Intestinal Parasite Infections and Fecal Steroid Levels in Wild Chimpanzees

Michael P. Muehlenbein*

Department of Anthropology, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53211

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ABSTRACT Immune-endocrine interactions have been evaluated much less frequently in nonhuman primates, and this may be due, in part, to logistical and ethical concerns regarding trapping and sampling of endangered species, especially apes. Using noninvasive fecal collection methods, the present study evaluates possible relationships between fecal steroid levels and gastrointestinal parasite infections in the Ngogo chimpanzee community in Kibale National Park, Uganda. Because both testosterone and cortisol exhibit immunosuppressive effects in vitro and in other animal models, it was hypothesized that both testosterone and cortisol would be positively associated with gastrointestinal parasite infections in these animals. When placed in a mixed model simultaneously, both testosterone ($F = 4.98$, $df = 1$, $P = 0.033$) and cortisol ($F = 5.94$, $df = 1$, $P = 0.020$) were positively associated with total (helminth and protozoan) parasite richness (the number of unique intestinal parasite species recovered from hosts' fecal samples). It is possible that androgens and corticoids alter the ability of a host to mount an effective immune response against concomitant infection with multiple parasitic species. The utility of fecal samples for assessing immune-endocrine interactions is discussed. Am J Phys Anthropol 130:546–550, 2006.

Immune-endocrine interactions are a subject of interest for evolutionary biologists, partly due to the important life-history trade-offs that are evident in the function of various hormones and immune factors (Ketterson and Nolan, 1992; Casto et al., 2001; Muehlenbein, 2004; Muehlenbein and Bribiescas, 2005). Diversion of metabolic energy to support immune function during infection potentially reduces the energy available for reproduction and/or secondary sexual characteristics, and testosterone's interactions with immune function may act as important mechanisms for regulating energy allocation between survivorship and reproductive effort (Folstad and Karter, 1992; Wedekind and Folstad, 1994; Sheldon and Verhulst, 1996; Muehlenbein, 2004; Muehlenbein and Bribiescas, 2005). Therefore, evaluating hormone-mediated immune functions are valuable not only from a clinical perspective (i.e., determining disease susceptibility), but also from an evolutionary one (i.e., determining how and why testosterone levels become optimized in an animal under certain ecological conditions).

The effects of both testosterone and cortisol on immune functions are generally considered suppressive. For example, testosterone can alter the CD4+/CD8+ T-cell ratio and increase suppressor T-cell populations (Weinstein and Bercovich, 1981), reduce T-helper cell function (Grossman et al., 1991; Wunderlich et al., 2002), and impair macrophage activity (Chao et al., 1994). Similarly, cortisol can inhibit inflammation (Elenkov and Chrousos, 1999), affect cytokine production (Turnbull and Rivier, 1999), and increase monocyte apoptosis (Norbiato et al., 1997). Because cortisol can also suppress hypothalamic-pituitary-gonadal function (Doerr and Pirke, 1976; Bambino and Hsueh, 1981), cortisol and testosterone are often inversely associated with one another (Aakvaag et al., 1978; Sapolsky, 1995).

The majority of work on immune-endocrine interactions has involved birds, rodents, and reptiles. With some exceptions, immune-endocrine interactions have been evaluated much less frequently in nonhuman primates, although their phylogenetic relationships with humans warrant such work. Some investigators utilize captive macaques and marmosets to elucidate the effects of activation of the hypothalamic-pituitary-gonadal axis on development of the immune system (Gould et al., 1998; Mann et al., 1998). Others observed an inverse association between cortisol and total lymphocyte levels in wild female baboons (Alberts et al., 1992), as well as an inverse association between cortisol and insulin-like growth factor I in wild male baboons (Sapolsky and Spencer, 1997). Immune-endocrine interactions have not been investigated in apes under natural infection conditions, largely due to logistical and ethical concerns regarding trapping and/or anesthetization and sampling of endangered species.

With the recent advent and validation of noninvasive methods for measuring hormone levels in urine and fecal samples (Whitten et al., 1998), such work with apes has become more feasible. However, in the absence of blood samples, the only proxy for immune function which can be assessed in these animals is parasitic output in stool

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*Correspondence to: Michael P. Muehlenbein, Department of Anthropology, University of Wisconsin-Milwaukee, 3413 North Downer Ave., Milwaukee, WI 53211. E-mail: mpm1@uwm.edu

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or urine. This is complicated by the fact that it is unknown if parasite excretion reflects the immune status of a host. Parasite egg/cyst/larvae abundance in any given fecal sample may not directly correlate with the number of parasites in a host at any given time. Although certainly not a direct measure of immune status, parasite richness (i.e., the number of unique intestinal parasite species recovered from a host's fecal sample) may reflect the ability of the host to control infections with multiple parasites at any given time.

To evaluate relationships between fecal steroid levels and gastrointestinal parasite richness, fecal samples were collected and analyzed for testosterone, cortisol, and gastrointestinal parasites in the world's largest habituated population of wild chimpanzees, the Ngogo community in Kibale National Park, Uganda. It was hypothesized that both testosterone and cortisol would be positively associated with gastrointestinal parasite infections in these animals.

MATERIALS AND METHODS

Study site and subjects

Ngogo is in Kibale National Park in western Uganda, and is maintained by the Makerere University Biological Field Station. The Ngogo study area is approximately 25 km^2 and contains a mix of mature, regenerating, and swamp forest, *Acanthus* scrub, and other vegetation types (Ghiglieri, 1984; Struhsaker, 1997). The field site is devoid of domestic herbivores and pets, human observation of chimpanzees is restricted to park caretakers and researchers, and latrines and garbage pits are used for disposal of human waste and refuse at the research camp. All adult and adolescent male chimpanzees are well-habituated and are observable within 5–10 m on the ground. At the time of this study (July–September, 2002), the Ngogo community had 24 adult males, 14 adolescent males, and a total of about 150 members.

Sample collection

One hundred fecal samples were collected opportunistically from 22 adult and 13 adolescent male chimpanzees. Between 1–5 samples were obtained from each animal immediately following defecation, thus ensuring positive matching of individuals with all of their fecal samples collected. All care was taken to avoid collecting portions of samples which may have been contaminated by soil or pooled water. Blood and mucus were not observed in any fecal mass collected, nor did color or consistency differ significantly between masses.

Most samples, but not all, were collected before 11 AM. Although there are concerns about the effects of diurnal variation on fecal hormone levels in smaller-bodied primates such as *Callithrix jacchus* (Sousa and Ziegler, 1998) and *Cebus apella nigritus* (Lynch et al., 2002), this is probably of less concern in larger-bodied animals such as *Pan troglodytes*, due to longer gut retention time (Milton and Demment, 1988; Whitten et al., 1998; Muehlenbein et al., 2004). Diurnal effects on parasite output in chimpanzees are unknown.

For 10 days of the 3-month study period, the males were associated with a single swollen parous female. During this time, 19 samples were collected from 12 individual males. Though it was possible that the presence of a receptive female or the act of mating could have resulted in elevated hormone levels in these males, there were no such significant changes (see Muehlenbein et al., 2004).

Parasitological analyses

Upon collection, a portion of each sample was immediately preserved in Para-Pak plastic transport vials (Meridian Diagnostics, Cincinnati, OH), prealiquoted with 10% neutral-buffered formalin. Samples were examined for all known intestinal parasites, using the formalin-ethyl acetate sedimentation technique (Ash and Orihel, 1991). Intestinal parasite infections were described in terms of prevalence and "richness," defined here as the number of unique intestinal parasite species recovered from hosts' fecal samples. Given the short study period, seasonal variation in parasite output could not be assessed. Furthermore, because of the sampling protocol, sex differences could not be evaluated in the present study.

Endocrinological analyses

Using a portable Coleman oven placed atop a kerosene stove in the field, a portion of each fresh fecal sample was dried on an aluminium dish for approximately 2 hr at 100°C (Seraphin, 2000). Following desiccation, each sample was individually packaged with silica gel, and was transported back to the Laboratory of Reproductive Ecology and Environmental Toxicology at Emory University (Atlanta, GA), where extractions and radioimmunoassays for testosterone and cortisol were performed. Specific details of assay procedures are described elsewhere (Muehlenbein et al., 2004). In brief, extractions were made on Sep-Pak VAC C18 columns (Water Corp., Milford, MA), and extraction recovery, measured by the addition of I^{125}-labeled steroid to fecal samples prior to extraction, averaged 65% for testosterone and 72% for cortisol.

The testosterone assay used reagents from the Equate Testosterone RIA kit (Binax, South Portland, ME), with antibody raised in rabbit against testosterone. Secondary antibody was a PEG goat anti-rabbit antibody solution. Sensitivity was 6 ng/dl. Cross-reactivity was 1.7% for dihydrotestosterone and <0.1% for all other steroids. Accuracy was tested by the addition of steroid standards to a chimpanzee extract. The mean percentage of observed concentration to expected values in the Equate testosterone assay was 91.4 ± 5.0% (n = 6). Internal controls were run in every assay, and consisted of human serum controls (male and female) provided with the kit (Equate) along with clinical serum standards (BioRad 1 and 2) and chimpanzee fecal extracts. The intra-assay coefficient of variation averaged 2.7 ± 1.1% for the male serum control (n = 5) and 4.4 ± 1.4% for duplicates of chimpanzee fecal extracts (n = 19). Interassay coefficients of variation were 4.2% for the Equate female serum control (4.8 ng/dl), 4.6% for the Equate male serum control (55.0 ng/dl), 4.2% and 7.2% for BioRad controls 1 (4.7 ng/dl), and 2 (58.2 ng/dl), respectively, and 10.1% for three chimpanzee samples assayed in two separate assays.

The cortisol assays were a modification of the Diagnostic Products Corporation Double Antibody I^{125} cortisol kit (DPC KCOD, Los Angeles, CA) for serum determinations, with antibody raised in rabbit against cortisol. The antibody-precipitating solution was polyethylene glycol (PEG) goat anti-rabbit gamma globulin. The sensitivity was 2.2 ng/ml. Cross-reactivity was 3.9% for cortisone, 3.6%

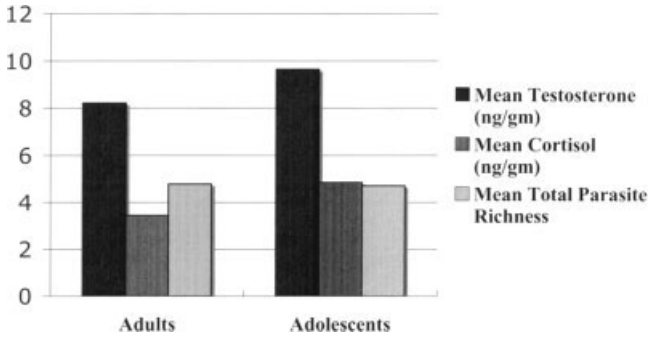

Fig. 1. Summary of parasite and hormone results for Ngogo chimpanzee sample.

for 6β-hydroxycortisol, 1.1% for corticosterone, and <1% for all other steroids. Accuracy, tested by the addition of cortisol standards to a chimpanzee extract, averaged 96.8 ± 2.6% (n = 5). Internal controls were run in every assay and consisted of clinical serum standards (BioRad 1, 2, and 3) and chimpanzee fecal extracts. The intra-assay coefficient of variation averaged 2.2 ± 2.1% for the BioRad controls (n = 4) and 11.6 ± 1.0% for duplicates of the chimpanzee fecal extracts (n = 12). Interassay coefficients of variation were 11.5%, 8.7%, and 5.3% for BioRad controls 1, 2, and 3, respectively.

Statistical analyses

Repeated-measures analysis using a mixed modeling approach was performed with SAS/STAT software to examine the relationship between hormone levels and intestinal parasite richness (SAS Institute, Inc., 2001). Mixed modeling allowed the use of all data points, including individuals with missing observations, and avoided the need for averaging testosterone levels for individuals and sampling intervals. It also allowed the examination of within-subject effects of continuous variables and controlled for time-variant and fixed within-subject covariates, such as hormones and age, respectively. Furthermore, the mixed model needed to take into account the fact that the hormonal data consisted of repeated measures at up to five unequally spaced time intervals. This was accomplished by using a time-series covariance structure that did not assume equal spacing. Potential correlations between the variables of interest (parasite richness and hormones) should theoretically remain stable during a short data-collection period (but obviously not for a long one). Therefore, the data were fit with a compound symmetry covariance structure which assumes that correlations remain constant. The level of significance was always set at 0.05.

RESULTS

Twelve taxa of intestinal species (5 helminth and 7 protozoan) were recovered from the samples. The four most prevalent species were *Troglodytella abrassarti* (97.3%), *Oesophagostomum* sp. (81.1%), *Strongyloides* sp. (83.8%), and *Entamoeba chattoni* (70.3%). Detailed parasitology results are reported elsewhere (Muehlenbein, 2005). The mean number of unique parasite species recovered from the total sample was 4.75 (4.77 for adults, and 4.71 for adolescents).

Individual testosterone levels are reported in Muehlenbein et al. (2004). The mean testosterone level for each adult animal (N = 22 animals) was 8.22 ng/g (range, 2.23–14.52; SD, 3.40). The mean cortisol level for each adult animal was 3.45 ng/g (range, 0.57–7.57; SD, 1.98). The mean testosterone level for each adolescent animal (N = 13 animals) was 9.65 ng/g (range, 4.42–15.44; SD, 3.43). The mean cortisol level for each adolescent animal was 4.85 ng/g (range, 1.82–17.77; SD, 4.13). Average cortisol and testosterone levels did not differ significantly between the adult and adolescent samples (analyses not shown). Figure 1 shows mean values for helminth/protozoan richness as well as average testosterone and cortisol levels for the adult and adolescent samples.

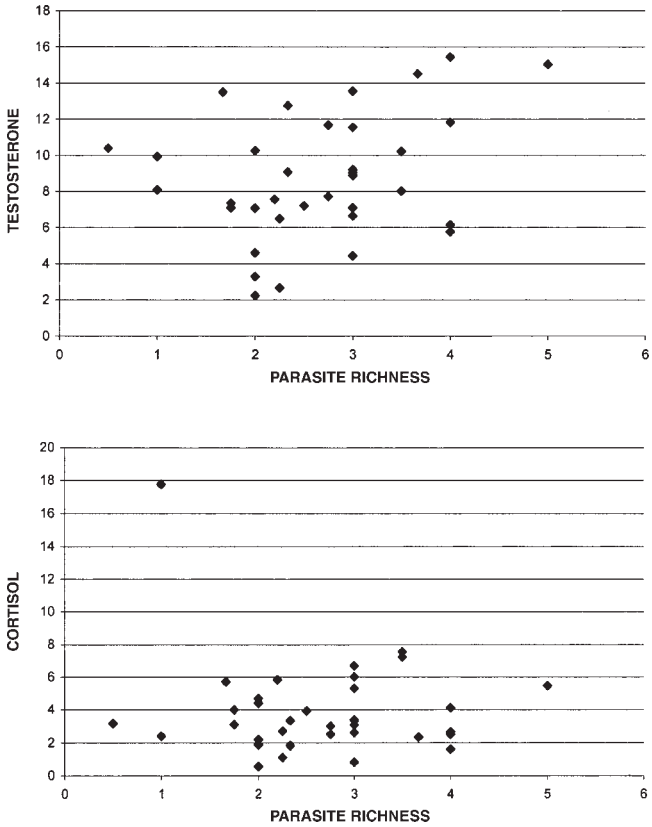

Fig. 2. Total (helminth and protozoan) parasite richness by mean testosterone and cortisol for each animal. For graphic representation, parasite richness was summed across samples from each animal, and was subsequently divided by number of samples obtained from that particular animal.

Within the total sample of adult and adolescent animals (N = 35 animals, 100 total fecal samples), testosterone and cortisol were borderline positively associated ($F = 3.92$, df = 1, $P = 0.056$). When placed in the mixed model simultaneously, both testosterone ($F = 4.98$, df = 1, $P = 0.033$) and cortisol ($F = 5.94$, df = 1, $P = 0.020$) were positively associated with total (helminth and protozoan) parasite richness. These associations were no longer significant when either testosterone or cortisol was removed from the model (testosterone, $F = 2.64$, df = 1, $P = 0.113$; cortisol, $F = 3.59$, df = 1, $P = 0.067$). Figure 2 shows mean testosterone and cortisol for each animal by parasite richness. In this case, parasite richness was summed across all samples for each animal, and was further divided by the number of times an animal was sampled.

DISCUSSION

The current study attempted to identify associations between fecal steroid levels and gastrointestinal parasite infection within a large population of wild, habituated chimpanzees. Both testosterone and cortisol were positively associated with parasite richness in this sample. Interestingly, the model was only significant when both testosterone and cortisol were included. Because both cortisol and testosterone can inhibit immune function, future studies which attempt to assess the immunomodulatory actions of one of these hormones need to include measurements of the other as well. It is possible that the two steroids function synergistically to modulate immune functions. It is also possible that many of the reported cases of testosterone-mediated immunosuppression in the past literature are due, in part, to cortisol's effects.

Androgens and corticoids possibly alter the ability of the primate host to mount an effective response against concomitant infection with multiple parasitic species. It may be that the higher an animal's average steroid level, the greater the likelihood of acquiring multiple infections, or rather, the more difficult it is to mount a successful immune response against multiple invaders. The correlative nature of the current study, however, cannot provide direct evidence for this. In addition, the truncated nature of this study cannot account for any changes in endocrine-parasite associations due to seasonal fluctuation in hormones or parasite levels. Another potential limitation is that intestinal parasite excretion may not be an accurate indicator of host immune status. Parasite egg/cyst/larvae intensity in any given fecal sample may not directly correlate with the number of parasites in the host at any given time. Parasite excretion can vary dramatically within and between individuals. In the present study, parasite infection intensities are not reported for just such reasons. However, "richness" or the number of unique infections at any given time is probably a better proxy of mucosal immunity. It is reasonable to assume that it is relatively more difficult for a host to control infections from multiple parasitic species than a single species at any given time.

Elevated testosterone and/or cortisol levels may contribute to suppressed immunity, making a host less effective at controlling multiple infections. It is also probable that steroids which function to promote anabolic or catabolic functions prevent energy usage elsewhere, e.g., for immunocompetence (Wedekind and Folstad, 1994; Sheldon and Verhulst, 1996; Muehlenbein, 2004; Muehlenbein and Bribiescas, 2005). Less energy available for mucosal immunity may translate into increased susceptibility to multiple intestinal infections. Furthermore, an increased risk of morbidity and mortality due to immunosuppression and elevated metabolic expenditure may both be costs associated with maintaining elevated testosterone levels in some individuals, particularly dominant males in the group. For example, dominance rank and fecal testosterone levels were directly associated in the same Ngogo chimpanzees (Muehlenbein et al., 2004). Parasite richness was also directly associated with both testosterone level ($F = 4.98$, $df = 1$, $P = 0.033$) and dominance rank ($r = 0.441$, $P = 0.045$) (analyses not shown). Combined, these results are consistent with the supposition that testosterone is an endocrinological moderator which may balance the competing demands of increased reproductive success (afforded by testosterone-mediated aggressive behavior and dominance status) with increased susceptibility to parasitic infection.

Because hormones influence and regulate immune functions and reproductive behaviors and physiology, measuring changes in hormone levels and determining how they interact with immune measures, including intestinal parasite richness, may help us understand the optimization of hormone levels under varying environmental and social conditions. Given the logistical difficulties in directly sampling populations of wild nonhuman primates, noninvasive measures through urine and feces should continue to be utilized. Furthermore, potential relationships between steroids, immune measures, or disease risk factors, and even behavioral measures, should be evaluated in other primate species, in both sexes, and under varying environmental and seasonal conditions.

Although the correlative data do not allow for drawing causative conclusions regarding the effects of steroids on immunity to gastrointestinal parasites within the chimpanzees, other studies eluded to the suppressive effects of testosterone on mucosal immunity. For example, testosterone treatment was associated with reduced expulsion of the nematode *Nipostrongylus brasilnesis* in female soft-furred rats (Tiuria et al., 1995). Likewise, testosterone treatment was associated with increased susceptibility of female mice to *Strongyloides ratti* infection (Watanabe et al., 1999). The immune responses to gastrointestinal parasites are complex and consist of a combination of leukocytes, the complement cascade, cytokines, and antibodies (Urban et al., 1992; Else and Finkelman, 1998; MacDonald et al., 2002). Further elucidation of the effects of steroids on these immune mechanisms in nonhuman primates would be potentially beneficial for clinical and evolutionary purposes alike.

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