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

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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 anthelmintic 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...
invasively 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: Löhricht et al., 2018; Wu et al., 2018), urinary markers of oxidative stress can be assessed to monitor health aspects in rodents (mice: Gyrásová 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 status 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 (Corthesy, 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 & Quinna, 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, 2004). 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; Kosarak 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: Paramástiri et al., 2007) and humans (Kang et al., 2018; Scholten 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.
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 IgA 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 IgA levels as a measure for parasite infections, and thus the value of IgA 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 IgA levels will change after deworming and change again after re-infection if IgA levels are a reliable predictor of mucosal immunity. To further evaluate the potential impact of HPA-axis activity on IgA levels, we first used an adrenocorticotropic hormone (ACTH) challenge (artificial stimulation of the HPA-axis) to confirm that IgA is responsive to the stimulation of adrenocortical activity as shown for fecal glucocorticoid metabolites (fGCM; Heistermann et al., 2006). 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 IgA measurements as a general physiological marker of parasite burden and mucosal immunity.

As IgA is responsive to acute activation of the HPA-axis (e.g., Lantz et al., 2018), we physiologically validated the measurement of IgA 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 IgA.

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 IgA 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 fIgA 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 (Djume-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 coproscopically 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 2600g 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 2000g, 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-antihelmintic 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). FgA 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. FgA 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 IGCM levels reached their maximum, fecal IgA levels had already declined.

3.2 Individual variation in fIgA levels

FgA levels were highly variable between individuals across the whole study period (Figure 2). Values ranged between 2.0 and
255.2 (µg/g feces) across females and between 8.9 and 183.0 (µg/g feces) across males. Within individual variation in flgA 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 flgA 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 flgA and fGCM levels

FlgA 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 flgA 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.
Abbreviation: Edf, effective degrees of freedom.

Woof & Mestecky, 2015), reflected nematode infection status in a group of Barbary macaques (generalized additive model, $f_{\text{IgA}}$: $R^2 = 0.25$, deviance explained = 30.5%, $N = 340$ samples; $f_{\text{GCM}}$: $R^2 = 0.46$, 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             | SE | p value | Edf | SE | 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 ($P_{\text{fIgA}} = 0.023$; $P_{\text{fGCM}} = 0.006$, Table 1, Figure 4), levels changed significantly over the study period ($P_{\text{fIgA}} = 0.019$; $P_{\text{fGCM}} = 0.005$, 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 levels increased 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; Perntaner 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 IgA 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 IgA 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 IgA responsiveness to the anthelmintic treatment: The effect of IgA level changes with parasite infection status may be blurred by the responsiveness of IgA to HPA-axis activation. Based on our simultaneous measurement of IgA 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 slgA (Campos-Rodríguez et al., 2013; Jarillo-Luna et al., 2007). For example, as in our study, ACTH administration also induced a strong IgA response in a chimpanzee that mirrored fGCM results (Lantz et al., 2018; Murray et al., 2013). Moreover, as in our study, measures of IgA 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; Viene 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,
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-Albior, 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 (Gesquière 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 (Metcalfe & 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.

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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 (equal); 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); data curation (supporting); formal analysis (supporting); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (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 (equal); formal analysis (supporting); funding acquisition (supporting); investigation (equal); methodology (supporting); resources (supporting); supervision (supporting); writing original draft (equal); writing review and editing (equal); Julia Ostner formal analysis (supporting); funding acquisition (supporting); investigation (equal); methodology (equal); 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/ALKB7H

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