Short Communication Gajah 53 (2021) 43-46

Using GnRH Vaccine During Post Musth in Captive Asian Elephants

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Introduction

During musth in elephants, testosterone levels increase severalfold (Ganswindt *et al*. 2002) and cortisol secretion is elevated due to possible stress (Yon *et al*. 2007). A positive correlation between testosterone and cortisol in musth bulls has been noticed, which indicates a possible role of the adrenal glands in modulating or facilitating their secretion (Brown *et al*. 2007).

Captive bull elephants in musth are difficult to manage and control, due to increased aggression and disobedience presumably caused by the elevated testosterone and cortisol levels (Lincoln & Ratnasooriya 1996). In general, serum testosterone and cortisol in male Asian elephants, could vary between 0.1–29.4 ng/ml and 4.6–98.7 ng/ml respectively (Brown 2006). Musth can occur at any time of the year (Eisenberg *et al*. 1971), but in a given elephant it occurs around the same period annually and may last up to 16 weeks (Rajaram 2006).

Musth in captive elephants can be divided into pre-musth, full musth and post-musth periods. During pre musth, the temporal glands become swollen, discharge begins and frequent urination and penile erection together with increased serum testosterone are noted while during full musth, these signs become more obvious with a further increase in serum testosterone (Rajaram 2006). In this report, we identify post-musth as the period after urine dribbling ends, until the elephant is released from the musth stable for its regular activities based on indication of complete obedience and absence of aggression.

Elephant keepers lose income when their elephants are in musth and several keepers have been killed while trying to release males too early in post-musth. Hormonal management of post-musth could prevent such occurrences. Musth can be prevented or postponed by using GnRH vaccine (Somgrid *et al*. 2016a; Piyadsa *et al*. 2017). It probably acts via suppressing testicular function by interrupting the hypothalamo-pituitary gonadal axis, leading to decreased production of testosterone (Somgrid *et al*. 2016a, 2016b) though the mechanism with regard to cortisol is not clear.

We monitored serum testosterone and cortisol levels of four captive male elephants in post-musth to assess whether administering GnRH vaccine could enable releasing them earlier than usual from their musth stables.

Materials and methods

Four adult captive bull Asian elephants (*Elephas maximus*) Saliya, Mangala, Bibila and Raja, which were privately owned, were used in the study. They had come into musth and stopped urine dribbling but still showed temporal gland secretion, hence were identified as being in post-musth. Their regular musth period, including pre- and post-musth, and the dates of sampling are given in Table 1.

The first GnRH injections were given 3–4 weeks prior to the anticipated date of release from the musth stables. Intramuscular injections of 4 ml (1.6 mg per dose) of GnRH (BOPRIVA®bovine immune castration vaccine, Zoetis GMS Australia) were administered on the given dates to 3 of the 4 animals (except Raja) and blood samples collected (Fig. 1). To control for diurnal variation in testosterone (Brown *et al*. 2010) blood samples were collected between 6 and

Manuscript Editor: Prithiviraj Fernando

Table 1. Details of elephants and their musth period.

Name	Age (years)	Usual musth period*	
		Months	Duration (months)
Saliya	44	Oct – Mar	6
Mangala	43	Nov – May	7
Bibila	26	Sep – Apr	8
Raja	40	Nov – May	7

*from pre-musth to release from the musth stable

8 am. Plasma was separated and was stored at –20ºC until analysis. Serum testosterone and cortisol were assessed using a Roche cobas e 411 analyzer at a commercial medical laboratory (Durdens Medical Laboratory).

The 'usual musth period' is from pre-musth to release from the musth stableIn spite of administering three doses of GnRH vaccine, Saliya's temporal gland secretion continued. Therefore on 3rd May 2019 he was taken away from the musth stable, given a bath and stabled about 800 m away from Raja who was in post-musth with temporal gland secretion. Behavioural monitoring of Saliya was continued at the new stable.

Results

Serum testosterone levels of Saliya were initially very low and continued to be low through the GnRH vaccine injections and afterwards (Table 2). He also continued to have temporal gland

Figure 1. Male being administered the GnHH vaccine.

secretions. After he was released from the musth pen, the fluctuation in his cortisol levels decreased, temporal gland secretion immediately stopped and he became obedient.

Both Mangala and Bibila had initially elevated levels of both testosterone and cortisol, which gradually reduced with the vaccine injections (Table 2). After the 2nd dose, both became obedient and were released from the musth stable for regular activities.

Raja had low serum testosterone and cortisol levels but was throwing his food and occasionally disobeying the keeper's commands, possibly due to the presence of Saliya in close proximity. Therefore, he was released from the musth stable for regular activities after the first sampling.

Discussion

Testosterone and cortisol in both Mangala and Bibila showed a continued decrease with GnRH vaccine injections. Saliya's testosterone levels were not impacted by GnRH, possibly because it was already low. GnRH vaccine is usually given before pre-musth to prevent or delay the occurrence of musth (Somgrid *et al*. 2016a, 2016b). However, our results suggest that the testosterone feed back regulatory mechanism can be disrupted even if GnRH vaccine is administered during post-musth. Such administration may help the early release of captive elephants from restrictions imposed in musth.

Levels of testosterone and cortisol are correlated in musth (Brown *et al*. 2007). However, serum cortisol tends to vary much more than testosterone as it is influenced by factors other than musth (Brown 2006). Saliya was in apparent post-musth and had low serum testosterone levels, but continued to have elevated cortisol levels. The discrepancy may have been due to the stress caused by the proximity of Raja who was also in post-musth. Raja was much bigger and heavier than Saliya hence likely to be dominant over him. The observed reduction in Saliya's cortisol levels upon moving him away from Raja supports this assumption. The observations on Saliya are also consistent with association between musth bulls being stressful (Claassens 2010), and that stress could be reduced by keeping musth males away from each other, especially from dominant males (Lincoln & Ratnasooriya 1996).

Table 2. Hormone levels and GnRH vaccine injection dates of the four bulls.

Elephant	Date	Testosterone (ng/ml)	Cortisol (nmol/l)	Comment
Saliya	7.3.2019	0.07	15.2	1st vaccine
	22.3.2019	0.05	50.5	2nd vaccine
	5.4.2019	0.06	11.2	3rd vaccine
	17.4.2019	0.06	30.6	
	3.5.2019	0.08	42.0	Released from musth stable
	6.6.2019	0.03	17.9	
	18.7.2019	0.08	20.1	
Mangala	5.4.2019	>15.00	10.4	1st vaccine
	22.4.2019	3.87	44.4	2nd vaccine
	6.5.2019	0.13	24.5	3rd vaccine
Bibila	6.2.2019	>15.00	33.0	1st vaccine
	17.2.2019	0.62	27.9	2nd vaccine
	1.3.2019	0.20	7.5	3rd vaccine
Raja	22.7.2019	1.40	14.2	
	5.8.2019	1.50	10.2	

The cortisol levels observed were much lower than reported elsewhere in Asian elephants during musth (Brown 2006; Claassens 2010). This may be due to the management of the study subjects being better and less stressful compared to others, and/or related to the analytical methods. Serum testosterone levels (0.03 to >15 ng/ml) observed were comparable with Jainudeen *et al*. (1972).

It would be interesting to study the variation in cortisol levels during musth in both captive and wild elephants. Our results indicate that human diagnostic laboratories could be used to determine serum testosterone and cortisol levels in elephants, which would facilitate conducting of such studies.

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