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Faecal Testosterone Concentration in Sri Lankan Bull Elephants During Musth

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Abstract. We evaluated the use of non-invasive faecal testosterone analysis to differentiate between musth and non-musth in captive Asian elephants. Twenty male elephants were studied, including five in 'musth' from the Pinnawala Elephant Orphanage (PEO), five privately owned males in musth, and 10 males not in musth from the PEO. Musth was identified by temporal gland swelling and urine dribbling. Faecal samples were collected and analysed using a 125I testosterone radioimmunoassay (RIA) kit. Faecal testosterone levels in elephants in musth were significantly higher than those not in musth but were not different between PEO and privately owned elephants in musth.

Introduction

Musth, a regular, annual three-to-four-month period of elevated serum testosterone level in sexually mature male elephants, is associated with heightened sexual activity and is characterised by increased aggressive behaviour (Glaeser *et al*. 2022). Musth plays a crucial role in the reproductive dynamics and social behaviour of wild elephants (Lincoln & Ratnasooriya 1996).

Understanding the hormonal changes associated with musth is essential for managing captive elephant populations (LaDue *et al*. 2022). Privately owned captive male elephants coming into musth around the same time period, limits their availability for cultural processions in the country (Katupotha & Kodituwakku 2018). A number of guidelines have been developed for managing captive elephants in musth and early detection, determining the duration and predicting cessation of musth are considered important for management (Duer *et al*. 2016; Brown *et al*. 2020).

However, obtaining blood samples for monitoring, is difficult when males are approaching musth and during musth (Fontes 2017). However, non-invasive faecal testosterone analysis has been reported to be useful in predicting musth (Ghosal *et al*. 2013).

Materials and methods

The study involved a total of 20 adult elephants, consisting of five males in 'musth' from the Pinnawala Elephant Orphanage (PEO), five privately owned males in 'musth' and ten males not in musth from the PEO.

Both PEO and privately owned elephants in musth were mostly kept isolated, tethered day and night and managed individually. The males that were not in musth were allowed to remain within the herd at the PEO.

The behaviour of the elephants was monitored from January 1, 2022, to January 1, 2024. The observations focused on identifying signs of musth, such as temporal gland swelling and secretion (Fig. 1), and urine dribbling. An elephant was classified as being in musth if these signs were consistently observed. Elephants that did not exhibit these signs were considered to be not in musth.

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Figure 1. A tusker in musth, secreting a thick, tar-like substance from its temporal glands, a characteristic sign of elevated testosterone levels and heightened aggression.

Single faecal samples were collected from each of the study elephants upon observed defecation. Approximately 100 g of faecal matter was collected for each sample. Using sterile gloves, samples were taken from the centre of multiple boli in one dung pile and homogenised. The samples were placed in polythene bags, labelled with the elephant ID and the date of collection, transported on ice to the laboratory and stored at -20°C until analysis.

Samples were then dried in an oven and ground into a fine powder using a mortar and pestle. A 0.2 g subsample was combined with 5 ml of 90% ethanol in a test tube, briefly vortexed, and suspended in a 90°C water bath for 20 minutes. Ethanol was added periodically to prevent drying. Samples were then centrifuged for 20 minutes at 1500 rpm, the supernatant transferred to a vial, and the process repeated with an additional 5 ml of 90% ethanol. Combined extracts were dried down and reconstituted in 1 ml of methanol, vortexed for approximately 2 minutes, and stored at –20°C.

Testosterone concentrations were measured using a 125I testosterone RIA kit (Weerasekera *et al*. 2020), with a detection limit of 0.2 ng/g faecal powder. Inter- and intra-assay coefficients of variation were maintained below 12.4% (Weerasekera *et al*. 2020).

For validation of the technique, 10,000 cpm of 125I testosterone was added to a 0.2 g sample of dried elephant faeces from a non-study male elephant which was not in musth and incubated at room temperature for 1 hour, followed by methanol extraction. The recovery of radioactivity was measured using a gamma counter. Serial dilutions of faecal extracts from elephants in musth and non-musth were tested against a standard curve using a 125I testosterone RIA kit and assay accuracy was calculated.

Results

Mean faecal testosterone was significantly higher ($t = 7.37$, $p < 0.0001$), in elephants in musth compared to those that were not in musth (Table 1). No significant difference was observed between those in musth from PEO and privately owned elephants in musth ($P > 0.05$).

Discussion

Our study aimed to evaluate the efficacy of non-invasive faecal testosterone analysis in distinguishing between captive male elephants in-musth and not-in-musth. The results show that faecal testosterone concentrations were significantly higher in elephants in musth, corroborating findings from previous studies.

Table 1. Average faecal testosterone concentrations in musth and non-musth bull elephants.

Group	Institution	N	Mean ± SE (ng/g)	Range (ng/g)
Musth	PEO	5	9.88 ± 1.12	5.66 – 12.05
	Privately owned	5	10.48 ± 0.82	8.08 – 12.68
	Overall	10	10.18 ± 0.66	5.66 – 12.68
Non-musth	PEO	10	3.78 ± 0.56	1.77 – 7.67

No significant difference was observed between the faecal testosterone concentrations of elephants from PEO and privately owned elephants in musth, suggesting that the hormonal response measured by faecal testosterone is consistent across different captive environments. This finding underscores the reliability of non-invasive faecal testosterone analysis as a method for monitoring musth, regardless of the elephant's captive situation.

The method for measuring faecal testosterone used in this study demonstrated strong robustness. The validation process, which included radioactivity recovery and assay accuracy checks, ensured the reliability and accuracy of our results.

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