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Assessment of faecal cortisol in captive elephants of Tamil Nadu state: An indicator of stress

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Abstract
A study was undertaken to assess the faecal cortisol levels in captive elephants of Tamil Nadu state. Fresh dung samples from 25 temple elephants, 7 elephants in mudumalai camp and 18 elephants in Anamalai camp were collected in 80% methanol added air tight containers. The overtaxing extraction method was used for extraction of glucocorticoids from the fecal samples. Subsequent to extraction process, cortisol was estimated by Enzyme Linked Immunosorbent Assay (ELISA) technique. Fecal cortisol concentration in temple elephants ranged from 195 ng/g to 550 ng/g with a mean fecal cortisol concentration of 392±24.20 ng/g. Similarly, the faecal cortisol concentration in camp elephants of Mudumalai ranged from 125 ng/g to 300 ng/g with a mean fecal cortisol concentration of 169.57±22.48 ng/g and in Anamalai camp, the fecal cortisol values ranged from 140 ng/g to 196 ng/g with a mean fecal cortisol concentration of 159.11±3.93 ng/g. Faecal cortisol can be used to indirectly assess stress in captive and wild animals, including elephants. The difference in faecal cortisol levels can be attributed to the different managemental practices followed in the captive elephants.

Keywords: Captive elephants, faecal cortisol, stress, management

Introduction
Asian elephants are regarded as a flagship species, and in India, myth and culture is strongly entwined with them. In addition to a sizable population of wild elephants, India also houses a sizable number of elephants in captivity. Elephants are migratory animals, and they spread out over several hundred square kilometres in search of new foraging sites. But under captivity, the whole situation is different. They become accustomed to living in small spaces and are typically reclusive, especially in temples.

Most captive elephants in Tamil Nadu are kept in temples. The way that animals react to being kept in captivity varies greatly. Elephants' health status may occasionally be impacted by factors that cause long-term stress. The welfare of elephants is always impacted by stress, which is why animal welfare-related measures are currently seen as important (Tarlow and Blumstein, 2007) [1]. The absence of stress may be a sign of animal welfare. The stress response involves a number of hormones, including prolactin, glucocorticoids, catecholamines, and ACTH. When things get stressful, either cortisol or corticosterone is released (Schwarzenberger, 2007) [2]. They may be utilized as a non-invasive method to measure elephant stress since they can act as an index of the stress response.

Applications for non-invasive physiological stress measurements in conservation biology, wildlife management, animal husbandry, behavioural ecology, and biomedicine are numerous (Touma and Palme, 2005) [3]. Faecal cortisol is usually determined in order to indirectly assess stress in captive and wild animals, including elephants. In this study, the amount of faecal cortisol in Tamil Nadu camp elephants and temple elephants was measured.

Materials and Methods
The captive elephants comprised of temple elephants and elephants of Arignar Anna Zoological Park. Fresh dung samples were collected from 25 elephants of various temples, seven elephants of Mudumalai camp and 18 elephants of Anamalai camp in 80% methanol added air tight containers. The vortexing extraction method was used for extraction of glucocorticoids from the fecal samples (Wasser *et al*. 2000) [4]. Well-mixed wet feces (0.6 g) was placed in a capped tube, containing 2.00 ml of 90% methanol, vortexed for 30 min and then the tubes were carefully centrifuged for 20 min at 2500 rpm. The supernatant material was diluted in phosphate buffer saline and stored at -80 $^{\circ}$C for subsequent use. Subsequent to extraction process, cortisol was estimated by Enzyme Linked Immunosorbent Assay (ELISA) technique.

ELISA Kit specific to cortisol (UBI Magiwell Enzyme Immunoassay Cortisol Catalog No. SH 101) was used for analysis. With the help of ELISA reader the absorbance values of standards and samples were analyzed and the cortisol concentrations of the samples were obtained by plotting them on the logit-log paper. In addition, the details on feeds offered, management practices like deworming, vaccination and exercise were also recovered. The statistical analysis of the data was carried out using one way ANOVA, as per the standard procedures.

Results and Discussion

Fecal cortisol concentration values in 25 temple elephants ranged from 195 ng/g to 550 ng/g and the mean fecal cortisol concentration in temple elephants was 392±24.20 ng/g (Table 1). The faecal cortisol concentration in camp elephants of Mudumalai ranged from 125 ng/g to 300 ng/g with a mean fecal cortisol concentration of 169.57±22.48 ng/g (Table 2). Similarly, in Anamalai camp the fecal cortisol values ranged from 140 ng/g to 196 ng/g with a mean fecal cortisol concentration of 159.11±3.93 ng/g (Table 2). Statistical analysis revealed significant variations between the temple elephants and the camp elephants (Table 3).

Some of the causes of the apparent variations in the fecal cortisol of temple elephants include separation from the group or the herd with different feeding and husbandry practices implemented, lack of necessary housing space, increased noise, distance between the elephants and visitors, etc. This was supported by the findings of Morgan and Tromborg (2007) [5], stating the various sources of stress in captive animals were artificial lighting, exposure to aversive sounds, arousing odours, restricted movements, forced proximity to humans. Similarly Li *et al.* (2007) [7] opined that small living space might impose physiological stress. Laws *et al.* (2007) [6] observed a significant increase in fecal cortisol after transportation. However, it should be noted that at the time of collection during this study period there was no history of elephant transportation recorded and the increase in the fecal cortisol level could be attributed to other causes.

Among the temple elephants (Table 1) it was found that the elephant from the Srirangam temple had the lowest cortisol level, whereas the elephant from Tiruverkadu temple had the highest fecal cortisol level. In this study, it was noted that among all the temple elephants that were studied, only the Srirangam temple elephant had two big well maintained enclosures. One enclosure with mud flooring was exclusively used for the elephant to rest during nights and the other enclosure was used for the day time. The mahout was also staying within this enclosure. An overall better management of this captive elephant is probably one of the reasons for its low fecal cortisol level.

On the other hand, the elephant from Tiruverkadu, on perusal of history from the temple authorities, it was observed that it had an accident involving the left for leg. Though it recovered, there was restricted movement resulting in more stereotypic behaviour, probably resulting in increased fecal cortisol level. However, more research is needed in also studying the management practices, the bonding effect of mahout and the captive temple elephants etc.

When compared with the temple elephants, the elephants of camps (Mudumalai camp and Anamalai camp) had lower levels of fecal cortisol concentration. This might be attributed to better maintenance, absence of stress causing factors which are highly variable in feature, large enclosure space, quality food, less proximity to public, provision for wallowing, water ponds, social enrichment etc, which was in agreement with the findings of Varma *et al.* (2008) [8].

Table 1: Fecal cortisol values of temple elephants

S. No	Temples under study	Faecal Cortisol ng/g
1.	Srirangam	195
2.	Alwarthirunagari	200
3.	Mayiladuthurai	202
4.	Tiruchendur Elephant I	205
5.	Tirukurungudi Elephant II	208
6.	Mannargudi	224
7.	Kumbakonam	320
8.	Tirunelveli	320
9.	Erraitaithirupathi	400
10.	Thiruparakundram	402
11.	Sriperumbudur	409
12.	Rameswaram elephant I	420
13.	Thirukolur	430
14.	Rameswaram elephant II	430
15.	Tiruchendur elephant II	440
16.	Thiruvidaimarudur	450
17.	Palani	460
18.	Selayur	460
19.	Madurai	490
20.	Alagarkovil	500
21.	Thirupananthal	515
22.	Trichy	520
23.	Uppiliappankovil	520
24.	Thirukurungudi elephant I	530
25.	Tiruverkadu	550
	Mean faecal cortisol value	392±24.20

Table 2: Faecal cortisol level in camp elephants

S. No	Mudumalai camp	Anamalai camp
1.	125	140
2.	139	140
3.	140	140
4.	150	141
5.	163	141
6.	170	150
7.	300	151
8.		160
9.		160
10.		163
11.		164
12.		164
13.		164
14.		165
15.		165
16.		165
17.		195
18.		196
Mean faecal cortisol value	169.57±22.48	159.11±3.93

Table 3: Mean faecal cortisol level in captive elephants of different regions

Places under study	Faecal cortisol level ng/g	F-value
Temples (N = 25)	392.00±24.20^{b}	35.485**
Anamalai camp (N = 18)	159.11±3.93^{a}	
Mudumalai camp (N = 7)	169.57±22.48^{a}	

Means bearing different superscripts in a parameter differ significantly

** Highly significant (p<0.01)

Table 4: Miscellaneous factors

Features	Camp elephants	Temple elephants
Feeding	• Ragi (10-16 kg) • Cooked rice (2-8 kg) • Horse gram (2-4 kg) • Jiggery (100g) • Salt + mineral mixture (150 + 100 g) • Adlibitum feeding of forest vegetation	• Cooked rice (3-7 kg) • Ragi (2-10kg) only in few temples • Horse gram or Bengal gram/green gram (1 -2 kg) • Jaggery (50 g) • Salt + Mineral mixture (50 + 50 g) • Green grass 100-150 kg • Tree fodder (coconut leaves, banana stem) • Fruits and vegetables
Exercise	+++ 8 – 10 km daily	++ 4 – 6 km daily
Deworming	Regularly carried out	Regularly carried out
Vaccination	Vaccinated against anthrax	Vaccinated against anthrax. In two temples extra FMD also done

Conclusion

In temple elephants lack of exercise was a feature observed since they comparatively walked for a shorter distance (Table 4). Husbandry practices recorded in captive elephants revealed less amount of exercise in terms of walking in temple elephants. Also most of the elephants were kept tied for a long time in solitary confinement. Lack of adequate enclosure size, modified feeding practices, absence of natural surroundings, and forced proximity to humans were observed during the study. Such activities could indirectly act as some of the causal factors for the highly significant rise in fecal cortisol of temple elephants. Some of the temple elephants in this study exhibited stereotypic behavior. This could be attributed to increase in serum cortisol levels.

In general, it is noteworthy to mention that the elevations in fecal glucocorticoids might reasonably be regarded as indicative of physiological stress response (Wasser *et al.* 2000) [4] as also reported by Laws *et al.* (2007) [6] that fecal cortisol metabolites could be a safe, practical and welfare indicator of adrenal activity in Asian elephants. Hence, assessment of fecal cortisol level as done in this study programme is useful for wide variety of conservation, management and biomedical investigations.

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