Received: 4 June 2021 | Revised: 30 August 2021 | Accepted: 4 September 2021

DOI: 10.1002/ajp.23329

RESEARCH ARTICLE

AMERICAN JOURNAL OF PRIMATOLOGY WILEY

Responsiveness of fecal immunoglobulin A to HPA-axis activation limits its use for mucosal immunity assessment

Verena Behringer[1] | Nadine Müller-Klein[2,3] | Christina Strube[4] | Oliver Schülke[3,5,6] | Michael Heistermann[1] | Julia Ostner[3,5,6]

[1]Endocrinology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

[2]Institute for Evolutionary Ecology and Conservation Genomics, Ulm University, Ulm, Germany

[3]Research Group Social Evolution in Primates, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

[4]Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine Hannover, Hanover, Germany

[5]Department of Behavioral Ecology, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, University of Göttingen, Göttingen, Germany

[6]Leibniz ScienceCampus Primate Cognition, Göttingen, Germany

Correspondence
Verena Behringer, Endocrinology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Kellenerweg 4, 37077 Göttingen, Germany.
Email: VBehringer@dpz.eu

Funding information
German Research Foundation (Deutsche Forschungsgemeinschaft,
Grant/Award Number: OS 201/6-1

Abstract

The assessment of mucosal immunity as a component of animal health is an important aspect for the understanding of variation in host immunity, and its tradeoff against other life-history traits. We investigated immunoglobulin A (IgA), the major type of antibody associated with mucosal immunity, in relation to changes in parasitic burden following anthelminthic treatment in noninvasively collected fecal samples in a semi-free ranging group of Barbary macaques (*Macaca sylvanus*). We measured IgA in 340 fecal samples of fourteen females and nine males. As IgA has been found to be responsive to stressors, we also related fecal IgA (fIgA) levels to fecal glucocorticoid metabolites (fGCM) measured in the same samples as part of a previous study. We found a high variability within and between individual fIgA levels over time. Running generalized additive mixed models, we found that fIgA levels were higher in males than in females, but did not change in response to the anthelmintic treatment and the resulting reduction in worm burden. Instead, fIgA level changes were significantly correlated to changes in fGCM levels. Our findings indicate that due to the strong responsiveness of fIgA to HPA-axis activity, the measurement of fIgA may have certain limitations with respect to reflecting gastrointestinal parasitic burden. Moreover, the responsiveness of fIgA to stressors interferes with the interpretation of IgA levels in fecal samples as a measure of mucosal immunity, at least in our study population of the Barbary macaques.

KEYWORDS
Health assessment, noninvasive samples, stress responses, parasite infection

1 | INTRODUCTION

Within the past decades, the assessment of an individual´s health status, the causes and consequences of variation in host immune defense, and the trade-off between investment in immunity and life-history decisions have attracted increasing interest in many disciplines such as biomedicine, epidemiology, evolutionary ecology, and evolutionary biology (Demas & Nelson, 2012; Laskowski et al., 2021; McDade et al., 2016; Nussey et al., 2014). To investigate immune challenges, it is mandatory to have methods at hand that enable the reliable assessment of immunity and/or health status in wildlife. Generally, markers for health assessment can be measured in blood or cerebrospinal fluid

Michael Heistermann and Julia Ostner share senior authorship.

(Bharucha et al., 2019; Kophamel et al., 2021), yet such invasive methods have limited feasibility in the context of wildlife and zoo research given that most animals, particularly large-bodied taxa, need to be anesthetized for the respective sampling procedures.

In the recent past, advancements have been made in the measurement of physiological markers of immune system activation from noninvasively collected urine and feces in wild and zoo-housed animals. For example, cell-mediated immune responses can be monitored by measuring neopterin in urine samples in nonhuman primates (*Macaca mulatta*: Fendrich et al., 1989; *M. mulatta*, *M. fascicularis*: Higham et al., 2015; *Pan troglodytes*: Löhrich et al., 2018; Wu et al., 2018), urinary markers of oxidative stress can be assessed to monitor health aspects in rodents (mice: Gyurászová et al., 2018; rats: Leeuwenburgh et al., 1999), dogs (Kendall et al., 2017), and chimpanzees (*P. troglodytes*: Thompson González et al., 2020), and urinary measurements of the urokinase plasminogen activator receptor allow monitoring of immune system activation and inflammation in rhesus macaques (*M. mulatta*: Higham et al., 2020).

Options for detecting immune status and health conditions from fecal samples are still limited. However, major sites of potential attack by invading pathogens are the bodies' mucosal surfaces, including the gastrointestinal tract (Woof & Kerr, 2006), making feces a potentially valuable source of insight into immune challenges. For example, in humans, fecal calprotectin levels are elevated during gut immune system activation (Mulak et al., 2019), a combination of fecal markers (e.g., lactoferrin, cathelicidins, osteoprotegerin) can be measured for health assessment in children (Pang et al., 2014), and parasite egg counts from feces are used to estimate parasite infections (Nonhuman primates: Gillespie et al., 2008; sheep: Pernthaner et al., 2006). The measurement of fecal neopterin has been linked to the occurrence and severity of local gastrointestinal diseases in humans (Husain et al., 2013), while its analysis in nonhuman primates has questioned its value as a biomarker for monitoring more systemic immune system activation processes (Higham et al., 2015).

One fecal measure that has recently been attracting increased attention as a potential biomarker for monitoring animal health state and well-being is the secretory form of immunoglobulin A (IgA) (Edwards et al., 2019; Gesquiere et al., 2020; Lantz et al., 2018; Staley et al., 2018). IgA belongs to a major class of antibody produced locally at all mucosal surfaces, including the gastrointestinal tract (Bosch et al., 2002; Mantis & Forbes, 2010; Woof & Kerr, 2006), and is assumed to reflect the functional status of the mucosal immune system (Corthésy, 2013). Its role is to protect the intestinal epithelium from enteric toxins and pathogenic agents, providing the 'first line of defense' by interfering with pathogen adherence, and modulating the gut inflammatory response to maintain intestinal homeostasis (e.g., Campos-Rodríguez et al., 2013; Chaney & Quinn, 2020; Walsh et al., 2011). Additionally, IgA influences the composition of the intestinal microbiota (Bruno et al., 2010; Mantis et al., 2011). IgA comprises 70% or more of the immunoglobulins produced by the mammalian body (Macpherson et al., 2008), and the daily energy used to produce IgA exceeds that of all other antibody classes combined, suggesting considerable benefits from IgA in immune defense at least from an evolutionary point of view (Woof & Kerr, 2006). Low levels of IgA are generally indicative of impaired mucosal immunity, intestinal function, and integrity (Campos-Rodríguez et al., 2013). Moreover, with respect to parasite infection, IgA is involved in eliminating infections and controlling parasite burden, for example in gastrointestinal nematode or *Giardia* spp. infections (de la Chevrotière et al., 2012; Langford et al., 2002).

For an overall animal health assessment, it would be useful to include a measure of mucosal immunity, because mucosal immunity is largely independent of systemic immunity (Woof & Kerr, 2006). Regarding wildlife, the measurement of fecal IgA (fIgA) could help to assess mucosal immunity in relation to parasite infections, providing a complementary physiological marker to those reflecting systemic immunity. IgA levels can be measured in minimally or noninvasively collected samples, including saliva, urine, and feces of many species, such as rodents (Bundgaard et al., 2012; Eriksson et al., 2004; Pihl & Hau, 2003; Taira et al., 2015), domestic animals (Carlsson et al., 2007; Heinrichs et al., 2013; Tress et al., 2006), equids (Krueger et al., 2019; Tombak et al., 2020), Asian elephants (*Elephas maximus*: Edwards et al., 2019; Kosaruk et al., 2020), roe deer (*Capreolus capreolus*: Escribano-Avila et al., 2013), Nonhuman primates (*Papio spp.*: Gesquiere et al., 2020; *P. troglodytes*: Lantz et al., 2018; *M. fascicularis*: Paramastri et al., 2007) and humans (Kang et al., 2018; Scholtens et al., 2008). However, previous studies using fIgA measurements as a measure of parasitic infections produced inconsistent results. For example, in mice, fIgA levels were negatively associated with parasite infections (*Heligmosomoides polygyrus* and *Eimeria* spp. burden; Clerc et al., 2018), whereas in goats and donkeys, fecal parasite egg count, a crude indicator of worm burden (Gassó et al., 2015; Nielsen et al., 2010), was positively correlated with IgA concentrations (de la Chevrotière et al., 2012; Tombak et al., 2020). In sheep, increased IgA levels were related to resistance to gastrointestinal nematode infection (Pernthaner et al., 2006). In baboons, fIgA concentrations were negatively correlated with egg counts of *Trichuris trichiura*, but not with parasite richness (Gesquiere et al., 2020). The inconsistencies in the reported association patterns of parasite infection and IgA level changes are likely related to species specificities, but may also be caused by differences in encountered stressors including food scarcity which differs between provisioned and wild populations.

The effect of stressors on the link between IgA and parasite resistance is conceivable given that numerous studies have shown that IgA levels also change during stress responses, and thus, are linked to the activation of the hypothalamic-pituitary-adrenal (HPA)-axis (Hau et al., 2001; Lantz et al., 2018; Pihl & Hau, 2003). During exposure to acute stressors, secretory IgA (sIgA) levels usually increase (Bosch et al., 2002), probably in response to the short-term stress-induced enhancement of immune function (Dhabhar, 2014). In contrast, during chronic stress, sIgA levels typically decline (Gleeson & Cripps, 2015; Viena et al., 2012), presumably as a result of the immunosuppressive effect of long-term elevations in GC concentrations (Sapolsky, 2000). Therefore, the modulation of sIgA production is associated with the duration as well as with the intensity of the stressor. Due to this link, IgA has recently been implemented in stress response research to evaluate the interplay between physiological stress and the immune system (Staley et al., 2018). In the research field of animal welfare, the combined measurement of GC and IgA secretion is becoming increasingly prominent as a more holistic measure to evaluate individual stress responses

(Carlsson et al., 2007; Edwards et al., 2019; Kosaruk et al., 2020; Staley et al., 2018). Given the strong association of IgA level changes and HPA-axis activity, the value of IgA as a measure of mucosal immunity needs revisiting. In this respect, studies carried out under controlled conditions, for example, during periods of anthelmintic treatments, may help to elucidate the usefulness of fIgA measurements as a general physiological marker of parasite burden and mucosal immunity.

In this study, we capitalized on the removal of helminthic parasites by anthelmintic treatment in a group of semi-free ranging Barbary macaques (*Macaca sylvanus*) (Müller et al., 2017; Müller-Klein et al., 2019a), to evaluate the feasibility of fIgA levels as a measure for parasite infections, and thus the value of fIgA as an indirect measure of mucosal immunity. In a previous study, we identified three nematode morphotypes, strongyle nematodes (mainly *Oesophagostomum*), *Capillaria* spp., and *Trichuris* spp. in the feces of our study animals. *Oesophagostomum* spp. were the most common parasites in our study population with a prevalence of >95%, while the prevalence of *Capillaria* spp. (37%) and *Trichuris* spp. (7%) was much lower (Müller et al., 2017). After anthelmintic treatment, the successful clearance of strongyle nematodes was found within two days posttreatment lasting at least three weeks (Müller-Klein et al., 2019a; Müller-Klein et al., 2019b).

To our knowledge, information on how these parasites affect health and fitness in Barbary macaques is lacking, however, nematode infections did not seem to cause obvious clinical symptoms in our study population (Müller-Klein et al., 2019b). In other species, nematode infections lead to transient or chronic infections associated with inflammation and tissue damage in the gut mucosa (Andreasen et al., 2015; Polderman & Blotkamp, 1995; Singla et al., 2012). For example, in chimpanzees and baboons, *Oesophagostomum* infections resulted in large numbers of mural colonic and mesenteric nodules (Terio et al., 2018). *Trichuris* spp. has been associated with reduced fertility in female yellow baboons (*Papio cynocephalus*: Akinyi et al., 2019), but male reproductive success is not correlated to parasite infection in wild red-fronted lemurs (*Eulemur fulvus rufus*: Clough et al., 2010). Since zoo-housed primates are often treated with anthelmintics as a preventative strategy, information about endoparasite loads and their health effects in zoo-housed primates are scarce (McPherson, 2013), but infections are known to cause diarrhea and death for example in zoo-housed lemurs (Rasambainarivo & Junge, 2010).

In this study and capitalizing on the routine deworming treatment, we predicted that fIgA levels will change after deworming and change again after re-infection if fIgA levels are a reliable predictor of mucosal immunity. To further evaluate the potential impact of HPA-axis activity on fIgA levels, we first used an adrenocorticotropic hormone (ACTH) challenge (artificial stimulation of the HPA-axis) to confirm that fIgA is responsive to the stimulation of adrenocortical activity as shown for fecal glucocorticoid metabolites (fGCM; Heistermann et al., 2006). We also examined the association between changes in fIgA and fGCM measured in the same samples across the study period. Based on previous studies showing an association between stress responses and IgA levels, we predicted that a significant portion of within- and between-individual variation in fIgA levels would be explained by variation in fGCM levels. This approach allowed us to evaluate what amount of variation in fecal IgA is due to glucocorticoid output.

2 | MATERIAL & METHODS

2.1 | Data set adrenocorticotropic hormone challenge

As IgA is responsive to acute activation of the HPA-axis (e.g., Lantz et al., 2018), we physiologically validated the measurement of fIgA by examining the IgA response to an adrenocorticotropic hormone (ACTH) challenge test, performed in two zoo-housed, adult, female Barbary macaques in August 2001. The ACTH challenge was part of a previous study on the validation of fGCM measurements in this species (Heistermann et al., 2006). The fecal samples available for this validation represented daily samples, three and five samples per individual, collected 2–6 days before ACTH injection (control samples), and all samples defecated by the two monkeys following ACTH administration for the next four days. All samples (10 and 12 samples for the two monkeys, respectively) had been collected within 30 min after defecation, and subsequently stored at −20°C until they were lyophilized and pulverized (Heistermann et al., 1995) for fGCM extraction and analysis, using a validated enzyme immunoassay (Heistermann et al., 2006). The remaining fecal powder was re-frozen at minus 20°C for >15 years and was now used for the analysis of fIgA.

2.2 | Ethics statement

This study was conducted noninvasively and data collection followed the Animal Behavior Society's guidelines for the treatment of animals in behavioral research and teaching and adhered to both the American Society of Primatologists Principles for the Ethical Treatment of non-human primates and the standards as defined by the European Union Council Directive 2010/63/EU on the protection of animals used for scientific purposes. Authorization for the anthelmintic treatment was given by the Veterinary Office of the district office of county Lake Constance. Anthelmintic treatment was performed as part of the routine procedures of Affenberg Salem as defined by the European Union Council Directive 1999/22/EC. The study was approved by the Animal Welfare Body of the German Primate Center (No. E9-16).

2.3 | Data set for fIgA and fGCM comparison

The fecal samples used for IgA analysis in response to the anthelmintic treatment were collected between June and December 2015 as part of a previous study on the physiological and social consequences of gastrointestinal infections in free-ranging Barbary macaques living in a 20-ha forested outdoor enclosure at Affenberg Salem, Germany (Müller et al., 2017; Müller-Klein et al., 2019a; Müller-Klein et al., 2019b). Fecal samples were collected

approximately once per week from adult males and females to characterize parasite burden, to examine the efficacy of the anthelmintic treatment, and fGCM quantification (for details Müller et al., 2017). Samples were stored on ice in the dark until transfer to a −20°C freezer at the end of the field day (i.e., within 8 h) and were transported frozen to the German Primate Center´s Endocrinology Laboratory for fGCM analysis (Müller-Klein et al., 2019a; Müller-Klein et al., 2019b). In the present study, we used these samples to quantify fIgA concentrations, which allowed us to investigate not only the response pattern of IgA to the deworming event but also the potential link between fIgA and fGCM responses.

Fecal samples had been lyophilized and pulverized for GCM extraction and the fecal powder was subsequently re-stored at either room temperature (ca. 21°C) or at −20°C. Unfortunately, for IgA analysis we could use only the frozen stored samples as initial tests showed that IgA was not detectable in samples ($N = 38$) stored at room temperature, suggesting marked IgA degradation in lyophilized feces stored for several years at room temperature. We are confident that the IgA measurements from samples stored long-term at −20°C provide valid information on IgA concentrations since the physiological validation test (see above) showed the predicted IgA response pattern to the ACTH challenge test (see results section) despite long-term frozen storage (>15 years). Moreover, IgA levels in aliquots of the same fecal samples ($N = 15$) that were stored as original neat feces and as lyophilized fecal powder for >5 years correlated strongly (Spearman correlation: rho = 0.946, $p < .001$, $N = 15$), indicating that long-term storage in a freeze-dried form does not alter relative IgA levels across samples. However, two-tailed pairwise *t* test comparisons showed that in native frozen fecal samples IgA levels were significantly higher than in frozen powdered samples ($T = 2.409$; $N = 15$, $p = .03$). Therefore, we used for our final data set only samples of individuals for which we had fecal sample powder stored frozen. Thus, this final data set for examining IgA response pattern to anthelmintic treatment included fecal samples from fourteen adult females (213 samples) and nine adult males (127 samples).

Anthelmintic treatment is carried out routinely as a veterinary care procedure to remove helminth infections in the Barbary macaques living at Affenberg Salem. All monkeys individually received Ivermectin (Diapec, Albrecht), a broad-spectrum anthelmintic compound (Djune-Yemeli et al., 2020; Heukelbach et al., 2004; Müller et al., 2017). The study animals were divided into two groups, a treatment group (eight females, five males) which received Ivermectin treatment in August 2015, and a control group (six females, four males) which did not receive the treatment at this time. Groups were matched for age (Müller et al., 2017). For population health management, all individuals (control and treatment group) received a second Ivermectin treatment in November 2015 (for detailed information on the treatment procedure Müller et al., 2017). Parasite analyses revealed the prevalence of three different morphotypes: strongyle nematodes (mainly *Oesophagostomum* spp.), *Capillaria* spp., and *Trichuris* spp. Coproscopic analysis indicated successful clearance of strongyle nematodes with individuals being coproscopally negative for at least three weeks. In contrast, the anthelmintic treatment was not fully effective to remove *Capillaria* spp. and *Trichuris* spp. (Müller et al., 2017).

2.4 | IgA extraction and analysis

For IgA extraction, we followed the extraction procedure described in Gesquiere et al. (2020) with some modifications. We extracted 0.1 g of pulverized fecal powder with 2 mL assay diluent (from Life Diagnostics, Inc. Monkey IgA ELISA kit; Cat# IGA-3) by mixing the samples for 15 min on a multitube vortexer. Samples were then centrifuged for 15 min at 2600*g* after which 750 µl of the supernatant was transferred into a glass tube and the rest of the extract discarded. The remaining fecal pellet was re-extracted with an additional 2 ml diluent as described above and 750 µl of this second extraction was combined with the 750 µl from the first extraction. The combined extract was finally centrifuged for 5 min at 2000*g*, the supernatant was transferred into a polypropylene tube, and samples were stored at −20°C until IgA analysis. Different from Gesquiere et al. (2020), we added a second extraction step after we found in an initial experiment on a subset of 18 samples that re-extraction increased IgA concentrations on average by 42% (range 32%–54%). In addition to improving overall extraction efficiency (c.f. Palme et al., 2013), this second extraction step also reduced between-sample variability (between-sample coefficient of variation decreased by 10% as a result of the second extraction) and thus resulted in individual IgA concentrations that were less affected by methodological variation.

In 340 fecal extracts, IgA was measured using the commercial monkey IgA ELISA kit (Life Diagnostics, Inc., Cat# IGA-3) previously used for IgA assessment in fecal samples of baboons (Gesquiere et al., 2020). For measurement, all extracts were diluted 1:20 with assay diluent provided by the kit, and samples were measured in duplicate according to the manufacturer´s instructions. In brief, we pipetted 100 µL of diluted extract or standard in each well. Plates were then incubated for 45 min on a microplate shaker at 100 rpm at room temperature in the dark. Plates were subsequently washed five times with the washing solution provided by the assay kit. 100 µL of enzyme conjugation reagent was then added into each well and the plates were incubated again for 45 min on the shaker. Following this second incubation step, the plates were washed a second time as described before, and 100 µL of tetramethylbenzidine substrate solution was pipetted into each well and the plates placed on the shaker for final incubation. The reaction was terminated within 10 min by adding 100 µL of the provided stop solution, and optical density was read at 450 nm.

Serial dilutions of fecal extracts from two female and two male samples gave displacement curves that run parallel to the IgA standard curve. Samples with a coefficient of variation (CV) value between duplicates of >10% were re-measured. Intra-assay coefficient of variation, determined as the average CV between duplicate determinations, was 7.7%, and interassay variability, calculated from the repeated measurement of a high- and low-value quality control provided with the kit, was 9.4% (high, $N = 13$) and 8.6% (low, $N = 13$).

2.5 | Statistical analyses

All statistical analyses were performed with R 4.0.3 (R Development Core Team, 2020). We ran Spearman correlation tests to examine the relationship between IgA concentrations in freshly lyophilized and long-term frozen lyophilized samples (see above), and to investigate the relationship between fGCM and fecal IgA levels (1) within an individual and (2) across individuals using individual IgA medians. In addition, a Spearman correlation was run to investigate the association between average IgA levels (µg/g feces) of the pre-anthelmintic treatment period and average egg count data (eggs per gram feces) during this period (Müller et al., 2017). To investigate the effect of anthelmintic treatment on fGCM and fIgA levels we fitted two identical general additive mixed models (GAMM; Wood, 2011) with Gaussian-identity link function using R. We used GAMM models because we expected that both markers would change during the study period in response to the treatment as well as with subsequent parasite reinfection (Müller-Klein et al., 2019a) in the treatment group but not in the group without treatment. We used the "gam" function in the R package "mgcv" version 1.8–27 (Wood, 2017). In the first model, log-transformed fIgA, and in the second model log-transformed fGCM levels were included as response variables. In both models, sex (females, males), and treatment (yes, no) were included as predictor variables. Group (treated, not-treated) was included as ordered factor. To model the changes in fIgA or fGCM levels, we included the smooth term of sample collection in interaction with group (treated or not) using the "by" argument (Wood, 2017). We also included a smooth term for the date of sample collection with a penalized cubic regression and monkey ID as a random factor to account for unequal and repeated sampling of individuals across the study period and parasitic treatment. The basis dimension, k was set to 5. We compared the full with the null model using "CompareML", with the null model only including the random factor monkey ID.

Model diagnostics included "gam.check". Model assumptions were assessed by visual inspections of a histogram, a qq plot of the residuals, and by plotting residuals against fitted values and basis dimensions. In both models, the k-index did not reveal any problems with model assumptions. Concurvity, the situation where a smooth term can be approximated by some combination of the others, was also not found to be an issue in either model. Significance for all tests was set at $p = .05$ level.

3 | RESULTS

3.1 | Physiological validation

Both female Barbary macaques responded to the ACTH challenge with an increase in fIgA and fGCM levels (Figure 1). FIgA levels were already increased in the first samples collected after ACTH administration, which was nine and eleven hours following the challenge, respectively. Peak concentrations of fIgA (1035.9 µg/g and 1744.5 µg/g, respectively) occurred in both individuals in the second sample after 22 and 29 h, post-ACTH administration, respectively, representing an 8- and 12-fold increase compared to pre-ACTH values. FIgA levels declined rapidly thereafter to reach pre-ACTH baseline values within 50 h after ACTH injection. FGCM levels showed an overall similar response to ACTH, but the temporal pattern slightly differed with peak fGCM concentrations being recorded later (i.e., after 46- and 45-hours post-ACTH, respectively). Moreover, when fGCM levels reached their maximum, fecal IgA levels had already declined.

3.2 | Individual variation in fIgA levels

FIgA levels were highly variable between individuals across the whole study period (Figure 2). Values ranged between 2.0 and

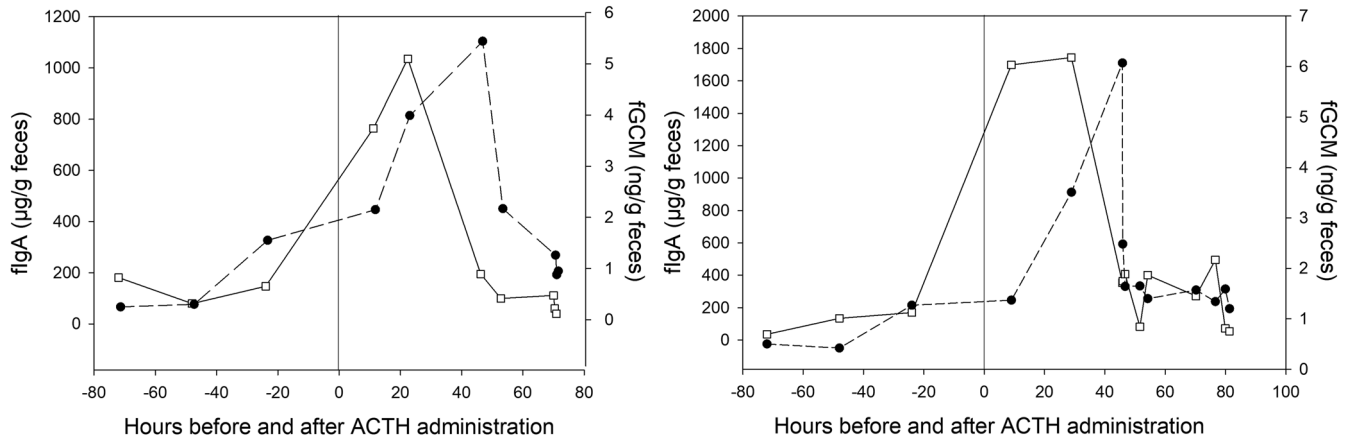

FIGURE 1 Fecal immunoglobulin A (fIgA, open square, solid line) and fecal glucocorticoid metabolite (fGCM, filled circle, dashed lines) concentrations measured before and after ACTH challenges in two female Barbary macaques

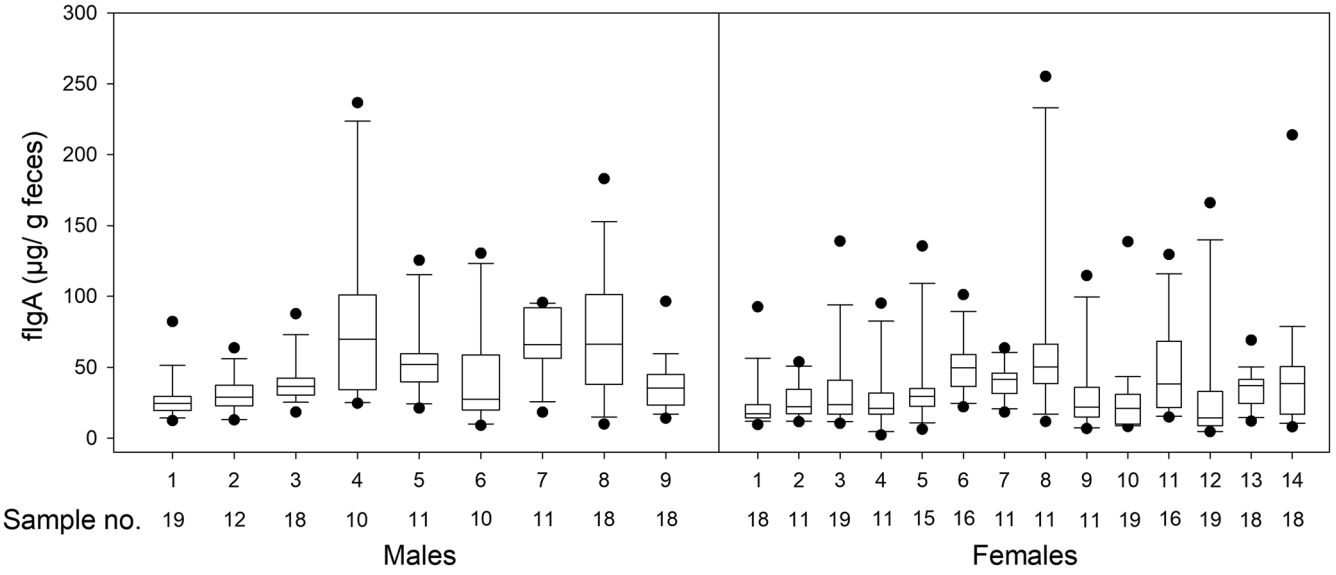

FIGURE 2 Box plots of fecal Immunoglobulin A (fIgA) concentrations of males and females including sample numbers per individual

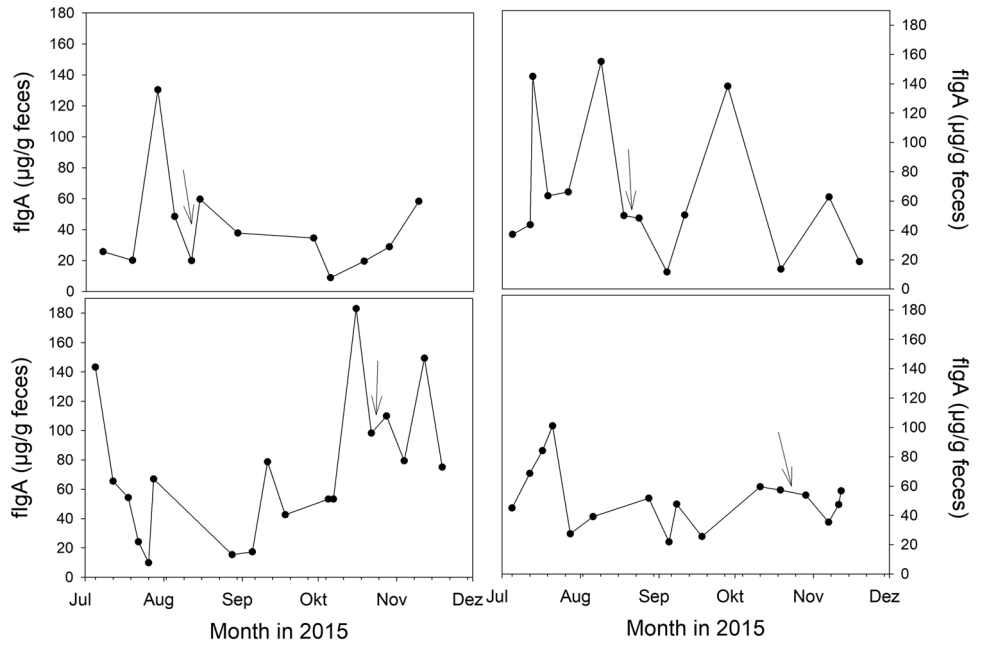

FIGURE 3 Four representative fecal Immunoglobulin A (fIgA) profiles (two males on the left, two females on the right side) of Barbary macaques over the course of the study period. Arrows indicate the time of deworming

255.2 (µg/g feces) across females and between 8.9 and 183.0 (µg/g feces) across males. Within individual variation in fIgA levels was also high in each of the study animals (four individual profiles are shown as examples in Figure 3).

Egg count (eggs per gram feces = EPG) varied between 0 and 475 across the 23 animals under study in the pre-anthelmintic treatment period. During the same period, average fIgA levels varied between 13.8 (µg/g feces) and 127.2 (µg/g feces). There was no significant correlation between the two variables (rho = −0.0332, $p = .876$, $N = 23$).

3.3 | Within- and between-sample correlation of fIgA and fGCM levels

FIgA levels correlated significantly positively with fGCM levels within a sample as well as when median values across animals were considered. However, within a sample, the positive correlation between fIgA and fGCM was rather weak (rho = 0.169; $p = .002$; $N = 340$) compared to the correlation across individual mean (rho = 0.533; $p = .009$; $N = 23$) or median (rho = 0.589; $p = .003$; $N = 23$) concentrations of the two measures.

TABLE 1 Estimates of parametric coefficients and effective degrees of freedom of smooth terms for fecal Immunoglobulin A (fIgA) and fecal glucocorticoid metabolite (fGCM) levels in Barbary macaques (generalized additive model, fIgA: $R^2 = 0.25$, deviance explained = 30.5%, $N = 340$ samples; fGCM: $R^2 = 0.46$, deviance explained = 49.8%, $N = 340$ samples)

Marker	fIgA			fGCM		
Parametric coefficients	Estimate	SE	*p* value	Estimate	SE	*p* value
Intercept	3.32	0.13	<0.001	5.59	0.11	<0.001
Sex (males)	0.35	0.15	**0.023**	0.37	0.13	**0.006**
Group (treated)	0.15	0.18	0.388	0.12	0.14	0.374
Treatment (yes)	−0.19	0.17	0.235	−0.05	0.07	0.467
Smooth terms	Edf		*p* value	Edf		*p* value
Date	3.06		**0.019**	3.51		**0.005**
Date* group (treated)	2.13		0.229	1.01		0.695

Abbreviation: Edf, effective degrees of freedom.

3.4 | Relationship of fIgA and fGCM levels in fecal samples of the treatment experiment

In both GAMM models the comparison of the full model with the null model was significant (fIgA: $p = .025$; fGCM: $p = .014$), indicating that the predictors in the full model explained variation in the response better than without them. In both the fIgA and the fGCM model, the same variables significantly predicted the respective biomarker concentration. Males had significantly higher levels than females (P_{fIgA}: = 0.023; Pf_{fGCM} = 0.006, Table 1, Figure 4), levels changed significantly over the study period (P_{fIgA}: = 0.019; P_{fGCM} = 0.005, Table 1, Figure 5), and this temporal pattern was similar in the two markers (Figure 5). The changes in both markers were independent of the anthelmintic treatment because the control and the treatment group did not differ significantly from each other over the study period (Table 1, Figure 5), indicating that the deworming event did not affect the concentrations of the two biomarkers. Additionally, fIgA levels did not correlate with fecal egg counts before treatment (see above).

4 | DISCUSSION

Assessing mucosal immunity as a component of animal health is an important aspect to better understand variation in immunity and the investment in maintaining immune function. In this study, we investigated whether changes in fIgA levels, the major type of antibody associated with mucosal immunity (Hand & Reboldi, 2021; Woof & Mestecky, 2015), reflected nematode infection status in a group of Barbary macaques. For this purpose, we used a routine veterinary anthelmintic treatment event. We found that fIgA concentrations over time were highly variable within and between individuals and were higher in males than in females. Unexpectedly, fIgA levels did not change in response to the anthelmintic treatment, despite its high efficacy in removing strongyle nematodes, the most prevalent parasites found in our study population (Müller-Klein et al., 2019a). We also found that fIgA level changes were closely linked and significantly correlated with changes in fGCM levels, measured concomitantly as an indicator of the animal´s physiological stress status. Because of the strong responsiveness of fIgA to HPA-axis activity, the measurement of fIgA levels may have limitations for the assessment of the degree of gastrointestinal parasitic infections, and consequently for the interpretation of IgA levels in fecal samples as a measure of mucosal immunity, at least in the study species.

Based on studies reporting significant correlations between parasitic burden/infection intensity and fIgA concentrations (e.g., Gesquiere et al., 2020; Pernthaner et al., 2006; Tombak et al., 2020), we expected fIgA levels to change significantly following an anthelmintic treatment that successfully removed the most abundant parasite (strongyle nematodes, prevalence = 98% pretreatment) in our study species. However, the temporal pattern in fIgA excretion during the six months study period was similar in treated and non-treated monkeys, and was also not different during the period of treatment and reinfection. One possible explanation for the lack of a significant change in fIgA levels to the deworming event is that not all parasite taxa were removed by the treatment. Ivermectin, the medication used to treat parasite infestations in the study species, is a broad-spectrum anthelmintic compound known to remove effectively many species of nematodes (Ottesen & Campbell, 1994), but is moderately effective against *Trichuris* and weakly effective when treating hookworm and *Enterobius* infections (Wen et al., 2008).

In our study, Ivermectin treatment was less effective in clearing nematodes of the genera *Capillaria* spp. and *Trichuris* spp., which had much lower prevalence though than strongyle nematodes (Müller-Klein et al., 2019a; Müller-Klein et al., 2019b). Given the absence of a clear fIgA response to strongyle nematode clearance, it could be argued that this morphotype may affect the secretion of IgA from the intestinal mucosa only a little. However, strongyle nematode egg count was positively correlated to IgA levels in plasma and feces of Soay sheep (*Ovis aries*) and donkeys (Pernthaner et al., 2006; Tombak et al., 2020; Watt et al., 2016), but this interrelationship did not appear in zebras (Tombak et al., 2020). At least *Trichuris* spp. infections were negatively correlated with fIgA levels in wild baboons (Gesquiere et al., 2020). Since in our data set only one individual was infected with *Trichuris* spp., a potential confound of a resisting *Trichuris* infection on IgA responsiveness can be excluded. Another explanation is that because *Trichuris* and *Enterobius* inhabit the large intestine, and strongyle nematodes inhabit the small intestine, the IgA released into the small intestine is (partially) degraded by the digestive enzymes, while the IgA released into the large intestine remains more or less unaffected, and could overlay an effect of deworming in the small intestine.

It is also possible that the IgA response to parasitic infections may not only be parasite-specific, but that also different parasite life stages might lead to different IgA level responses (Bosqui et al., 2015; Yang et al., 2020). Additionally, bacterial strain variation of the gut microbiota induces different IgA secretion patterns (Yang et al., 2020). This may add to the high variability in fIgA concentrations over time which are reported both within- and between animals of several species (Carlsson et al., 2007; Edwards et al., 2019; Pihl & Hau, 2003; Plangsangmas et al., 2020), including the Barbary macaque of our study. It is also possible that fIgA may reflect constitutive mucosal immunity, and therefore, the removal of one parasite may not affect overall its concentration.

Our data offer a second, not-mutually exclusive, explanation for the lack of a clear effect in fIgA responsiveness to the anthelmintic treatment: The effect of fIgA level changes with parasite infection status may be blurred by the responsiveness of fIgA to HPA-axis activation. Based on our simultaneous measurement of fIgA and fGCM levels in the same samples, we demonstrate that both compounds changed in parallel as shown by (1) a similar pattern of both measures in response to an ACTH challenge test, (2) significant positive correlations of the two measures across individuals, (3) similar sex-specific differences, and (4) comparable temporal patterns over the course of the entire study period. Our data are in line with numerous other studies showing that stress-induced activation of the HPA-axis also modulates the secretion of sIgA (Campos-Rodríguez et al., 2013; Jarillo-Luna et al., 2007). For example, as in our study, ACTH administration also induced a strong fIgA response in a chimpanzee that mirrored fGCM results (Lantz et al., 2018; Murray et al., 2013). Moreover, as in our study, measures of fIgA and glucocorticoids were also significantly correlated in Sichuan golden snub-nosed monkeys (*Rhinopithecus roxellana*: Huang et al., 2014). In pigs, salivary IgA increased in response to restraint stress (Muneta et al., 2010) and isolation (Escribano et al., 2015), whereas in dogs, the stress of noise and defense training decreased levels of IgA (Svobodová et al., 2014). Finally, secretory IgA is also recognized as a stress-sensitive marker in rodents (Bundgaard et al., 2012; Pihl & Hau, 2003), elephants (Edwards et al., 2019; Kosaruk et al., 2020), and humans (Campos-Rodríguez et al., 2013; Viena et al., 2012). Therefore, the assessment of IgA in saliva, urine, or feces has recently been proposed as a novel biomarker to evaluate the interplay between stress, the immune system, and animal welfare in wildlife species (Edwards et al., 2019; Kosaruk et al., 2020; Plangsangmas et al., 2020; Staley et al., 2018). However,

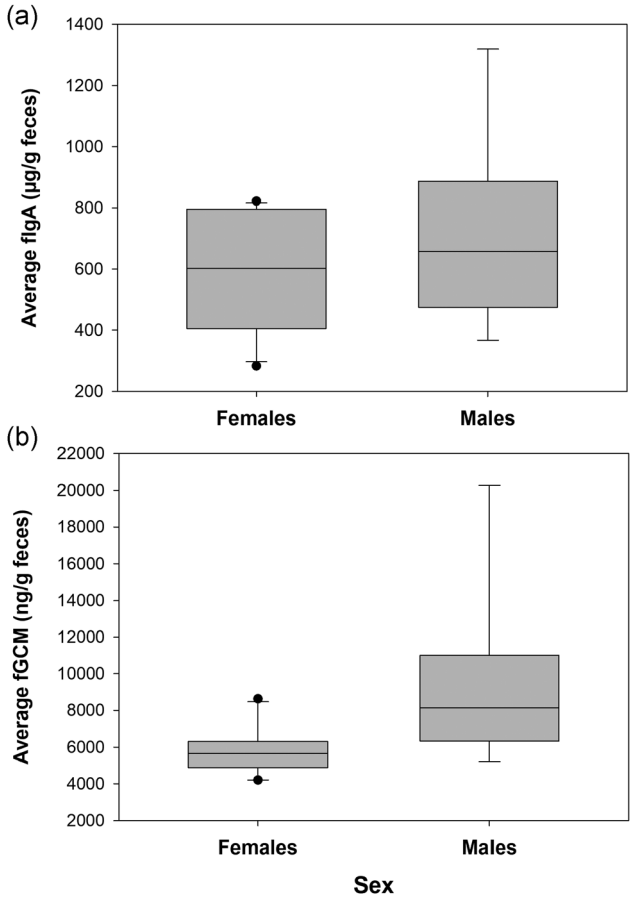

FIGURE 4 (a) Fecal Immunoglobulin A (fIgA) and (b) fecal glucocorticoid metabolites (fGCM) measurements of female (blue, $N = 14$) and male (red, $N = 9$) Barbary macaques. The boxes illustrate the 25th and 75th percentiles, bars indicate medians, and circles indicate outliers

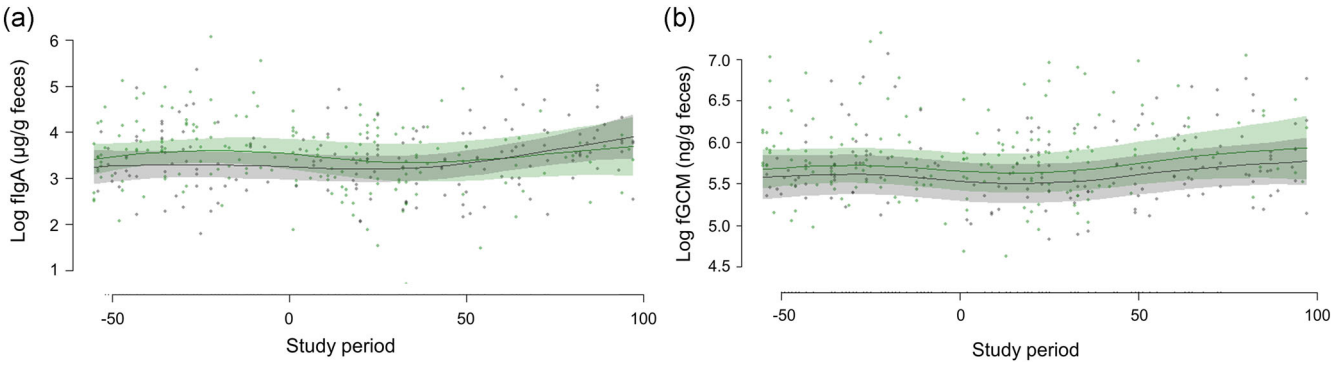

FIGURE 5 (a) Fecal Immunoglobulin A (fIgA) and (b) fecal glucocorticoid metabolite (fGCM) measurements of Barbary macaques with treatment (green) and without treatment (gray). The anthelmintic treatment is set to zero. Shaded areas represent bootstrapped 95% confidence intervals for expected biomarker levels

using IgA as a marker of well-being is hampered by the fact that on the one hand chronic stressors can suppress immune functions and reduce IgA production (Romero-Martínez & Moya-Albiol, 2017; Staley et al., 2018), while on the other hand, an acute disease can increase IgA levels (Edwards et al., 2019; Gupta et al., 2002; Yu et al., 2020). Therefore, the interpretation of IgA level changes is not always straightforward, similar to GC measurements, since both can increase in response to acute immune-related stressors (Kosaruk et al., 2020; Romero & Beattie, 2021; Tsujita & Morimoto, 1999). Additionally, it is important to consider within- and between-individual variation in IgA levels to understand the interaction of GCs and IgA, in particular the effect and duration of different stressors on the production of IgA (Kosaruk et al., 2020).

In contrast to other studies on nonhuman primates (Gesquiere et al., 2020; Huang et al., 2014; Lantz et al., 2018), we found significant sex differences in fIgA levels, with males having higher fIgA levels than females. A difference in parasite immunity between the sexes is expected if males and females differ in their tradeoffs between immunity and reproduction (Metcalf & Graham, 2018; Sheldon & Verhulst, 1996; Zuk & McKean, 1996). For example, parasite egg count (EPG feces) was higher in males during the mating season, and higher in females during the birth season in Japanese macaques (MacIntosh et al., 2010). Our sample collection period in the Barbary macaques covered mainly the birth and lactation season (Küster & Paul, 1984; Paul & Thommen, 1984), a period with high energetic demands for females (Gittleman & Thompson, 1988; Heldstab et al., 2017). Our finding that females have lower fIgA levels compared to males during this period may thus suggest that female Barbary macaques tradeoff mucosal immunity against the reproductive effort, whereas mucosal immunity in males is not constrained by energetics. Future studies should address whether this sex difference in fIgA concentrations persists also outside this period and whether it reverses during the mating season when males are expected to be more energetically challenged than females due to high levels of mating competition.

Our study was carried out on a semi free-ranging, provisioned population. Studies in mammals and birds in natural habitats have shown that differences in pathogen prevalence and available energy are correlated with differences in immune responses (Behringer et al., 2019; Borkow & Bentwich, 2008; Buehler et al., 2008; Fair et al., 2017). Moreover, natural environments differ from captive environments in the number and severity of stressors, in particular nutritional stressors. As the expression of IgA is a marker for immune system activity which is sensitive to stressors, it responds to available energy and HPA-axis functioning. Therefore, a different relationship between IgA and parasitic infestation than the one found in this study may be found in the wild.

In conclusion, our study suggests that the interplay of stress and immune responses on fIgA levels as well as the differences in the responsiveness of IgA to different parasite taxa challenge the usefulness of IgA to monitor parasite infection status. However, the incorporation of fIgA measurements in combination with monitoring clinical signs of illness and parasite infection in fecal samples can enhance our ability to detect changes in the immune and stress response of wild and zoo-housed individuals.

ACKNOWLEDGMENTS

We are grateful to Ellen Merz and Roland Hilgartner for the permission to conduct the study and Mamisolo Hilgartner and Bernhard Stark for their help with the anthelmintic treatment, as well as the Affenberg staff for their support. We thank Nicole Paschek, Navina Lilie, Zina Maria Morbach, Alice Bouchard, Breanne Boughan, Fabrizio del'Anna, Frauke Gärtner, Cassandra Gaylor, Georg Messerer, Lola Rivoal, Graciela Sanguino, Juliane Schindler, Andrea Schick, Aaron Skinner, Natalie Weil, Louise Wilson, and Rachel Voyt for their help. The study was supported by funding by the German Research Foundation (Deutsche Forschungsgemeinschaft (grant number OS 201/6-1)) and profited from discussions in the research unit "Sociality and Health in Primates" (FOR 2136). Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTERESTS

The authors declare they have no conflict of interests.

AUTHOR CONTRIBUTIONS

Verena Behringer conceptualization (equal); formal analysis (lead); funding acquisition (supporting); investigation (equal); methodology (equal); project ad-ministration (lead); validation (equal); visualization (lead); writing original draft (lead); writing review and editing (supporting). Nadine Müller-Klein data curation (lead); formal analysis (supporting); investigation (equal); methodology (supporting); funding acquisition (supporting); resources (lead); writing original draft (supporting); writing review and editing (supporting). Christina Strube formal analysis (supporting); funding acquisition (supporting); methodology (supporting); resources (supporting); supervision (supporting); writing review and editing (supporting). Oliver Schülke conceptualization (supporting); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (supporting); resources (supporting); supervision (supporting); visualization (supporting); writing original draft (supporting); writing review and editing (supporting). Michael Heistermann conceptualization (equal); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); validation (equal); visualization (supporting); writing original draft (equal); writing review and editing (supporting). Julia Ostner conceptualization (equal); data curation (lead); formal analysis (supporting); funding acquisition (lead); investigation (equal); methodology (supporting); project administration (equal); resources (equal); supervision (equal); writing original draft (supporting); writing review and editing (supporting).

DATA AVAILABILITY STATEMENT

The datasets presented in this study will be found in the online repository GRO. publications: https://doi.org/10.25625/ALKB7HReferences

ORCID

Verena Behringer http://orcid.org/0000-0001-6338-7298

REFERENCES

Akinyi, M. Y., Jansen, D., Habig, B., Gesquiere, L. R., Alberts, S. C., & Archie, E. A. (2019). Costs and drivers of helminth parasite infection in wild female baboons. *Journal of Animal Ecology*, *88*(7), 1029–1043.

Andreasen, A., Petersen, H. H., Kringel, H., Iburg, T. M., Skovgaard, K., Dawson, H., Urban, J. F., & Thamsborg, S. M. (2015). Immune and inflammatory responses in pigs infected with *Trichuris suis* and *Oesophagostomum dentatum*. *Veterinary Parasitology*, *207*(3–4), 249–258.

Behringer, V., Stevens, J. M. G., Wittig, R. M., Crockford, C., Zuberbühler, K., Leendertz, F. H., & Deschner, T. (2019). Elevated neopterin levels in wild, healthy chimpanzees indicate constant investment in unspecific immune system. *BMC Zoology*, *4*(1). http://doi.org/10.1186/s40850-019-0041-1

Bharucha, T., Gangadharan, B., Kumar, A., de Lamballerie, X., Newton, P. N., Winterberg, M., Dubot-Pérès, A., & Zitzmann, N. (2019). Mass spectrometry-based proteomic techniques to identify cerebrospinal fluid biomarkers for diagnosing suspected central nervous system infections. A systematic review. *Journal of Infection*, *79*(5), 407–418.

Borkow, G., & Bentwich, Z. (2008). Chronic parasite infections cause immune changes that could affect successful vaccination. *Trends In Parasitology*, *24*(6), 243–245.

Bosch, J. A., Ring, C., de Geus, E. J. C., Veerman, E. C. I., & Nieuw Amerongen, A. V. (2002). Stress and secretory immunity, *International Review of Neurobiology* (52, pp. 213–253). Elsevier. https://doi.org/10.1016/S0074-7742(02)52011-0

Bosqui, L. R., Gonçalves, A. L. R., Gonçalves-Pires, M., do, R. F., Custodio, L. A., de Menezes, M. C. N. D., Murad, V. A., de Paula, F. M., Pavanelli, W. R., Conchon-Costa, I., Costa-Cruz, J. M., & Costa, I. N. (2015). Detection of parasite-specific IgG and IgA in paired serum and saliva samples for diagnosis of human strongyloidiasis in northern Paraná state, Brazil. *Acta Tropica*, *150*, 190–195. http://doi.org/10.1016/j.actatropica.2015.07.026

Bruno, M. E. C., Rogier, E. W., Frantz, A. L., Stefka, A. T., Thompson, S. N., & Kaetzel, C. S. (2010). Regulation of the polymeric immunoglobulin receptor in intestinal epithelial cells by *Enterobacteriaceae*: Implications for mucosal homeostasis. *Immunological Investigations*, *39*(4–5), 356–382.

Buehler, D. M., Piersma, T., & Irene Tieleman, B. (2008). Captive and free-living red knots *Calidris canutus* exhibit differences in non-induced immunity that suggest different immune strategies in different environments. *Journal of Avian Biology*, *39*(5), 560–566.

Bundgaard, C. J., Kalliokoski, O., Abelson, K. S., & Hau, J. (2012). Acclimatization of mice to different cage types and social groupings with respect to fecal secretion of IgA and corticosterone metabolites. *In Vivo*, *26*, 883–888.

Campos-Rodríguez, R., Godínez-Victoria, M., barca-Rojano, E., Pacheco-Yépez, J., Reyna-Garfias, H., Barbosa-Cabrera, R. E., & Drago-Serrano, M. E. (2013). Stress modulates intestinal secretory immunoglobulin A. *Frontiers in Integrative Neuroscience*, *7*(68), 10.

Carlsson, H.-E., Lyberg, K., Royo, F., & Hau, J. (2007). Quantification of stress sensitive markers in single fecal samples do not accurately predict excretion of these in the pig. *Research in Veterinary Science*, *82*(3), 423–428.

Chaney, C., & Quinn, E. A. (2020). Salivary secretory immunoglobulin A variation between female varsity athletes and nonathletes. *American Journal of Human Biology*, *32*, 23405.

de la Chevrotière, C., Bambou, J.-C., Arquet, R., Jacquiet, P., & Mandonnet, N. (2012). Genetic analysis of the potential role of IgA and IgE responses against *Haemonchus contortus* in parasite resistance of Creole goats. *Veterinary Parasitology*, *186*(3–4), 337–343.

Clerc, M., Devevey, G., Fenton, A., & Pedersen, A. B. (2018). Antibodies and coinfection drive variation in nematode burdens in wild mice. *International Journal for Parasitology*, *48*(9–10), 785–792.

Clough, D., Heistermann, M., & Kappeler, P. M. (2010). Host intrinsic determinants and potential consequences of parasite infection in free-ranging red-fronted lemurs (*Eulemur fulvus rufus*). *American Journal of Physical Anthropology*, *142*(3), 441–452.

Corthésy, B. (2013). Multi-faceted functions of secretory IgA at mucosal surfaces. *Frontiers in immunology*, *4*(185), 2.

Demas, G. E., & Nelson, R. J. (2012). In Eds. Demas, G. E. & Nelson, R. J., *Ecoimmunology*. Oxford University Press.

Dhabhar, F. S. (2014). Effects of stress on immune function: The good, the bad, and the beautiful. *Immunologic Research*, *58*(2–3), 193–210.

Djune-Yemeli, L., Nana-Djeunga, H. C., Lenou-Nanga, C. G., Donfo-Azafack, C., Domche, A., Fossuo-Thotchum, F., Niamsi-Emalio, Y., Ntoumi, F., & Kamgno, J. (2020). Serious limitations of the current strategy to control Soil-Transmitted Helminths and added value of Ivermectin/Albendazole mass administration: A population-based observational study in Cameroon. *PLoS Neglected Tropical Diseases*, *14*(11), e0008794.

Edwards, K. L., Bansiddhi, P., Paris, S., Galloway, M., & Brown, J. L. (2019). The development of an immunoassay to measure immunoglobulin A in Asian elephant feces, saliva, urine and serum as a potential biomarker of well-being. *Conservation Physiology*, *7*(1), 077.

Eriksson, E., Royo, F., Lyberg, K., Carlsson, H.-E., & Hau, J. (2004). Effect of metabolic cage housing on immunoglobulin A and corticosterone excretion in faeces and urine of young male rats. *Experimental Physiology*, *89*(4), 427–433.

Escribano, D., Gutiérrez, A. M., Tecles, F., & Cerón, J. J. (2015). Changes in saliva biomarkers of stress and immunity in domestic pigs exposed to a psychosocial stressor. *Research in Veterinary Science*, *102*, 38–44.

Escribano-Avila, G., Pettorelli, N., Virgós, E., Lara-Romero, C., Lozano, J., Barja, I., Cuadra, F. S., & Puerta, M. (2013). Testing Cort-Fitness and Cort-Adaptation hypotheses in a habitat suitability gradient for roe deer. *Acta Oecologica*, *53*, 38–48.

Fair, P. A., Schaefer, A. M., Houser, D. S., Bossart, G. D., Romano, T. A., Champagne, C. D., Stott, J. L., Rice, C. D., White, N., & Reif, J. S. (2017). The environment as a driver of immune and endocrine responses in dolphins (*Tursiops truncatus*). *PLoS One*, *12*(5), e0176202.

Fendrich, C., Lüke, W., Stahl-Hennig, C., Herchenröder, O., Fuchs, D., Wachter, H., & Hunsmann, G. (1989). Urinary neopterin concentrations in rhesus monkeys after infection with simian immunodeficiency virus (SIVmac 251). *AIDS (London, England)*, *3*(5), 305–307.

Gassó, D., Feliu, C., Ferrer, D., Mentaberre, G., Casas-Díaz, E., Velarde, R., Fernández-Aguilar, X., Colom-Cadena, A., Navarro-Gonzalez, N., López-Olvera, J. R., Lavín, S., Fenández-Llario, P., Segalés, J., & Serrano, E. (2015). Uses and limitations of faecal egg count for assessing worm burden in wild boars. *Veterinary Parasitology*, *209*(1–2), 133–137.

Gesquiere, L. R., Habig, B., Hansen, C., Li, A., Freid, K., Learn, N. H., Alberts, S. C., Graham, A. L., & Archie, E. A. (2020). Noninvasive measurement of mucosal immunity in a free-ranging baboon population. *American Journal of Primatology*, *82*(2), e23093.

Gillespie, T. R., Nunn, C. L., & Leendertz, F. H. (2008). Integrative approaches to the study of primate infectious disease: Implications for biodiversity conservation and global health. *American Journal of Physical Anthropology*, *137*(S47), 53–69.

Gittleman, J. L., & Thompson, S. D. (1988). Energy allocation in mammalian reproduction. *American Zoologist*, *28*(3), 863–875.

Gleeson, M., & Cripps, A. W. (2015). Ontogeny of mucosal immunity and aging. In (Eds.) Mestecky, J., Strober, W., Russell, M. W., Kelsall, B. L., Cheroutre, H. & Lambrecht, B. N., *Mucosal Immunology*

(pp. 161–185). Academic Press. https://doi.org/10.1016/B978-0-12-415847-4.00011-2

Gupta, M., Johann-Liang, R., Bussel, J. B., Gersony, W. M., & Lehman, T. J. A. (2002). Elevated IgA and IgM anticardiolipin antibodies in acute Kawasaki disease. *Cardiology*, *97*(4), 180–182.

Gyurászová, M., Kovalčíková, A., Janšáková, K., Šebeková, K., Celec, P., & Tóthová, Ľ. (2018). Markers of oxidative stress and antioxidant status in the plasma, urine and saliva of healthy mice. *Physiological Research*, *67*, 921–934.

Hand, T. W., & Reboldi, A. (2021). Production and function of Immunoglobulin A. *Annual Review of Immunology*, *39*(1), 695–718.

Hau, J., Andersson, E., & Carlsson, H.-E. (2001). Development and validation of a sensitive ELISA for quantification of secretory IgA in rat saliva and faeces. *Laboratory Animals*, *35*(4), 301–306.

Heinrichs, A. J., Heinrichs, B. S., & Jones, C. M. (2013). Fecal and saliva IgA secretion when feeding a concentrated mannan oligosaccharide to neonatal dairy calves. *The Professional Animal Scientist*, *29*(5), 457–462.

Heistermann, M., Finke, M., & Hodges, J. K. (1995). Assessment of female reproductive status in captive-housed Hanuman langurs (*Presbytis entellus*) by measurement of urinary and fecal steroid excretion patterns. *American Journal of Primatology*, *37*(4), 275–284.

Heistermann, M., Palme, R., & Ganswindt, A. (2006). Comparison of different enzyme immunoassays for assessment of adrenocortical activity in primates based on fecal analysis. *American Journal of Primatology*, *68*(3), 257–273.

Heldstab, S. A., van Schaik, C. P., & Isler, K. (2017). Getting fat or getting help? How female mammals cope with energetic constraints on reproduction. *Frontiers in Zoology*, *14*(1), 29.

Heukelbach, J., Winter, B., Wilcke, T., Muehlen, M., Albrecht, S., Sales, F. A., de Oliveira, Kerr-Pontes, L. R. S., Liesenfeld, O., & Feldmeier, H. (2004). Selective mass treatment with ivermectin to control intestinal helminthiases and parasitic skin diseases in a severely affected population. *Bulletin of the World Health Organization*, *82*(8), 563–571.

Higham, J. P., Kraus, C., Stahl-Hennig, C., Engelhardt, A., Fuchs, D., & Heistermann, M. (2015). Evaluating noninvasive markers of nonhuman primate immune activation and inflammation. *American Journal of Physical Anthropology*, *158*(4), 673–684.

Higham, J. P., Stahl-Hennig, C., & Heistermann, M. (2020). Urinary suPAR: A non-invasive biomarker of infection and tissue inflammation for use in studies of large free-ranging mammals. *Royal Society Open Science*, *7*(2), 191825.

Huang, S., Li, L., Wu, J., Li, C., Bai, J., Sun, Y., & Wang, G. (2014). Seasonal variations in immunoreactive cortisol and fecal immunoglobulin levels in Sichuan golden monkey (*Rhinopithecus roxellana*). *Turkish Journal of Zoology*, *38*, 642–650.

Husain, N., Tokoro, K., Popov, J. M., Naides, S. J., Kwasny, M. J., & Buchman, A. L. (2013). Neopterin concentration as an index of disease activity in Crohn's disease and ulcerative colitis. *Journal of Clinical Gastroenterology*, *47*(3), 246–251.

Jarillo-Luna, A., Rivera-Aguilar, V., Garfias, H. R., Lara-Padilla, E., Kormanovsky, A., & Campos-Rodríguez, R. (2007). Effect of repeated restraint stress on the levels of intestinal IgA in mice. *Psychoneuroendocrinology*, *32*(6), 681–692.

Kang, L. J., Koleva, P. T., Field, C. J., Giesbrecht, G. F., Wine, E., Becker, A. B., Mandhane, P. J., Turvey, S. E., Subbarao, P., Sears, M. R., Scott, J. A., & Kozyrskyj, A. L. (2018). Maternal depressive symptoms linked to reduced fecal Immunoglobulin A concentrations in infants. *Brain, Behavior, and Immunity*, *68*, 123–131.

Kendall, A., Woolcock, A., Brooks, A., & Moore, G. E. (2017). Glutathione peroxidase activity, plasma total antioxidant capacity, and urinary F2- isoprostanes as markers of oxidative stress in anemic dogs. *Journal of Veterinary Internal Medicine*, *31*(6), 1700–1707.

Kophamel, S., Illing, B., Ariel, E., Difalco, M., Skerratt, L. F., Hamann, M., Ward, L. C., Méndez, D., & Munns, S. L. (2021). Importance of health assessments for conservation in noncaptive wildlife. *Conservation Biology*, *cobi*, 13724.

Kosaruk, W., Brown, J. L., Plangsangmas, T., Towiboon, P., Punyapornwithaya, V., Silva-Fletcher, A., Thitaram, C., Khonmee, J., Edwards, K. L., & Somgird, C. (2020). Effect of tourist activities on fecal and salivary glucocorticoids and immunoglobulin A in female captive Asian elephants in Thailand. *Animals: An Open Access Journal from MDPI*, *10*(10), 1928.

Krueger, K., Marr, I., Dobler, A., & Palme, R. (2019). Preservation of fecal glucocorticoid metabolites and immunoglobulin A through silica gel drying for field studies in horses. *Conservation Physiology*, *7*(1), 65.

Küster, J., & Paul, A. (1984). Female reproductive characteristics in semifree-ranging Barbary macaques (*Macaca sylvanus* l. 1758). *Folia Primatologica*, *43*(2–3), 69–83.

Langford, T. D., Housley, M. P., Boes, M., Chen, J., Kagnoff, M. F., Gillin, F. D., & Eckmann, L. (2002). Central importance of immunoglobulin A in host defense against Giardia spp. *Infection and Immunity*, *70*(1), 11–18.

Lantz, E. L., Lonsdorf, E. V., Heintz, M. R., Murray, C. M., Lipende, I., Travis, D. A., & Santymire, R. M. (2018). Non-invasive quantification of immunoglobulin A in chimpanzees (*Pan troglodytes schweinfurthii*) at Gombe National Park, Tanzania. *American Journal of Primatology*, *80*(1), e22558.

Laskowski, K. L., Moiron, M., & Niemelä, P. T. (2021). Integrating behavior in life-history theory: Allocation versus acquisition? *Trends in Ecology & Evolution*, *36*(2), 132–138.

Leeuwenburgh, C., Hansen, P. A., Holloszy, J. O., & Heinecke, J. W. (1999). Oxidized amino acids in the urine of aging rats: Potential markers for assessing oxidative stress in vivo. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *276*(1), R128–R135.

Löhrich, T., Behringer, V., Wittig, R. M., Deschner, T., & Leendertz, F. H. (2018). The use of neopterin as a noninvasive marker in monitoring diseases in wild chimpanzees. *EcoHealth*, *15*(4), 792–803.

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*(4), 353–364.

Macpherson, A. J., McCoy, K. D., Johansen, F.-E., & Brandtzaeg, P. (2008). The immune geography of IgA induction and function. *Mucosal Immunology*, *1*(1), 11–22.

Mantis, N. J., & Forbes, S. J. (2010). Secretory IgA: Arresting microbial pathogens at epithelial borders. *Immunological Investigations*, *39*(4–5), 383–406.

Mantis, N. J., Rol, N., & Corthésy, B. (2011). Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunology*, *4*(6), 603–611.

McDade, T. W., Georgiev, A. V., & Kuzawa, C. W. (2016). Trade-offs between acquired and innate immune defenses in humans. *Evolution, Medicine, and Public Health*, *2016*(1), 1–16.

McPherson, F. J. (2013). Normal blood parameters, common diseases and parasites affecting captive non-human primates. *Journal of Primatology*, *2*(2), 112.

Metcalf, C. J. E., & Graham, A. L. (2018). Schedule and magnitude of reproductive investment under immune trade-offs explains sex differences in immunity. *Nature Communications*, *9*(1), 4391.

Mulak, A., Koszewicz, M., Panek-Jeziorna, M., Koziorowska-Gawron, E., & Budrewicz, S. (2019). Fecal calprotectin as a marker of the gut immune system activation is elevated in Parkinson's disease. *Frontiers in Neuroscience*, *13*, 992.

Müller, N., Heistermann, M., Strube, C., Schülke, O., & Ostner, J. (2017). Age, but not anthelmintic treatment, is associated with urinary

neopterin levels in semi-free ranging Barbary macaques. *Scientific Reports*, *7*, 41973.

Müller-Klein, N., Heistermann, M., Strube, C., Morbach, Z. M., Lilie, N., Franz, M., Schülke, O., & Ostner, J. (2019). Physiological and social consequences of gastrointestinal nematode infection in a nonhuman primate b. *Behavioral Ecology*, *30*(2), 322–335.

Müller-Klein, N., Heistermann, M., Strube, C., Franz, M., Schülke, O., & Ostner, J. (2019). Exposure and susceptibility drive reinfection with gastrointestinal parasites in a social primate a. *Functional Ecology*, *1365-2435*, 13313.

Muneta, Y., Yoshikawa, T., Minagawa, Y., Shibahara, T., Maeda, R., & Omata, Y. (2010). Salivary IgA as a useful non-invasive marker for restraint stress in pigs. *Journal of Veterinary Medical Science*, *72*(10), 1295–1300.

Murray, C. M., Heintz, M. R., Lonsdorf, E. V., Parr, L. A., & Santymire, R. M. (2013). Validation of a field technique and characterization of fecal glucocorticoid metabolite analysis in wild chimpanzees (*Pan troglodytes*). *American Journal of Primatology*, *75*(1), 57–64.

Nielsen, M. K., Baptiste, K. E., Tolliver, S. C., Collins, S. S., & Lyons, E. T. (2010). Analysis of multiyear studies in horses in Kentucky to ascertain whether counts of eggs and larvae per gram of feces are reliable indicators of numbers of strongyles and ascarids present. *Veterinary Parasitology*, *174*(1–2), 77–84.

Nussey, D. H., Watt, K. A., Clark, A., Pilkington, J. G., Pemberton, J. M., Graham, A. L., & McNeilly, T. N. (2014). Multivariate immune defences and fitness in the wild: Complex but ecologically important associations among plasma antibodies, health and survival. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1779), 20132931.

Ottesen, E. A., & Campbell, W. (1994). Ivermectin in human medicine. *Journal of Antimicrobial Chemotherapy*, *34*(2), 195–203.

Pang, T., Leach, S. T., Katz, T., Day, A. S., & Ooi, C. Y. (2014). Fecal biomarkers of intestinal health and disease in children. *Frontiers in Pediatrics*, 2. https://doi.org/10.3389/fped.2014.00006

Paramastri, Y., Royo, F., Eberova, J., Carlsson, H.-E., Sajuthi, D., Fernstrom, A.-L., Pamungkas, J., & Hau, J. (2007). Urinary and fecal immunoglobulin A, cortisol and 11-17 dioxoandrostanes, and serum cortisol in metabolic cage housed female cynomolgus monkeys (*Macaca fascicularis*). *Journal of Medical Primatology*, *36*(6), 355–364.

Paul, A., & Thommen, D. (1984). Timing of birth, female reproductive success and infant sex ratio in semifree-ranging Barbary macaques (*Macaca sylvanus*). *Folia Primatologica*, *42*(1), 2–16.

Pernthaner, A., Cole, S.-A., Morrison, L., Green, R., Shaw, R. J., & Hein, W. R. (2006). Cytokine and antibody subclass responses in the intestinal lymph of sheep during repeated experimental infections with the nematode parasite *Trichostrongylus colubriformis*. *Veterinary Immunology and Immunopathology*, *114*(1–2), 135–148.

Pihl, L., & Hau, J. (2003). Faecal corticosterone and immunoglobulin A in young adult rats. *Laboratory Animals*, *37*(2), 166–171.

Plangsangmas, T., Brown, J. L., Thitaram, C., Silva-Fletcher, A., Edwards, K. L., Punyapornwithaya, V., Towiboon, P., & Somgird, C. (2020). Circadian rhythm of salivary immunoglobulin a and associations with cortisol as a stress biomarker in captive Asian elephants (*Elephas maximus*). *Animals: An Open Access Journal from MDPI*, *10*, 157.

Polderman, A. M., & Blotkamp, J. (1995). Oesophagostomum infections in humans. *Parasitology Today*, *11*(12), 451–456.

R Development Core Team. (2020). R: A language and environment for statistical computing. R foundation for statistical computing. http://www.R-project.org.

Rasambainarivo, F. T., & Junge, R. E. (2010). A 12-month survey of gastrointestinal helminth infections of lemurs kept in two zoos in Madagascar. *Journal of Zoo and Wildlife Medicine*, *41*(4), 638–642.

Romero, L. M., & Beattie, U. K. (2021). Common myths of glucocorticoid function in ecology and conservation. *Journal of Experimental Zoology Part A*, 2459. https://doi.org/10.1002/jez.2459

Romero-Martínez, Á., & Moya-Albiol, L. (2017). Stress-induced endocrine and immune dysfunctions in caregivers of people with eating disorders. *International Journal of Environmental Research and Public Health*, *14*, 1560.

Sapolsky, R. M. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*(1), 55–89.

Scholtens, P. A. M. J., Alliet, P., Raes, M., Alles, M. S., Kroes, H., Boehm, G., Knippels, L. M. J., Knol, J., & Vandenplas, Y. (2008). Fecal secretory Immunoglobulin A is increased in healthy infants who receive a formula with short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides. *The Journal of Nutrition*, *138*(6), 1141–1147.

Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, *11*(8), 317–321.

Singla, N., Singla, L. D., Gupta, K., & Sood, N. K. (2012). Pathological alterations in natural cases of *Capillaria hepatica* infection alone and in concurrence with *Cysticercus fasciolaris* in *Bandicota bengalensis*. *Journal of Parasitic Diseases*, *37*, 16–20.

Staley, M., Conners, M. G., Hall, K., & Miller, L. J. (2018). Linking stress and immunity: Immunoglobulin A as a non-invasive physiological biomarker in animal welfare studies. *Hormones and Behavior*, *102*, 55–68.

Svobodová, I., Chaloupková, H., Končel, R., Bartoš, L., Hradecká, L., & Jebavý, L. (2014). Cortisol and Secretory Immunoglobulin A Response to Stress in German Shepherd Dogs. *PLoS One*, *9*(3), e90820.

Taira, T., Yamaguchi, S., Takahashi, A., Okazaki, Y., Yamaguchi, A., Sakaguchi, H., & Chiji, H. (2015). Dietary polyphenols increase fecal mucin and immunoglobulin A and ameliorate the disturbance in gut microbiota caused by a high fat diet. *Journal of Clinical Biochemistry and Nutrition*, *57*(3), 212–216.

Terio, K. A., Lonsdorf, E. V., Kinsel, M. J., Raphael, J., Lipende, I., Collins, A., Li, Y., Hahn, B. H., Travis, D. A., & Gillespie, T. R. (2018). Oesophagostomiasis in non-human primates of Gombe National Park, Tanzania. *American Journal of Primatology*, *80*(1), e22572.

Thompson González, N., Otali, E., Machanda, Z., Muller, M. N., Wrangham, R., & Emery-Thompson, M. (2020). Urinary markers of oxidative stress respond to infection and late-life in wild chimpanzees. *PLoS One*, *15*(9), e0238066.

Tombak, K. J., Budischak, S. A., Hauck, S., Martinez, L. A., & Rubenstein, D. I. (2020). The non-invasive measurement of faecal immunoglobulin in African equids. *IJP: Parasites and Wildlife*, *12*, 105–112.

Tress, U., Suchodolski, J. S., Williams, D. A., & Steiner, J. M. (2006). Development of a fecal sample collection strategy for extraction and quantification of fecal immunoglobulin A in dogs. *American Journal of Veterinary Research*, *67*(10), 1756–1759.

Tsujita, S., & Morimoto, K. (1999). Secretory IgA in saliva can be a useful stress marker. *Environmental Health and Preventive Medicine*, *4*(1), 1–8.

Viena, T. D., Banks, J. B., Barbu, I. M., Schulman, A. H., & Tartar, J. L. (2012). Differential effects of mild chronic stress on cortisol and S-IgA responses to an acute stressor. *Biological Psychology*, *91*(2), 307–311.

Walsh, N. P., Gleeson, M., Shepard, R. J., Gleeson, M., Woods, J. A., Bishop, N. C., Fleshner, M., Green, C., Pedersen, B. K., Hoffman-Goetz, L., Rogers, C., Northoff, H., Abbasi, A., & Simon, P. (2011). Position statement. Part one: Immune function and exercise. *Exercise Immunology Review*, *17*, 6–63.

Watt, K. A., Nussey, D. H., Maclellan, R., Pilkington, J. G., & McNeilly, T. N. (2016). Fecal antibody levels as a noninvasive method for measuring immunity to gastrointestinal nematodes in ecological studies. *Ecology and Evolution*, *6*(1), 56–67.

Wen, L.-Y., Yan, X.-L., Sun, F.-H., Fang, Y.-Y., Yang, M.-J., & Lou, L.-J. (2008). A randomized, double-blind, multicenter clinical trial on the efficacy of ivermectin against intestinal nematode infections in China. *Acta Tropica*, *106*(3), 190–194.

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models: Estimation of Semiparametric Generalized Linear Models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *73*(1), 3–36.

Wood, S. N. (2017). Generalized additive models: An introduction with R (Second edition). CRC Press/Taylor & Francis Group.

Woof, J. M., & Kerr, M. A. (2006). The function of immunoglobulin A in immunity. *The Journal of Pathology*, *208*(2), 270–282.

Woof, J. M., & Mestecky, J. (2015). Mucosal Immunoglobulins, *Mucosal Immunology* (pp. 287–324). Elsevier. https://doi.org/10.1016/B978-0-12-415847-4.00017-3

Wu, D. F., Behringer, V., Wittig, R. M., Leendertz, F. H., & Deschner, T. (2018). Urinary neopterin levels increase and predict survival during a respiratory outbreak in wild chimpanzees (Taï National Park, Côte d'Ivoire). *Scientific Reports*, *8*(1), 13346.

Yang, C., Mogno, I., Contijoch, E. J., Borgerding, J. N., Aggarwala, V., Li, Z., Siu, S., Grasset, E. K., Helmus, D. S., Dubinsky, M. C., Mehandru, S., Cerutti, A., & Faith, J. J. (2020). Fecal IgA levels are determined by strain-level differences in *Bacteroides ovatus* and are modifiable by gut microbiota manipulation. *Cell Host & Microbe*, *27*, 467–475.

Yu, H. Q., Sun, B. Q., Fang, Z. F., Zhao, J. C., Liu, X. Y., Li, Y. M., Sun, X. Z., Liang, H. F., Zhong, B., Huang, Z. F., Zheng, P. Y., Tian, L. F., Qu, H.-Q., Liu, D. C., Wang, E. Y., Xiao, X. J., Li, S. Y., Ye, F., Guan, L., … Zhong, N. S. (2020). Distinct features of SARS-CoV-2-specific IgA response in COVID-19 patients. *European Respiratory Journal*, *56*(2), 2001526.

Zuk, M., & McKean, K. A. (1996). Sex differences in parasite infections: Patterns and processes. *International Journal for Parasitology*, *26*(10), 1009–1024.

How to cite this article: Behringer, V., Müller-Klein, N., Strube, C., Schülke, O., Heistermann, M., & Ostner, J. (2021). Responsiveness of fecal immunoglobulin A to HPA-axis activation limits its use for mucosal immunity assessment. *Am J Primatol*, 83, e23329. https://doi.org/10.1002/ajp.23329