unknown	
Adolescent/Juvenile	3	69	7		3		unknown	
Infant	3	71	4		1 or 2		unknown	
Unknown (not silverback)**				33		50		16

*Group composition estimated from limited observations and night nests.

**Unknown includes adult females, adolescents/juveniles, infants or sub-adult males in unhabituated gorillas.

2.3 Hormone analyses

We measured FGCMs using a 11ß-hydroxyetiocholanolone (3a,11ß-dihydroxy-CM) enzyme immunoassay which we have previously validated physiologically, biologically and immunologically for measuring FGCM output in our study species in the same laboratory

(Shutt, et al. 2012). Inter-assay coefficients of variation for these measurements were 9.2 % (high value quality control) and 15.1 % (low value quality control). We removed any samples with known complications (e.g., seeds discovered in the fecal matrix or alcohol evaporation).

FGCMs decrease over time in unpreserved gorilla feces and the temporal degradation pattern is best described by a polynomial fit, $Y = 0.0039x^2 - 0.0844x + 0.9976$, where x = time between defecation and preservation (Shutt, et al. 2012). We calculated the age of fecal samples collected from nests using the precise collection time and the mean time gorillas leave their nests (05:30 h: K. Shutt pers. obs.; A. Todd pers. comm.) and used this information to compensate for hormone degradation in samples not collected immediately. We obtained a corrected value from the original wet hormone content value using the equation

Corrected value = original wet hormone content value *100 / Y

We express all hormone data as hormone content per fecal wet mass. We found no diurnal variation in FGCMs (Shutt, et al. 2012) so used all samples for analysis.

2.4 Parasite analyses

To prepare samples for parasitological analyses, we homogenized each sample and strained it through a sieve into Falcon conical tubes (50 ml) to minimize the effects of feeding residues or varying water content in the feces on the egg quantification. We weighed the final sediment after centrifugation and re-suspended it up to 10 ml with 4% formaldehyde. We used a modified sedimentation procedure for quantification of strongylid nematodes (Pafčo et al., 2017). The determination of strongylid nematodes to species or even genus level based on egg morphology is generally unreliable and *Mammomonogamus* sp. is the only strongylid for which eggs can be easily distinguished by microscopy (Modrý et al., 2018). However, we could assign the thin-walled strongylid eggs to the genera *Necator* or *Oesophagostomum* based on the morphology of L3 larvae developed from the eggs and strongylid metabarcoding (Hasegawa et al., 2014; Pafčo et al., 2017, 2018, 2019). We, therefore, divided the strongylid eggs into two categories: *Necator/Oesophagostomum* spp. and *Mammomonogamus* sp.

2.5 Statistical analyses

To statistically evaluate Prediction 1 we used the PROC GENMOD procedure in SAS/STAT software (Version 9.4 of the SAS System for Windows. Copyright © 2002-2012 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA). We evaluated the association between FGCMs and *Necator/Oesophagostomum* spp. and *Mammomonogamus* sp. egg counts for Makumba group (fully habituated) and Mata group (under habituation) as they were well sampled. We chose a negative binomial model (Hilbe, 2007) to test the association between our independent variable, FGCM levels, and our two outcome variables, egg counts of *Necator*/*Oesophagostumum* spp. and *Mammomonogamus* sp. because of its robustness to over-dispersion. We tested Makumba and Mata groups separately due to differences in sampling methods. We tested for effects of age-sex category, sex and season for Makumba group in one model, and for effects of age-sex category (silverback vs. other) and season for Mata group in a second model. To account for season, we converted the date of sample collection to radian time by dividing each date by 365.25 (the number of days in a year) and then multiplying by 2*π before taking the sine and the cosine of the resulting values, and including these in the model. Although there were zero values in the outcome data (*Necator*/*Oesophagostumum* spp. and *Mammomonogamus* sp. egg counts), a zero-inflated negative binomial model failed to converge so we report the results of the negative binomial model. We report the incidence rate ratio (IRR) and the associated 95% confidence intervals.

To test Prediction 2 we conducted statistical analyses in *R 4.0.2* (R Core Team, 2021). We used the same approach we used previously to compare FGCM levels among gorilla groups at different habituation levels (Shutt et al., 2014). That is, we used a General Linear Mixed Model (GLMM) with negative binomial distribution to test the effect of the group/habituation status on strongylid egg counts using the *lme4* package (Bates et al., 2015). We used age-sex category as a random effect as the data were not identified to individual level in the group undergoing habituation and the unhabituated gorillas. We included season as it is known to influence strongylid nematode egg counts (Pafčo et al., 2017). We used Tukey post-hoc tests to test for differences in strongylid egg counts among the two habituated groups (Makumba, Mayele), the group undergoing habituation (Mata) and completely unhabituated gorillas using the *multcomp* package (Hothorn et al., 2008). We report descriptive data as means +/- SD and range.

3. RESULTS

3.1 Prediction 1

We did not find a significant relationship between FGCM levels and strongylid egg counts in Makumba (Table 2) or Mata groups (Table 3). We found no effect of sex on *Necator/Oesophagostumum* spp. egg counts in Makumba group, but egg counts were significantly associated with seasonal variation and significantly higher for infants than silverbacks (Table 2). In Mata group, silverbacks had significantly lower *Necator/Oesophagostumum* spp. egg counts than other age-sex categories, and *Necator/Oesophagostumum* spp. egg counts were also significantly affected by season (Table 3). We found no effect of sex, age-sex category or season on *Mammomonogamus* sp. egg counts in either group (Tables 2 & 3).

Table 2. Results of negative binomial analyses testing the association between FGCMs, sex, age and seasons and *Necator/Oesophagostumum* spp. and *Mammomonogamus* sp. egg counts in a habituated group of western lowland gorillas (Makumba group) at Bai Hokou in Dzanga-Sangha Protected Areas, Central African Republic.

Variables	***Necator/Oesophagostumum* spp.**		***Mammomonogamus* sp.**	
	Incidence Rate Ratio (95% CI)	P	Incidence Rate Ratio (95% CI)	P
Model Intercept	12.56 (3.80, 41.51)	<0.001	4.81 (0.52, 44.07)	0.164
FGCMs	1.12 (0.87, 1.42)	0.376	0.91 (0.59, 1.41)	0.675
Sex				
Male/Female	0.33 (0.05, 2.05)	0.235	2.73 (0.16,47.54)	0.488
Age-sex category compared to silverback				
Adult females	0.91 (0.57, 1.45)	0.683	0.58 (0.25, 1.32)	0.192
Sub-adult males	3.12 (0.49, 19.9)	0.228	0.51 (0.03, 9.48)	0.649
Adolescents/Juveniles	3.40 (0.63, 25.4)	0.141	0.36 (0.02, 6.50)	0.487
Infants	1.60 (1.09, 2.35)	0.017	0.72 (0.36, 1.46)	0.314
Seasonal variation				
Sine	2.01 (1.68, 2.41)	<0.001	1.09 (0.81, 1.46)	0.577
Cosine	0.81 (0.70, 0.96)	0.014	0.78 (0.58, 1.04)	0.090
Dispersion	2.05 (1.79, 2.34)		6.30 (3.80, 10.44)	

Table 3. Results of negative binomial analyses testing the association between FGCMs, rank (silverback vs. other group members) and seasons and *Necator/Oesophagostumum* and *Mammomonogamus* egg counts in a group of western lowland gorillas undergoing habituation for ecotourism (Mata group) at Bai Hokou in Dzanga-Sangha Protected Areas, Central African Republic.

Variables	***Necator/Oesophagostumum* spp.**		***Mammomonogamus* sp.**	
	Incidence Rate Ratio (95% CI)	P	Incidence Rate Ratio (95% CI)	P
Model Intercept	8.46 (1.09, 65.87)	0.041	4.44 (0.15, 132.85)	0.390
FGCMs	1.34 (0.84, 2.12)	0.221	0.99 (0.45, 2.06)	0.913
Age-sex category compared to silverback				
Silverback vs. others*	0.46 (0.31, 0.68)	<0.001	0.87 (0.46, 1.64)	0.663
Seasonal variation				
Sine	2.33 (1.81, 2.99)	<0.001	1.07 (0.68, 1.68)	0.768
Cosine	1.30 (0.99, 1.69)	0.053	1.22 (0.81, 1.84)	0.340
Dispersion	1.64 (1.42, 2.01)		3.35 (2.12, 6.97)	

* includes adult females, adolescents, juveniles and infants

3.2 Prediction 2

We found significant differences between gorilla groups and a significant influence of season on *Necator/Oesophagostumum* spp. egg counts (Table 4). Tukey post-hoc tests showed that *Necator/Oesophagostumum* spp. egg counts were significantly higher in the group undergoing habituation (Mata, p = 0.001) and one fully habituated group (Makumba, p = 0.031) than in unhabituated gorillas (Table 5). We found no significant influences on *Mammomonogamus* sp. egg counts (Table 4).

Table 4: Results of a General Linear Mixed Model testing the association between group ID, and seasonal variation and *Necator/Oesophagostumum* and *Mammomonogamus* egg counts in western lowland gorillas at Bai Hokou in Dzanga-Sangha Protected Areas, Central African Republic.

Variables	***Necator/Oesophagostumum* spp.**		***Mammomonogamus* sp.**	
	χ	P	χ	P
Model Intercept	610.9	<0.001	62.47	<0.001
Group*	12.84	0.005	5.07	0.167
Season	20.31	<0.001	0.05	0.823

* Makumba group, Mayele group, Mata group, unhabituated gorillas

Table 5: Strongylid infections (fecal egg counts) in western lowland gorillas in Dzanga-Sangha Protected Areas, Central African Republic.

Group	**Level of habituation**	**N**	***Necator/Oesophagostumum* spp.**		***Mammomonogamus* sp.**	
			mean +/- SD	range	mean +/- SD	range
Makumba	Fully habituated	257	27.3±28.6	1-188	2.9±4.2	1-24
Mayele	Fully habituated	50	24.2±25.9	1-114	3.4±3.2	0-16
Mata	Undergoing habituation	74	37.5±50.9	1-232	2.4±3.6	1-23
Unknown	Unhabituated	30	16.2±15.3	1-62	3.4±5.2	0-24

4. DISCUSSION

We evaluated the relationship between FGCM levels and the intensities of strongylid infections (*Necator/Oesophagostumum* spp. and *Mammomonogamus* sp.) within and across groups of gorillas at different habituation levels to test the hypothesis that increased glucocorticoid levels compromise the immune system, and therefore the host's ability to regulate the intensities of parasite infections. Within groups, we did not find a significant relationship between FGCM levels and strongylid infections in the two groups that we tested (contra Prediction 1). However, across groups, egg counts of *Nectator/Oesophagostomum* spp. were lowest in unhabituated animals and highest in the group undergoing habituation, matching the previously reported pattern in FGCM levels (Shutt et al., 2014), and supporting Prediction 2. Thus, we find support for the hypothesis across groups, but not within groups.

There can be several reasons for lack of the direct relationship between FGCM levels and strongylid infections either in the group of gorillas habituated for ecotourism or in the group of gorillas undergoing habituation in DSPA, Central African Republic. Defolie et al. (2020) found that one-third of studies testing the relationship between parasites and glucocorticoids found no such relationship. They provided three explanations for this pattern that may apply to our study. First, limitations in study design, such as small sample sizes and low statistical power. Our study design was limited by the number of gorilla groups available and the logistics of sampling. Makumba was the only fully habituated group in which all individuals could be sampled individually and repeatedly and Mata was the only group under habituation, with very limited sampling possibilities. Moreover, western lowland gorilla groups are relatively small, and our study is also limited by the number of the individuals in each group (10 individuals in each group tested for Prediction 1).

Second, host-parasite coevolution may explain the absence of a relationship between parasites and glucocorticoids (Defolie et al. 2020). A parasite can have no or limited effect on the host, and taxa considered parasites might in fact be commensals. Moreover, the relationship between glucocorticoid hormone and macroparasites such as helminthes are delayed or minor compared with those by bacteria (O'Dwyer et al. 2020). The existence of host-specific strongylids in the gorillas suggests co-evolution between gorillas and their strongylid nematodes (Pafčo et al., 2018, 2019), which may limit parasite infection (Allison, 1982; Toft et al., 1991). Prevalence of strongylid nematodes reaches 100% (Pafčo et al., 2017), gorillas live in a contaminated environment and thus they keep encountering the infection throughout their life. They may therefore develop tolerance to parasites, or highly targeted immune responses towards parasites leading to low intensities of infection (Schoenle et al. 2019; St Juliana et al., 2014).

Third, and finally, methodological and analytical differences may explain differences in the results of tests of parasite-hormone relationships (Defolie et al. 2020). This may explain why we found support for our hypothesis across groups, but not within groups. This discrepancy may occur because FGCM levels fluctuate more rapidly than parasite infection. For example, contacts made with gorillas undergoing habituation elicit significant FGCM responses, which accumulate in the days following contacts (Shutt et al., 2014). However, fluctuation in parasite infection over time is much slower and complicated by the delay between infection with a parasite and detection of the parasite via the recovery of the parasite form in the feces (the pre-patent period). Immature parasites or unfertilized females can be present in the host and affect host health before parasite stages can be detected non-invasively in feces.

Further, the results could be impacted by variables included in the model. For example, radian time seems to be a best approach to modeling season (Gillespie et al., 2013), however, a significant number of studies includes season wet vs. in the model (e.g. Arlet et al., 2015; Friant et al., 2016; Lynch et al., 2002;) or do not include such variable at all, meaning that the association could be influenced by chosen variable. Various factors influence both strongylid infections and FGCMs and the effects of these variables may confound any association between FGCM levels and strongylid egg numbers in feces. We found no relationship between FGCMs and age-sex category, sex, season, mean daily temperature or rainfall in the gorillas (Shutt et al., 2014) using the same data we analyze here, but other factors such as food availability, predation risk and other environmental conditions could affect glucocorticoid homeostasis (Presley et al., 1996; Monello et al., 2010), however, these data are difficult to obtain especially in the wild animals. In contrast, *Necator*/*Oesophagostumum* spp. are strongly affected by seasonal variation and host age-sex category (this study, Pafčo et al. 2017), suggesting that environmental factors or host traits have a stronger influence on strongylid infections than on FGCM levels.

The relationship we found between age-sex category and parasite infection is well documented in other non-human primates (e.g., Miller, 1960; Müller-Graf et al., 1996). Our findings are in accordance with other studies of both lowland and mountain gorillas, in which younger gorillas are more susceptible to strongylid nematodes than older gorillas (Ashford et al., 1996; Lilly et al., 2002; Masi et al., 2012). These age differences may be caused by adults developing immunity after repeated exposure to infection resulting in lower infection intensities (Lilly et al., 2002; Woolhouse, 1998). Our results also reflect observed patterns of seasonality in parasite infection in other non-human primates (MacIntosh et al., 2010; Setchell et al., 2010; Masi et al., 2012; Trejo-Macías and Estrada, 2012), and support our previous study (Pafčo et al., 2017). However, the unknown prepatent period for the parasites we studied complicates the evaluation of seasonal variation, which is connected to development of the parasite stages in the external environment (Anderson, 2000). Ecological factors, such as seasonal influence on parasite richness, might be more relevant than physiological factors as fecal glucocorticoid levels, which was shown also in wild white-handed gibbons (Gillespie at el., 2013).

Although we did not find direct relationship between FGCM levels and strongylid infections, the results of our comparison across groups suggest that the animals in the habituation process are more susceptible to diseases or have a reduced ability to regulate parasite infection. The differences in *Necator/Oesophagostumum* spp. among the groups match the differences in FGCM levels. Elevated glucocorticoids contribute to immune suppression in humans and animals (Råberg et al., 1998; Sapolsky, 1998; Sapolsky et al., 2000), and some studies demonstrate a specific immunosuppressive effect of steroid hormones resulting in increased parasite infections (Klein, 2004; Zuk and McKean, 1996). Theoretically, parasites could be also introduced to gorillas by humans due to close contact (Sak et al., 2013), but the observed transmission patterns of strongylid nematodes between gorillas and humans in DSPA seem to be due to sharing a habitat rather than close contact during the habituation or ecotourism (Pafčo et al., 2019). According our results, the parasite infections start to increase when a gorilla group undergoes habituation and is contacted more often by human observers, when they also have higher FGCMs, then both parasites and FGCMs decrease when the group is habituated and ignores humans. However, both FGCMs and intensities of parasite infections stay elevated in long-term habituated groups compared to unhabituated animals, which have the lowest parasites and FGCM levels. With a small number of groups, these across-group patterns may simply reflect chance variation across groups, but this seems unlikely, because samples from unhabituated gorillas are likely to be from several gorilla groups. Sample collection from the beginning of habituation until full habituation would help to further understand the association between FGCM levels and parasite infections in a gorilla group. However, this would take up to eight years and it is extremely difficult to follow groups at the beginning of the habituation process.

5. CONCLUSIONS

Glucocorticoid-parasite interactions are of great interest to evolutionary biologists, as they represent life-history trade-offs between endocrine and immune function processes. For conservationists, it is important to monitor the effects of human pressure on the target species, and to mitigate any negative effects as far as possible. This includes studies of the effect of habituation and subsequent ecotourism disturbance on the physiology of the animals involved. We evaluated the relationship between parasite infections and glucocorticoid hormones in wild groups of western lowland gorillas inhabiting the same environment but at different levels of habituation. We found limited support for the hypothesis that elevated glucocorticoids reduce a host’s ability to control the extent of parasitic infections but found differences in *Necator*/*Oesophagostumum* egg counts among groups at different habituation levels, matching differences in FGCM levels. Further long-term studies are needed to better understand the potentially negative effects of increased glucocorticoids in endangered animals as a result of habituation and ecotourism activities. Such studies will enable the development and application of appropriate adaptive mitigation strategies (e.g., Shutt et al., 2014), increasing the positive conservation impact of ecotourism. Our results also show the impact of season and age on strongylid nematodes, supporting previous studies (Pafčo et al. 2017)

ACKNOWLEDGMENTS

We thank the World Wildlife Fund and the Administration of Dzanga-Sangha Protected Areas in the Central African Republic for assistance with obtaining permits and for collaborative research support, and the Ministre de l’Education Nationale, de l’Alphabetisation, de l’Enseignement Superieur, et de la Recherche for granting research permission and sample transport permits. Special thanks to all the staff of the Primate Habituation Programme for logistical support and assistance, and especially the BaAka trackers in the field. We thank P. Kiesel and A. Heistermann for expert help with hormone analysis.

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