Measurement of concentrations of faecal glucocorticoid metabolite in Free-Ranging African Elephants within the Kruger National Park

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MEASUREMENT OF CONCENTRATIONS OF FAECAL GLUCOCORTICOID METABOLITES IN FREE-RANGING AFRICAN ELEPHANTS WITHIN THE KRUGER NATIONAL PARK

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ABSTRACT

During the past several years, non-invasive monitoring of steroid metabolites in faeces of elephants has become an increasingly popular technique to generate more information about the causal relationship between hormones and behaviour in both living elephant species. This is important knowledge which can be used to optimise local conservation and wildlife management by finding new strategies for better elephant population management and control. In this context, however, information about an actual involvement of the hypothalamic-pituitary-adrenal axis during assumable stressful events is still limited, especially for wildlife populations. One difficulty in discovering such information is often the lack of reliable data for hormone baseline levels. Therefore, the aim of this study was to determine baseline concentrations of faecal glucocorticoid metabolites that could be expected within age classes and between seasons in African elephants (Loxodonta africana) in the Kruger National Park (KNP). A total of 374 faecal samples were collected from randomly located family herds in the southern KNP between May 2002 and August 2005. The samples were analysed for immunoreactive concentrations of faecal glucocorticoid metabolites using a validated enzyme immunoassay for 3α,11β-oxy-cortisol metabolites (3α,11β-Oxo-CM). All samples were grouped according to the estimated age class of the subject using a field method based on bolus diameter, and regarding the ecological season collected. No significant differences in faecal 3α,11β-oxy-CM concentrations were found across age classes (H1, = 7.54; p = 0.057), but the mean 3α,11β-oxy-CM concentration of samples collected in the dry season (n = 196) was significantly higher than in the wet season (n = 178) (u = 15206.50; p = 0.032), which indicates a possible physiological stress situation due to a decline in food quantity and quality. The information generated in this study represents a reliable data set for baseline concentrations of faecal glucocorticoid metabolites for elephants within the KNP and can be used to measure the stress-related effects of translocations, management actions and the impact of chosen land use activities.

Keywords: glucocorticoid metabolites, faecal, baseline, African elephant, enzyme immunoassay
Faecal glucocorticoid metabolites in African elephants

MATERIALS AND METHODS

Study area and population
The study area is located in the Kruger National Park (KNP) in South Africa. The KNP covers an area of approximately 19 000 km² and is home to a large population of African elephants. The study area is divided into several sections, including the Sabie-Sand river system, the Lowveld, and the Lebombo region. The climate is sub-Saharan, with distinct wet and dry seasons. The average annual rainfall during the study period was 527 mm, which is within the known average annual rainfall range of 500–700 mm for this region, and therefore would not have unduly influenced environmentally induced feeding stress.

Sampling
A matriarch of a family herd (median size 12) was fitted with a VHF radio collar and this group was followed on foot as and not to bias sampling close to roads. Samples were collected as soon as possible after an individual had defecated, from individuals in the collared group as well as from family herds (median size 13) located within the study area. We tried to exclude individual bias by collecting samples throughout each feeding period used by a family herd. When there were obvious signs that a herd had detected our presence, such as a definite orientation towards or away from us, no samples were collected, in order to avoid the collection of stress-induced samples. All samples were collected between 07:00 and 11:00 am. Rubber gloves were used to collect approximately 100 grams of faecal material that was then placed in a labelled plastic bag. The time lapsed between defecation and the freezing of a sample was standardised to a maximum of two hours. Each sample was marked with the date of collection, a GPS coordinate, and the estimated age class of the subject using a field method based on the bulbus diameter as described by Wimberger (2001).

Faecal samples were collected over the four ecological seasons as classified by Zambatis (2002), namely early dry (May–July); late dry (Aug–Oct); early wet (Nov–Jan) and late wet (Feb–April). This allowed us to determine, through concentrations of glucocorticoid metabolites, whether environmental factors, which change between seasons, have any possible influence on physiological stress. Samples (n = 374) were collected between May 2002 and August 2005. The average annual rainfall during the study was 527 mm, which is in the known average annual rainfall range of 500–700 mm for this region, and therefore would not have unduly influenced environmentally induced feeding stress.

Faecal extraction and hormone assays
Faecal samples were extracted according to the procedure described by Mertl et al. (2000). In brief, 0.5 g faeces plus 1 ml water and 4 ml methanol were vortexed for 30 minutes and centrifuged at 2000 g for 15 minutes. A quantity of 1 ml of the supernatant was mixed with 5 ml ethyl ether and 0.25 µl of a 5% NaHCO₃ solution, and centrifuged at 2000 g for 15 min. The aqueous phase was frozen at −20 °C overnight and then the ether was decanted and dried down under a stream of N₂. Following evaporation, the samples were reconstituted in an assay buffer and taken to assay.

Faecal extracts were measured for immunoreactive glucocorticoid metabolites using an enzyme immunoassay for 3α,11α-oxo-cortisol metabolites (3α,11α-CM) (Möstl et al. 2002), which has previously been shown to provide reliable information on adrenocortical function in the African elephant (Ganswindt et al. 2003; 2005). Briefly, 50 µl aliquots of standards, quality controls, and diluted faecal extracts were pipetted in duplicate into microtitre plate wells. A total of 100 µl of biotinylated label and antisera (raised in a rabbit against 5β-Androstane-3α-ol-11-one-17-CMO) were added and the plates incubated overnight at 4 °C. Following incubation, the plates were washed and incubated with streptavidin–peroxidase conjugate and 3,3′-diaminobenzidine tetrahydrochloride, and the plates were read at 450 nm.

In this paper we provide a quantitative baseline measure of concentrations of faecal glucocorticoid metabolites, after taking age and seasonal effects into account, in order to start establishing baseline levels for elephants in the Kruger National Park (KNP).
were washed four times and 250 μl (4.2 μM) of streptavidin horseradish peroxidase conjugate added to each well. Following incubation in the dark for 45 min at 4 °C, plates were washed again, before 250 μl (69.4 nmol) tetramethylbenzidine was added and plates further incubated (45 min; 4 °C). The reaction was terminated by adding 50 μl of 2 M H₂SO₄ and the absorbance measured at 450 nm (reference filter: 620 nm) with an automated plate reader. Sensitivities of the assays at 90 % binding were 3.0 pg/well and intra- and interassay coefficients of variation, determined by repeated measurements of high and low value quality controls ranged between 3.0 % and 12.5 %, respectively.

Statistical analysis

The age class data, as well as the data from the ecological season, was tested for normality using the Shapiro-Wilks W test. The data sets were not normally distributed and subsequently subjected to non-parametric statistical methods. An ANOVA was performed to test for the possible effect of age and season on concentrations of faecal glucocorticoid metabolite levels. The computer program STATISTICA (StatSoft, 1995) was used for all statistical analyses. All tests were two-tailed, with the level of significance set at 0.05. In cases of all pair-wise multiple comparison procedures, the α-level was adjusted by applying the procedure described by Holm (1979).

RESULTS

No significant variation in concentrations of faecal glucocorticoid metabolites ($H = 7.54, N = 374, P = 0.057$) was found across age classes (Table 1). Therefore, the age classes were combined for further analysis. Although no significant differences in concentrations of faecal glucocorticoid metabolites were found between the four ecological seasons (Table 2), there was a statistically significant difference ($u = 15206.50; p = 0.032$) between the wet season (n = 178) and dry season (n = 196), after the early and late period of both the dry and wet seasons were combined (Figure 1).

DISCUSSION

This study provides new information on baseline concentrations of faecal glucocorticoid metabolites that could be expected within age classes and between seasons in African elephants in the southern Kruger National Park. Our results show that the variability in baseline concentrations of faecal glucocorticoid metabolites ($3α,11α$-CM levels) in African elephant faeces is dependent on seasonal changes, rather than on the age class of the subject. In the present study faecal $3α,11α$-CM levels differ significantly between seasons, but no differences were found across age classes. Although the seasonal effect found seems to be rather small, future studies using methods of faecal hormone analysis to determine the effect of a potential stressor should account for seasonal effects, especially between the wet and dry season.

The fact that no significant variation in faecal $3α,11α$-CM levels was found across age classes confirm findings by Ganswindt et al. (2005), who also reported no age effects on $3α,11α$-CM levels in a group of African elephant bulls (n = 52, age range: 18–49 years) living in the Samburu and Buffalo Springs National Reserves, Kenya. In the same study, Ganswindt and colleagues described a small but clear elevation in $3α,11α$-CM levels in longitudinal hormone profiles of African elephant bulls at the end of a long dry period (Ganswindt et al. 2005). A correlation between season-dependent rainfall and adrenal endocrine function (highest concentrations of faecal glucocorticoid metabolites in the dry season) was further described for female elephants by Foley et al. (2001). The present study confirms a possible influence of a dry period on increased glucocorticoid excretion, because the mean concentration of faecal glucocorticoid metabolites of samples collected in the dry season was significantly higher than in the wet season. The elevation of concentrations of faecal glucocorticoid metabolites during the dry season could be an indication of increased physiological stress due to a decline in food quantity and quality. This was a suggestion already made by Codron et al. (2006), who reported that the percentage of nitrogen in elephant faeces from the southern KNP showed a dramatic increase from the dry to the wet season. An elevation of nitrogen in faeces is known as a useful indicator of nutritional status.

We could unfortunately not investigate differences in concentrations of faecal glucocorticoid metabolites between sexes due to safety considerations for the observers (distance away from the herd), and visual impairment caused by vegetation structure. However, further studies should examine the difference in $3α,11α$-CM levels between the sexes after intense sampling from cows and bulls within both seasons, because sex could be a co-factor for the variability in baseline concentrations of faecal glucocorticoid metabolites. Greyling (2004) found that during periods of resource limitation, males showed significantly lower levels of faecal minerals, together
with higher levels of fibre, than adult females. These results indicate that males could be maintaining diets of poorer quality than females and consequently be nutritionally more stressed than females during the dry season.

Further studies should also investigate the difference in concentrations of faecal glucocorticoid metabolites between elephants from different areas, which would finally allow a comparison of mean concentrations of faecal glucocorticoid metabolites across elephant management regions within the KNP (Whyte et al. 2005). This would aid in creating a baseline for the entire park; that would be per season, per region and between different elephant densities. Finally, this information could also be applied more universally, e.g. if a significant correlation with average monthly rainfall can be shown, whether a direct correlation exists between increasing physiological stress and vegetation damage.

CONCLUSION

A validated method (EIA) was applied in assessing the level of physiological stress in free-ranging elephant herds. The method has been shown to be practical and enables long-term monitoring of ethological or environmental factors without interfering with the result.

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