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Fecal cortisol as an indicator of stress in free-ranging and captive Asian elephants of Odisha

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Abstract
An investigation was conducted to study the stress level through non-invasive fecal cortisol analysis in free-range and captive Asian elephants in Odisha covering three seasons namely, summer (April-June), rainy (July-September) and winter (November-January). The locations in Odisha include Chandaka Wildlife Sanctuary, Bhubaneswar; Satkosia Wildlife Sanctuary, Angul and Similipal Biosphere Reserve, Mayurbhanj for free-range and Nandankanan Zoological Park, Bhubaneswar for the captive ones. The fecal cortisol concentrations (ng/ml) were estimated using ELISA from freshly collected fecal samples. The mean cortisol concentrations (ng/ml) showed no significant ($P \geq 0.05$) difference between locations, whether free-range or captive, in summer or rainy while, significant ($P \leq 0.05$) differences between locations were recorded only in winter. A comparative analysis made among seasons for each of the locations revealed the highest value in summer followed by winter and rainy, the differences being significant ($P \leq 0.05$), except that the rainy and winter values for Chandaka WLS did not show statistical significance ($P \geq 0.05$). The findings in the present study indicated the fact that it was the weather condition along with locality, and no other possible stressor, that affected the cortisol levels in the elephants, be they captive or free-ranging.

Keywords: Fecal cortisol, indicator, stress, free-ranging, captive Asian elephants of Odisha

Introduction
Asian elephants form an integral part of the culture, heritage, tradition and religion in most parts of Asia including India, and as such, regarded as the 'National heritage animal of India'. It is quite natural to believe that the gracious animal is subject to adversities like increased anthropogenic pressure, shrinking feed resources, and increased parasitic infestation- factors contributing to intimidating stress on the part of the animal. It is believed that stress could alter animal behaviour, reduce disease resistance, and affect population performance (Millspaugh and Washburn, 2004) [1]. As a physiological response to stress, activation of the hypothalamic-pituitary-adrenal axis (HPA axis) leads to an increase in glucocorticoid secretion, essential for stress adaptation (O'Connor *et al*., 2000 [2]; Mostl & Palme, 2002 [3]). The fecal glucocorticoid metabolite (FGM) assays provide a more accurate assessment of long-term glucocorticoid levels as they reflect an average level of circulating glucocorticoids over a time period, rather than a point sample (Harper and Austad, 2000) [4]. Moreover, being non-invasive, they do not disturb the animal and provide an accurate assessment of stress without the bias of capture-induced increases in glucocorticoids (Harper and Austad, 2000 [4]; Millspaugh *et al*., 2001 [5]; Touma *et al*., 2003 [6]). Seasonal variation in baseline and elevated glucocorticoid levels have been observed in many wild, free-living vertebrates (Romero, 2002) [7]. The study aims at assessing physiological stress level through non-invasive fecal cortisol estimation in free-ranging and captive Asian elephants and the influence of seasonality upon stress.

Materials and methods
Study locations and duration

The field study was carried out in Odisha, India involving Chandaka Wildlife Sanctuary (WLS), Bhubaneswar, Khordha; Satkosia Wildlife Sanctuary, Angul and Similipal Biosphere Reserve (BR), Mayurbhanj for free-ranging Asian elephants, and in the Nandankanan Zoological Park (ZP), Bhubaneswar, Khordha for captive ones. The trial was carried out covering three naturally classified seasons namely, summer (April-June), rainy (July-September) and winter (November-January).

Sample collection

Approximately 50 g of fresh faeces was collected from each captive elephant shortly after the animal had defecated. In case of the wild elephants, fresh (<12 h since defecation) fecal samples were collected as it was not always possible to get fresh faeces. Using a disposable rubber glove, a sample from the middle of the bolus was removed to avoid cross-contamination with urine or contamination with other fecal samples in the area. Fecal samples were collected in plastic zip-lock bags by mixing of 2-3 central portions of the voided materials of each elephant (Vimalraj and Jayathangaraj, 2012)[8] and were immediately placed on ice inside the icebox. The fecal samples were frozen at -20°C within one hour of collection and stored until analysis.

Preparation of samples and estimation

Frozen samples were thawed at room temperature before vortexing extraction for extraction of glucocorticoid in fecal samples as per the procedure by Wasser *et al*. (2000) [9]. A quantity of 0.6 g well-mixed wet faeces was placed in a capped tube containing 2 ml 90% methanol. The tubes were vortexed for 30 minutes for proper mixing of the sample in methanol using BR-2000 Vortexer (BIO-RAD). This was followed by careful centrifugation of the tubes at 2500 rpm for 25 minutes. The supernatant material was then collected in 2 ml micro-centrifuge tubes and stored at -30 °C for further analysis. The concentration of cortisol in fecal extracts was estimated using ELISA kit specific to cortisol (Sigma-Aldrich Cortisol ELISA, catalog no. SE120037). The procedure followed for ELISA (Enzyme Linked Immuno-Sorbent Assay) was as specified by the kit and after reading the concentrations were expressed in ng/ ml.

Temperature-Humidity Index (THI)

The meteorological data for the study locations were obtained from the Government of India Meteorological Centre, Bhubaneswar and the THI calculated is presented in Table 1. The temperature-humidity index (THI) was calculated from ambient temperature (Ta in °F) and Relative Humidity (RH in %) using the Buffington formula (LPHSI, 1990) [10]:

$$\text{THI} = \text{Ta}\ (°\text{F}) - [(0.55 - 0.0055 \times \text{RH}) \times (\text{Ta} - 58)]$$

Statistical analysis

The data were subjected to analysis as per the methods suggested by Snedecor and Cochran (1998) [11].

Results and Discussion

The fecal cortisol concentrations (ng/ml) for the free-ranging and captive Asian elephants in different seasons and locations are presented in Table 2. Fecal cortisol concentrations (ng/ml) showed no significant ($P \geq 0.05$) difference between locations, whether free-range or captive, in summer or rainy. Significant ($P \leq 0.05$) differences between locations were recorded only in winter, in that, Satkosia WLS and Similipal BR had similar ($P \geq 0.05$) concentrations, which were significantly ($P \leq 0.05$) higher than that for Chandaka WLS or Nandankanan ZP, the latter two being similar ($P \geq 0.05$). This could probably be due to the colder ambient temperatures in Similipal BR and Satkosia WLS leading to cold stress in the elephants. The comparable mean values between Satkosia and Similipal as observed could be ascribed to the similarity in the average weather conditions between the locations. Differences in agro-climatic conditions have been shown to influence cortisol levels between sanctuaries (Vimalraj and Jayathangaraj, 2012)[8].

Comparing between the free-ranging and captive elephants, it was found that, while no significant ($P \geq 0.05$) difference was found in summer or rainy, significant ($P \leq 0.05$) differences were noticed in winter. No significant ($P \geq 0.05$) difference was observed between Chandaka WLS and Nandankanan ZP, while each of these locations showed a significantly ($P \leq 0.05$) lower concentration than Satkosia WLS or Similipal BR, in winter. There was no difference between the captive elephants at Nandankanan ZP and free-ranging elephants at Chandaka WLS, which could be due to the fact that both the locations are situated in the same bio-geographical region.

A comparison was made between the seasons for each of the free-ranging and captive locations. For each of the locations, the highest value was recorded in summer followed by winter and rainy, the differences being significant ($P \leq 0.05$), except that the rainy and winter values for Chandaka WLS did not show statistical significance ($P \geq 0.05$). The highest value in summer could be attributed to the higher THI of the locations in summer during the period of study causing heat stress. Fowler and Mikota (2006) [12] reported increase in both hydric and nutritional stress during drought, triggering clinical disease in free-ranging hosts by compromising host immunity. Rainy season revealed the lowest stress in terms of low cortisol level probably due to abundance of food and water during this part of the year along with lower THI compared to summer months.

The cortisol levels were found higher in winter than rainy in each of the study locations, implying that winter season was more stressful than rainy. A higher cosrtisol level in winter might be attributed to cold stress coupled with nutrient deficiency in the dry season. Studies have documented elevated glucocorticoid levels in wildlife species (Millspaugh *et al*., 2001[5]; Yousef *et al*., 1971[13]; Huber *et al*., 2003[14]). Besides cold stress, nutrient deficiency in the dry season could be another reason for the elevated cortisol level. Rangel-Negrin *et al*. (2009)[15] viewed that seasonal variation in fecal cortisol level between seasons in the herbivores could be due to variations in food availability. Codron *et al*. (2006) [16] observed that, fecal nitrogen, as an indicator of nutritional status, increased from dry to wet season in African elephants, implying seasonal changes in nutritional quality. Nutrient deficiency during dry season leading to elevated glucocorticoid levels in wild animals had been reported by several workers (Harvey *et al*., 1984 [17]; Vimalraj and Jayathangaraj, 2012 [8], DelGiudice *et al*., 1992 [18]; Saltz and White, 1991 [19]).

Conclusion

The stress level estimated in the present study tended to be lower in the captive compared to the free-ranging elephants. Moreover, the stress level was the highest in summer and lowest in rainyseason revealing a major influence of seasonality on stress. The findings in the present study indicated the fact that it was the weather condition along with locality, and no other possible stressor, that affected the cortisol levels in the elephants, be they captive or free-ranging. These findings may help for taking steps towards better stress management in Asianelephants andas baseline data in taking up future studies in this regard.

Conflict of interests: The author declares that there is no conflict of interest regarding the publication of this paper.

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Table 1: Temperature-Humidity Index

Season	Month	Fortnight	Bhubaneswar (Chandaka and Nandankanan)	Angul (Satkosia)	Mayurbhanj (Similipal)
Rainy	July	I	94.66	92.70	93.79
		II	91.65	91.58	90.76
	August	I	91.84	92.23	90.98
		II	92.69	91.72	92.81
	September	I	92.46	91.73	93.48
		II	91.69	92.18	90.79
Winter	November	I	66.49	59.81	62.09
		II	62.62	57.31	60.32
	December	I	59.97	53.86	59.96
		II	55.20	50.42	51.27
	January	I	57.03	50.44	53.65
		II	55.70	47.80	49.29
Summer	April	I	103.39	97.19	96.00
		II	103.12	97.36	97.75
	May	I	99.87	97.81	99.06
		II	98.57	100.4	96.13
	June	I	98.73	97.07	98.47
		II	94.90	94.28	92.74

Table 2: Mean fecal cortisol concentrations (ng/ml) in free range and captive elephants

Season	Free range			Captive	P value
	Chandaka	Satkosia	Similipal	Nandankanan	
Summer	1147.27^{A} ± 40.16	1255.91^{A} ± 34.70	1234.83^{A} ± 26.55	1169.96^{A} ± 45.06	> 0.05
Rainy	437.31^{BC} ± 44.68	420.77^{C} ± 39.31	312.08^{C} ± 26.56	360.83^{C} ± 60.40	> 0.05
Winter	555.30^{bB} ± 41.65	831.91^{aB} ± 41.12	835.00^{aB} ± 43.32	593.43^{bB} ± 37.72	< 0.01
Overall	713.29 ± 78.74	836.20 ± 85.29	793.97 ± 93.38	708.07 ± 86.61	> 0.05
P value	< 0.01	< 0.01	< 0.01	< 0.01	

^{a,b} Means along rows differ significantly ($P \leq 0.05$)
$^{A, B, C}$ Means along columns differ significantly ($P \leq 0.05$)

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