

Faecal Cortisol Levels in Captive Asian Elephants: Associations with Facility Features, Physical Attributes and Foot Disorders

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ABSTRACT

Captive Asian elephants (*Elephas maximus*) are often subjected to chronic stress due to restricted movement, limited exercise, monotonous routines and confinement, all of which can compromise their health and welfare. Assessing stress through non-invasive biomarkers such as faecal cortisol provides valuable insights into their physiological well-being under different management systems. This study evaluated faecal cortisol concentrations in 46 captive elephants maintained under various management systems across Tamil Nadu, India. Physical parameters, body condition scores, housing facilities and foot disorders were recorded. Foot disorders were classified into nail, cuticle, sole, pad lesions and structural deformities. Faecal cortisol concentrations were estimated using High-Performance Liquid Chromatography. The mean faecal cortisol level was 243.96 ± 17.08 ng/g, ranging from 85.14 to 553.24 ng/g. Cortisol concentrations varied significantly between locations, with elephants from Tirunelveli showing higher levels (317.91 ± 50.0 ng/g) compared to those from Trichy and Thanjavur (193.79 ± 34.78 ng/g). A significant negative correlation was observed between faecal cortisol and body condition score, while no correlation was found with age or body weight. Elephants with structural deformities exhibited significantly higher cortisol concentrations (413.33 ± 69.80 ng/g; $p < 0.01$) than those without. The findings suggest that chronic foot disorders, suboptimal housing and inadequate management practices contribute to elevated stress levels in captive elephants. Regular monitoring of faecal cortisol, combined with improved husbandry and foot care, can enhance welfare and inform targeted management strategies for captive elephant conservation in India.

Key words: Captive Asian elephants, Faecal cortisol, Foot disorders, Stress.

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INTRODUCTION

The Asian elephant (*Elephas maximus*) is the largest land mammals in Asia, ranges from India eastward through Southeast Asia and are found in 13 countries. The species is primarily threatened by habitat destruction, poaching and fragmentation and the population continues to decline, with only 40,000-50,000 estimated to remain in worldwide (Choudhury *et al.*, 2008). Elephants have adapted to a wide variety of environments in captive conditions. The health status of captive elephants depends on various factors including body mass index, husbandry management, nutrition and infectious and non-infectious diseases (Sadler, 2001). Studies on captive elephant health and diseases are far less and hence evolving remedial measures to promote health and welfare remains challenging one. One of the most important objectives in captive condition is maintaining psychological and physiological wellbeing of elephants. Changes in environmental, social, physical, physiological and psychological factors in captive conditions can modify the homeostasis of individual elephants and lead to a range of health issues that could contribute to significant morbidity and mortality in elephants.

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Many studies have revealed the stressful situation triggered in captive elephants by several stimuli including food quality and quantity, transportation, housing facilities and prolonged health issues like unhealed wound, foot diseases and disorders (Viljoen *et al.*, 2008). In captive situation, elephants are subjected to chronic stress due to monotonous routines, a lack of interaction and being confined to small areas and adverse climatic changes (Mumby *et al.*, 2015). The stress if persist for a prolonged period, the animal loses its adaptive value and adversely impacts the health and longevity of the species by impairing endocrine and immune functions, degrading body mass and causing reproductive failure (Kumar *et al.*, 2019). A prolonged high concentration of cortisol level during chronic stress may have deleterious effect on bone formation and accelerate bone loss (Marcilla *et al.*, 2012), this may decrease the individual fitness by impairing the immune and endocrine functions (Pokharel *et al.*, 2017; Kumar *et al.*, 2019). Faecal glucocorticoid metabolites are used as a non-invasive approach to measuring the cortisol as a marker of stress and health of elephants (Mumby *et al.*, 2015). The present study reports the cortisol level of captive elephants in Tamil Nadu, India and to suggest measures that can strengthen the management of health and welfare of captive elephants.

MATERIALS AND METHODS

The study was carried out in 46 captive elephants maintained in different parts of Tamil Nadu (India). Out of this, 24 captive Asian elephants were assessed during a health and welfare campaign for Tamil Nadu temple elephants. Remaining 22 captive elephants were privately maintained elephants and were assessed during their periodical health and veterinary examinations. The age of elephants in this study ranged from 11 years to 62 years. All these captive animals were under captive feeding programme and fed with green grass, coconut leaves, seasonal fruits and vegetables, sugar cane and food bolus mixed with ragi, horse gram, rice, jaggery and salt.

Study on Facility Features, Physical Attributes and Foot Disorders

Body condition score system was designed based on the direct visual observation of physical characters of elephants. This was modified from the methods developed by Wijeyamohan *et al.* (2014) and Morfeld *et al.* (2016). Ten characters of body

region, *viz.*, head temporal depression, scapula (shoulder blade), thoracic region, flank area immediately in front of pelvis, lumbar vertebrae just behind ribs and in front of pelvis, pelvic bone (external angle of the ilium) and rump, axillary fat (immediately behind joint of humerus and scapula), brisket fat (between forelegs at base of neck), tail and skin were chosen and measured for ranking. The body condition score with a simple numeric scale of BCS=1 to BCS=5 was used (Morfeld *et al.*, 2016). This 1 to 5 score index represents that the BCS=1-2 equates to "underweight/thin", BCS = 3 to "Ideal/normal" and BCS = 4-5 to "overweight/ obese".

Data included basic information about animals such as elephant age, sex, feeding, watering and exercise (daily walking distance) and information about the health influencing factors such as housing (indoor and outdoor space, floor, roof), time spent in indoor area and tethering; animal keeping conditions were collected. The housing facilities and physical factors such as age, body weight, body condition score were also correlated with stress markers.

All the elephants were also clinically assessed for foot disorders. Out of 46 elephants investigated, 80.4% revealed the presence of different types of foot lesions and 19.6% elephants did not have any lesions, *i.e.*, elephants with and without foot disorders. After clinical examination, based on the lesions present in the foot, the elephants were grouped into three categories: those with nail disorders, those with cuticle disorders and those with sole and pad disorders. The elephants with joint or angular deformities along with associated lameness or changes in gait were categorized into a group as elephants with structural deformities of the limb. The classification of foot disorders is depicted in Table 1. The various type of foot disorders was correlated with stress markers.

Faecal Sample collection and Faecal Extraction

Fresh dung samples (0.5 gm) from 46 elephants were collected in air tight containers containing 5 mL of 80% methanol and were shifted to laboratory for further extraction on the next day itself to avoid variations. The faecal extraction was carried out according to Nishanth *et al.* (2013); 0.5 gm of methanol stored dung samples were vortexed for 30 min and then the samples were centrifuged at 1500 x g for 15 min. After centrifugation, a quantity of 1 mL of supernatant was mixed with phosphate buffer saline and stored at -21°C until further process.

Table 1: Classification of foot disorders in elephants based on the lesions presented

Nail disorders	Cuticle disorders	Sole and pad disorders
<ul style="list-style-type: none"> • Cracked nail • Split nail • Overgrown nail • Ingrown nail • Infected toe nail • Abscess in split nail • Crowding of nail 	<ul style="list-style-type: none"> • Cuticle feathering • Overgrown cuticle • Fluid pocket behind the cuticular growth • Hangnails (cuticular splitting) • Abscess in inter-digital area • Cuticular erosions 	<ul style="list-style-type: none"> • Deep fissures in a pad • Difference in shades of pad tissue • Cracked heel • Excessive sole growth with debris • Cracked sole

Estimation of Faecal Cortisol

Faecal extracts were measured for cortisol metabolites by High Performance Liquid Chromatography (HPLC) according to the procedure described by Ganswindt *et al.* (2003) and Veeraselvam *et al.* (2021) with some modifications. Cortisol (Cerilliant®) obtained from the Merck was used as standard solution. Standard stock solution was availed with a concentration of 1 mg/mL methanol. From the stock solution, the concentration of 10 µg, 5 µg, 2.5 µg, 1 µg, 0.5 µg, 0.25 µg, 0.125 µg, 0.1 µg, 0.05 µg and 0.025 µg were prepared to setup a standard calibration; 10 µL of these concentrations was injected into HPLC and the standard calibration curve for cortisol was obtained by plotting concentrations verses mean of the peak areas obtained for their respective standard.

The obtained faecal extract was directly passed through the HPLC millipore filter (0.45 µ) vials. The separation of cortisol metabolites was carried out using a C-18 column (particle size 5 mm x Length 150 mm); the flow rate 1.0 mL/min was maintained at ambient temperature. A mixture of 70% methanol and 30% water was utilized a mobile phase. The obtained peaks were plotted on the standard HPLC curve and multiplied by the dilution factor to quantify the cortisol concentration.

Statistical Analysis

The data were pooled and taken for the assessment. The housing facilities were taken for correlating with stress level of elephants. The physical parameters such as age, body weight, body condition score and foot disorders were correlated with stress markers. One way ANOVA was also used to examine the faecal cortisol concentration in relation to the age group, facility available and foot disorders. Kruskal-Wallis's test (K-W test) was used for comparing the body condition score of individual elephants with age, housing facilities, feeding pattern of elephants. Pearson correlation (*r*) was used to test the relationship between faecal cortisol and body condition score (Kumar *et al.* 2019). All statistical analyses were carried out using IBM® SPSS® ver.23.0 for windows.

RESULTS AND DISCUSSION

Captive elephants, in particular, are more prone to chronic stress due to restricted movement, limited exercise and reduced behavioural and feeding opportunities (Morgan and Tromborg, 2007; Bansiddhi *et al.*, 2019). In elephants, faecal cortisol levels are widely used for the assessment of stress, as elevated glucocorticoid concentrations have been

reported in stressful situations (Bahr *et al.*, 2000; Issac *et al.*, 2017). Cortisol can be measured in plasma, saliva, faeces, and urine samples (Salaberger *et al.*, 2016). Among these, faecal cortisol metabolite measurement is a well-established non-invasive biomarker of stress and an indicator of animal health (Hadinger *et al.*, 2015). This method has been successfully applied to quantify stress in both captive and free-ranging elephants (Ganswindt *et al.*, 2003; Kumar *et al.*, 2014).

Faecal Cortisol Concentrations of Elephants in Different Locations and Facilities

Faecal cortisol concentration was assessed in 46 elephants. The overall mean ± SE value of faecal cortisol concentration was 243.96±17.08 ng/g and it ranged between 85.14 ng/g to 553.24 ng/g. The elephants located in Trichy and Thanjavur had the low concentration of 193.79 ± 34.78 ng/g and elephants from Tirunelveli had a higher concentration of 317.91 ± 50.0 ng/g (Table 2). These levels were in agreement with the findings of 195 to 550 ng/g recorded in 25 temple elephants by Nishanth *et al.* (2013). The elevated cortisol levels in elephants from the Tirunelveli region may be attributed to the fact that most of these elephants were kept in open areas under trees without proper housing facilities, were chained by the legs and around the body and were highly exposed to human disturbances. This observation is further supported by Kumar *et al.* (2014), who reported significant differences between housing facilities and the body condition of individual animals. Elevated faecal cortisol levels in captive elephants may result from inadequate enclosure space, exposure to loud sounds, disturbances caused by visitors, varying husbandry practices, and unusual behaviours arising from mishandling by mahouts. Similar observations were made by Young *et al.* (2004) and Morgan and Tromborg (2007), who attributed stress in captive elephants to restricted movement, aversive sounds, artificial lighting, translocation, and exposure to unfamiliar surroundings. Furthermore, Hadinger *et al.* (2015) reported that inappropriate captive environments, poor diet quality and human disturbances adversely affect the physiology of animals. Cortisol release, in general, is a normal physiological response to stressors in mammals, including Asian elephants (Mumby *et al.*, 2015).

The mean faecal cortisol concentrations of elephants 23, 18, and 5 maintained in temple, private and rehabilitation centers were 231.71±19.40 ng/g, 275.12±32.61 ng/g and 188.67±45.60 ng/g, respectively, but no significant differences (*f* =1.408; *p* value = 0.253) were observed

Table 2: Faecal cortisol concentration of elephants in different locations

Location	Number of elephants	Faecal cortisol level (ng/g)
Elephant rehabilitation camp, Tekkampatty	24	231.72±19.00
MR Palayam elephant rehabilitation centre	4	232.85±51.7
Private		
Tirunelveli	10	317.91±50.0
Trichy & Thanjavur	8	193.79±34.78



between them. Most temple elephants were housed in well-maintained enclosures with mud flooring and received regular feeding, unlike private elephants, which lacked such organized management practices. These differences may explain the lower mean faecal cortisol concentrations observed in temple elephants.

A significant increase in faecal cortisol levels has been observed following translocation and transportation (Young *et al.* 2004; Laws *et al.*, 2007). In the present study, there was no history of elephant transportation. Therefore, the increases observed in faecal cortisol levels could be attributed to other factors. Dantzer *et al.* (2014) reported that glucocorticoid responses in animals can be influenced by numerous factors, including individual factors (metabolic rate, gut microbes, and parasites), social factors (group size), physiological factors (reproductive and stress status), and ecological factors (adverse climatic conditions and food availability). Captive elephants, especially those in solitary conditions, may experience certain stress levels, which could have contributed to the elevated cortisol levels observed in this study.

In the present study, most of the temple elephants were made to stand in the temple premises for devotees and visitors to receive blessings, and they were housed in small enclosures. These factors could have contributed to elevated stress hormone levels. This is in agreement with Morgan and Tromborg (2007), who reported that captive elephants experience higher stress due to monotonous routines, limited interactions and confinement to small areas. Captive elephants have also been found to be more prone to chronic stress because of restricted movement, lack of exercise, and limited behavioural and feeding opportunities (Bansiddhi *et al.*, 2019)

Faecal Cortisol Concentration in Relation to Physical Parameters and Age Groups

Faecal cortisol showed a negative correlation with age, body weight, and body condition score of the study animals (Table 3). A highly significant difference ($p < 0.01$) was observed between faecal cortisol and body condition score.

Table 3: Faecal cortisol concentration of elephants in relation to physical parameters

Physical characters	No.	'r' value	p value
Faecal cortisol Vs Age	46	- 0.078	0.605
Faecal cortisol Vs Body Wt.	46	-0.032	0.834
Faecal cortisol Vs BCS	46	-0.439	0.002**

Faecal cortisol concentration in elephants had no significant difference (p value = 0.474) among the three age groups, viz., 10 to 25 years ($n=9$), 26 to 40 years ($n=20$) and >40 year ($n=17$). The corresponding mean values of faecal cortisol recorded were 284.03 ± 32.1 , 241.97 ± 26.4 and 225 ± 29.8 ng/g. These results are consistent with the findings of Pokharel *et al.* (2017), who reported significantly higher faecal glucocorticoid metabolite (fGCM) concentrations in elephants with lower BCS, suggesting that poor body condition can elevate physiological stress levels in these animals. Similarly, Seltmann *et al.* (2020) demonstrated both seasonal and age-dependent variations in BCS as reflected by faecal glucocorticoid profiles.

Faecal Cortisol in Relation to Foot Disorders

The overall mean faecal cortisol concentrations did not differ significantly (f value = 0.453; $p=0.504$) between the groups of elephants with ($n=37$) and without ($n=9$) foot disorders (251.83 ± 19.8 Vs. 223.77 ± 78.71 ng/g). However, the faecal cortisol concentrations varied among different groups of elephants based on the type of foot disorders. Elephants with structural deformities in foot were found to have the higher cortisol level of 413.33 ± 69.80 ng/g. The faecal cortisol concentration was found to be lower in elephants with nail disorders (158.24 ± 25.41 ng/g). Values in elephants with nail disorders, sole and pad disorders, cuticular disorders and mixed disorders did not differ significantly when compared with the elephants without disorders, except the groups of elephants which had structural deformities showed highly significant ($p < 0.01$) difference (Table 4). Foot affections may persist over long periods and contribute to stress in elephants. Elephants kept in captive conditions were found to have an increased probability of developing foot problems

Table 4: Faecal cortisol in relation with various foot disorders in Asian elephants

Groups	Number of elephants	Faecal cortisol ($\mu\text{g/g}$) Mean \pm SD	F-value	P value
Elephants without foot disorders	9	223.77 ± 26.23	0.944	0.010
Elephants with nail disorders	8	158.24 ± 25.41		
Elephants with sole and pad disorders	6	196.56 ± 48.03		
Elephants with cuticular disorders	7	287.41 ± 28.50		
Elephants with structural deformities disorders	3	$413.33 \pm 69.80^{**}$		
Elephants with mixed disorders	13	270.35 ± 35.87		

**Highly significance ($p < 0.01$)

(Veasey, 2006). Fowler and Mikota (2006) observed that chronic stress was evident in captive elephants, particularly in those with prolonged foot abnormalities and foot disorders.

The present study found that captive Asian elephants with foot disorders generally exhibited elevated faecal cortisol levels. However, when cortisol values were pooled across foot disorder types, there were no significant differences between elephants with and without foot problems, aligning with the findings of Bansiddhi *et al.* (2019). Notably, cortisol concentrations were higher in elephants with structural foot deformities, which concurred with the findings of Ganswindt *et al.* (2010). Elephants with minor nail cracks and cuticular feathering had low cortisol, while those with chronic foot disorders showed cortisol levels nearly double compared to those with minor affections. This supports Bansiddhi *et al.* (2019) who reported higher faecal glucocorticoid concentrations in elephants with major wounds. Elevated stress hormone levels can impair immunity and wound healing, contributing to delayed recovery (Kumar *et al.*, 2019). As foot disorders are a major health concern in captive elephants and are associated with increased stress hormones (Panagiotopoulou *et al.*, 2016), periodic monitoring of cortisol is essential to mitigate their adverse effects on elephant health and welfare.

CONCLUSION

The present study demonstrates that faecal cortisol concentration is a reliable non-invasive biomarker for assessing stress in captive Asian elephants. Captive elephants with foot disorders, especially structural deformities, exhibited higher cortisol levels, indicating that chronic foot problems contribute significantly to physiological stress. Housing conditions, management practices, and exposure to human disturbances further influenced cortisol concentrations, with elephants kept in inadequate or open enclosures showing higher stress levels compared to those in well-maintained temple enclosures. Minor foot affections, such as nail cracks or cuticular feathering, were associated with lower cortisol levels, while chronic foot disorders amplified stress responses. Overall, monitoring faecal cortisol, along with regular assessment and proper management of foot health, is essential to mitigate stress and improve the welfare and well-being of captive elephants

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