**The influence of pZP vaccination on stress in female African elephants (*Loxodonta Africana)* in the Greater Makalali Game Reserve.**

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**Makalali Game Reserve 3rd March - 10th July**

**Abstract**

*In contrast to other parts of Africa where elephants are still endangered, elephant numbers in South African wild-life parks have risen to levels where they threaten biodiversity.* *One option currently being used for managing elephant population growth in the Greater Makalali Game Reserve is immunocontraception using the Porcine Zona Pellucida (PZP) vaccine. The anticipated effect of pZP immunocontraception is repeated episodes of sexual receptiveness not leading to pregnancy (Rasmussen and Schulte 1998). As a result, pZP vaccination is expected to result in repeateelevated stress levels in pZP vaccinated cows.*

*The aim of this study was therefore to examine whether there is a correlation between the presence of an adult bull close to a matriarchal herd and indices of stress in the mature cows within that herd, and whether there is a correlation between progesterone concentrations i.e. the phase of the cycle of a pZP vaccinated elephant and circulating corticosteroid levels.*

*During a 4 month period (March-June), behavioural observations were performed on, and feacal samples were collected from, pZP immunocontracepted cows in the Greater Makalali Game Reserve, Limpopo, South Africa. A total of 58 samples from 19 different cows were analyzed for 11-oxo-cortisol by EIA: Wasser et al. (2000) reported fecal 11-oxo-cortisol levels to be a* *reliable measure of the glucocorticoid stress response in African elephants.*

*In the current study, fecal 11-oxo-cortisol didn’t vary with respect to the presence or absence of an adult bull, suggesting there is no significant stress response to the presence of a bull near a matriarchal herd. Nevertheless, the approach described provides a useful basis for further studies on the influence of pZP vaccination and resulting increased frequency of bull presence, on stress in elephant cows.*

**Introduction and background**

In large parts of Africa, African Elephants (*Loxodonta Africana*) are still poached for their ivory, meat and skin. This poaching, allied to encroachment into traditional elephant range lands by the growing human population, threatens the existence of the species.

By contrast, a combination of good park management and secure fencing in South Africa has virtually eliminated poaching and led to elephant population growth exceeding 5% per year (Hall-Martin A.J. et al. 1989).On the other hand, by feeding on the leaves, branches, bark and roots of over 200 species of grass, tree and bush, elephants are destructive feeders and demolish vegetation in a way that tends to change woodlands into grassland (Chromatogr 1993, Whyte IJ. et al. 2003) and thereby threatens species dependent on a woodland habitat.

The need for elephant population management is not a recent phenomenon. As early as 1966, the problem was acknowledged and a program was set up to limit further population growth. Professional hunters were employed to cull up to 10% of the elephants in the Kruger Park each year. The annual culls continued until the media helped to propagate the assumption that the southern African elephant population was in danger of extinction and that culling was both cruel and unnecessary (Rasmussen L.E. et al. 1998).

This was the reason that in 1995 it was decided that to suspend the elephant cull. Since the last cull in 1994, the elephant population in Kruger National park has grown from 7,000 animals to at least 12,500 in 2006 (Borchert 2007). Not only does this increase in elephant density threaten to harm ecosystems and biodiversity, but the elephant threatens to become a victim of its own ‘success’ since a drought could result in a mass die-off. To solve the problem of elephant over-population in southern African parks, various measures have been proposed or tested; hese include translocation, increasing the size of the park and various types of contraception.

The major disadvantages of translocation are that it is costly (approximately $8000 per elephant) with profound implications for animal welfare not only because the elephants need to be sedated and transported large distances by truck, but also because one cannot be sure if an entire family group is being moved; sometimes members of a herd are accidentally left behind (Whyte IJ. et al. 2003). Furthermore, there are not many places left to relocate the animals (Dublin HT et al. 1983, Whyte IJ. et al. 2003). Moreover, it appears that removing animals from the population, either by culling or by translocation, leads to a so called "reproductive rebound". By removing some animals, more food is made available for the remaining animals which therefore reproduce more successfully such that the population grows even faster (**Ramakrishnan U. 2002**).

A third possible solution, besides culling or translocation, is creating larger protected areas for the elephants to live in. By opening the fences to abutting parks, elephants can migrate to areas where they are relatively scarce. This is already happening at various locations: Kruger has opened the fences to parks in Zimbabwe and Mozambique. Unfortunately, due to a shortage of available habitat and the time taken to organize the merging of parks across national boundaries, the ‘trans-frontier’ park initiative alone will not be sufficient.

A fourth option for managing elephant population growth is contraception. This can be achieved by means of surgical methods such as castration or vasectomy, although these approaches have the disadvantage of being invasive and irreversible, while castration is likely to influence the behaviour of both the treated animal and those with which it interacts. Castration and vasectomy are particularly invasive in elephants because the testes are located intra-abdominally. Another way to achieve contraception is via steroid hormone administration, for example in the form of androgens or progestagens. An obvious advantage over the surgical methods is that this would be reversible, but it requires immobilization of the animals to administer hormone releasing implants, and will significantly influence behaviour and may even have contraceptive effects on animals that eat either elephant flesh or faeces.

*Immunocontraception:*

The introduction of immunocontraceptive techniques has provided a new means of elephant population control. Immunocontraception is based on the same principles as disease prevention by vaccination. In this instance, however, vaccination stimulates the immune system to produce antibodies against endogenous molecules that play an essential role in reproductive cyclicity or conception (Perdok et al. 2007). There are a variety of immunocontraceptive vaccines including a vaccine against porcine Zona Pellucida (pZP). The zona pellucida is an extracellular matrix that surrounds the oocytes of all mammals and plays critical roles in regulating sperm binding, penetration and fertilization (Kirkpatrick 1991). Porcine ZP is commonly chosen as the active ingredient of ZP vaccines because it can be easily harvested from the ovaries of slaughtered pigs, while the antibodies induced cross-react with ZP epitopes in many target species (Fayrer-Hosken et al. 1997, 1999; Barber and Fayrer-Hosken 2000a, 2000b).

The exact mechanism of infertility induced by ZP vaccination may differ between species and individuals. In general, however, it is assumed that antibodies raised against ZP proteins either directly block sperm–ZP binding or disrupt ZP formation and thereby indirectly inhibit the ability of sperm to bind and penetrate an oocyte (Muller et al. 1997; Miller et al. 2000; Kirkpatrick and Rutberg 2001);

The advantages of pZP immunocontraception over hormonal contraception are (1) that the animals will continue cycling after administration, (2) that they don't need to be handled or sedated (no hormone implant needs to be placed) and (3) there is no disruption of fetal development and/or lactation. The latter is important since, in a species with a long non-seasonal gestation, it is almost impossible to avoid injecting some pregnant animals (since elephant gestation is nearly 2 years it is likely that many cows will be pregnant or lactating at the moment of vaccination); abortion, dystocia or birth of abnormal or weakened offspring would all be unacceptable side-effects. (Turner, Liu, et al. 1996; Fayrer-Hosken et al. 1999; Kirkpatrick and Rutberg 2001; Delsink et al. 2003).

The use of reproductive steroid hormones as contraceptives has proven unacceptable in wildlife because of marked effects on behaviour, such as separation of treated animals from the family herd (Fayrer-Hosken et al. 2000; Kirkpatrick and Rutberg 2001).

It is generally assumed that pZP vaccination will not alter reproductive hormone secretion (Powel and Monfort 2001) and that treated females will therefore experience normal ovarian cycles (Barber and Fayrer-Hosken 2000). However, while some studies have indeed recorded normal oestrous cyclicity following ZP vaccination (Fayrer-Hosken et al. 2000; Kirkpatrick and Rutberg2001), others have recorded abnormalities such as:

• altered ovarian function in horses (Kirkpatrick et al. 1992) and deer (Miller et al. 2001)

• altered cyclicity in primates (Nettles 1997) and deer (Muller et al. 1997)

• reduced ovulation rate in horses (Kirkpatrick et al. 1992)

• decreased estrogen production in baboons (Miller et al. 2001) and horses (Kirkpatrick et al. 1997)

• altered ovarian structure in primates (Nettles 1997)

• follicular inflammation in deer (McShea et al. 1997)

• acyclicity in horses (Muller et al. 1997)

The effects on cyclicity tend to become more severe with the duration of elevated anti-pZP antibody titres. However, it is not clear whether failure to cycle is advantageous or disadvantageous for health and behaviour.

*Social behaviour*

With regard to social behaviour, African elephants live in stable groups consisting of related adult females and their offspring. Young bulls leave the herd shortly after reaching sexual maturity and go off to live in bull groups or in solitude (Rasmussen and Schulte 1998). Adult males only really interact with the matriarchal herds when a female is in oestrus and ready for mating (Moss 1983). A consequence of pZP contraception is that the number of offspring born into a herd will decrease or stop; as yet, it is not clear how this will affect group stability or behaviour (Rasmussen and Schulte 1998; Fayrer-Hosken et al. 1999).

The other major change expected after pZP vaccination is more oestrous cycles, and therefore interaction with adult bulls. The oestrous cycle of an elephant cow lasts 12 to 18 weeks (Rasmussen and Schulte 1998). During this cycle the cow has a 2- 10 day period of sexual receptivity when she will show oestrous behaviour, accept mating and may conceive (Moss 1983; Rasmussen and Schulte 1998). A female elephant announces her sexual receptivity in advance through chemical, auditory and behavioural signals, increasing the likelihood that a desirable bull will present himself for mating; bulls will travel great distances to find an oestrous female (Rasmussen and Schulte 1998). In the event of pregnancy, the cow will not cycle again for at least another two years, the length of gestation (Rasmussen and Schulte 1998). Because sexually receptive periods usually end with mating and pregnancy (Rasmussen and Schulte 1998), repeated oestrous cycles are not a normal feature of wild elephant reproduction. It is, therefore, not clear how an increase in the number of oestrous cycles due to immunocontraception will affect male behaviour, or how much the disruption caused by more frequent bull attention will affect the matriarchal groups, or whether it will lead to higher stress levels in the cows.

The aim of this study was tolook for a correlation between the presence of a bull with a matriarchal herd and indices of stress in the mature cows of that herd. It was anticipated thatthe presence of a bull would result in more stress for the mature cows.

The second aim of the study was todetermine whether there was a correlation between the progesterone concentration (i.e. phase of the cycle) of pZP vaccinated elephants and their stress levels. It was expected that a cow in oestrus would receive more visits from bulls and that the resulting stress would be reflected by higher glucocorticoid concentrations in the faeces.

**Materials and Methods:**

*Study site*

The study elephants were in the GMPGR which is located on the Lowveld plain, 300–500 m above sea level close to the foothills of the Drakensberg Mountains in South Africa’s Limpopo Province (30.49°S, 24.00°E). The reserve is divided into different sections (PNO, PSO, Garonga, Twines, 26, MEA, Lufafa and M5), each with a different owner.

The Makhutswi River, which originates in the Drakensberg Mountains and is a tributary of the Olifants River, bisects the park. The main vegetation type is *Combretum apiculatum* Mixed Bushveld (Acocks 1988). GMPGR is located in a summer rainfall area with a mean annual precipitation of 450 mm (Druce D J 2000). The summer starts in October and ends in March, these are also the months with the highest rainfall. Temperatures vary between 3 ºC in winter and 36ºC in summer (Druce 2004).

There are several artificial waterholes in the park, the filling of which is regulated to control animal movements so that there is not too much stress on specific areas since this could lead to destruction of vegetation.

*The elephant population:*

In 1996, the Kruger National Park performed an initial trial of pZP immunocontraception in elephants. In 1994 and 1996, GMPGR received two herds of elephants from KNP (13 and 24 animals respectively) and embarked on a follow-up study on the efficiacy of pZP immunocontraception. This study has now been running for approximately 10 years.

In January 2006, the population at Makalali had grown to 73 elephants, comprising 28 females aged ≥8 years distributed over four herds of 8–22 animals, and 14 independent adult males. In May 2000, all the adult females aged >12 years (18 animals) were vaccinated with 600 μg pZP + 0.5 ml of Freund’s Modified Adjuvant (FMA; Sigma Chemical Co., St Louis) (Delsink A.K. 2002). However, since births have been recorded in females as young as 9–10 years, the breeding population was classified as females ≥8 yr for subsequent vaccinations. By July 2003, a total of 23 cows had been vaccinated In July 2004, it was decided not to vaccinate pre-pubertal cows or those that had not yet produced offspring until after the birth of their first calf (Delsink 2004). In December 2007, one female (‘Smelly’) in which vaccination was deliberately stopped in 2004 after 5 years of repeated vaccination produced a calf, thereby indicating that medium term repeated PZP vaccination is reversible in at least some cases.

At present, the population consists of 74 elephants; 60 individuals spread over 4 herds led respectively by the matriarchs Kwatile, Holey ear, Queeny and Yvonne, and 14 free-roaming adult bulls. In each herd, one female is fitted with a radio collar that sends a GPS signal twice a day (Delsink et al. 2007).



*Table 1. The number of cows vaccinated between 2000 and 2005. See Appendix 2 for more detailed information on the vaccination schedule.*

*Tracking*

Observations were mainly performed during game drive hours, i.e. between 5:00 and 11:00 in the morning and between 15:00 and 21:00 in the afternoon/evening from Tuesday until Saturday. Elephants were located by means of traditional tracking methods i.e. the presence and freshness of spoor and dung deposits. The majority of observations were conducted from the vehicle, except where conditions were more conducive to off-road viewing on foot (e.g. if elephants were in the river) (Delsink A K 2006).

*Animal identification*

Animals were identified according to individual characteristics including sex, height, unique ear patterns comprising nicks, tears and holes, the size and shape of tusks and other distinguishing features such as growths, lumps and scars (Delsink A.K. 2006; Moss C 1996; Poole J 1996). These features were recorded on individual ID-sheets. During an observation, the herd was first ID-ed on the basis of the matriarch or another distinctive group member. Subsequently, individuals were ID-ed using the ID sheet database for the appropriate herd. If ID sheets were not available for the individual, pictures were taken on the scene and the identity confirmed later.

*Behavioural Observations*

During every sighting of a herd, behavioural observations and external factors that could induce stress were recorded using an ethogram *(Appendix 1)*. Behavioural observations that were recorded included: herd mood, herd activity and female mock charges. External factors that were noted were: the presence of a bull, interaction of the bull with females, the mood of the bull, the habitat,the weather, the presence of a helicopter or aeroplane and whether herds were gathered together.

Since the individuals of a herd adjust their behaviour to the other members of the herd it was possible to assign a general herd activity and mood. The herd mood was classified as relaxed, skittish or aggressive and the herd activity as feeding, drinking, bathing, mobile, resting or any other activity, which was specified.

Specific behaviours displayed by a cow that had a possible correlation with stress were added to the ethogram. These behavioural observations included: female mock charges, trumpeting, temporal gland secretion and dust kicking. The female mock charges were classified as headshaking, ear-flapping or charging towards a vehicle or other animal. According to Buss et al. (1976) temporal gland secretion is usually associated with stress and excitement. Temporal gland secretion is characterized by a watery, faint-to-musky smelling secretion that stains the cheeks below the orifice of the gland. Trumpeting is the sound produced by an elephant blowing through its nostrils hard enough to make the trunk resonate, while usually holding it straight down or curved slightly backwards. The sound can be modulated from a short blast to a prolonged reverberating cry. Headshaking is the rapid rotation of the head from side to side, causing the ears to slap against the face with the sound of a snapping towel.

The presence of a bull was recorded when the bull was within the visual field of the herd The bull’s mood was classified as skittish, relaxed or aggressive, and his interaction with females as testing scent, chasing or consort behaviour. Consort behaviour is characterised by affinitive behaviour, proximity and attempts to prevent other males from copulating with a female. (Rossum et al. 200?). Testing scent was recorded when the bull was touching a cow’s genetalia with his trunk and subsequently putting his trunk in his mouth.

*Sample collection:*

Fresh faecal samples were collected from as many identified females above the age of 8 years as possible, ideally approximately twice a week. Within 1 hour after defecation, a sample was recovered from the centre of a faecal bolus, mixed and transferred to a vial. Date of collection and animal ID were noted. The vials were transported in a cooler box on ice and then transferred to and stored in a freezer until analysis. According to Wasser et al. (1996) intra-sample variation in faecal hormone concentrations can be substantially reduced by extracting well mixed faecal powder from freeze-dried samples taken from the central portion of the wet sample.

*Analysis of glucocorticoid metabolites*

A generally accepted and commonly applied method for estimating stress in wild animals is to measure faecal glucocorticoid concentrations combined with behavioural observations. Detection of glucocorticoids in blood, while more sensitive, requires sedation, may itself be stressful and may miss more chronic stress because of the pulsatile excretion pattern of glucocorticoids into the blood(Harper and Austad, 2000; Monfort et al., 1993; Windle et al. 1998) .

Wasser et al. (2000) found that the peak in faecal cortisol metabolites was reached approximately 30 hours after an adrenocorticotropin hormone (ACTH) challenge. Moreover, ACTH injection produced a four to five-fold rise in serum cortisol followed the expected 30h later by a comparable rise in fecal cortisol metabolites, measured using an I-125 corticosterone assay (Wasser et al. 1996, 2000), thereby confirming that faecal corticosterone concentrations provide a reliable measure of the glucocorticoid stress response in the African elephant.

The faecal hormone analysis was performed via the Section of Reproduction of the University of Pretoria’s Department of Production Animal Studies. First, faecal samples were lyophilised (freeze dried), pulverised and sieved through a mesh to remove undigested faecal matter, as described by Fieβ et al. (1999). Approximately 0.05 g of the faecal powder was extracted by vortexing for 15 min with 80% methanol in water (3 ml). Following centrifugation for 10 min at 3300 g, supernatants were transferred to a glass tube ready for hormone analysis.

Faecal extracts were measured for immunoreactive glucocorticoid metabolites using an enzyme immunoassay for 3α,11oxo-cortisol metabolites (3α,11oxo-CM) (Möstl *et al*. 2002), which has previously been shown to provide reliable information on adrenocortical function in the African elephant (Ganswindt *et al*. 2003; 2005). Briefly, 50 μl aliquots of standards, quality controls, and diluted faecal extracts were pipetted in duplicate into microtiter plate wells. A total of 100 μl of biotinylated label and antiserum (raised in a rabbit against 5β-Androstane-3α-ol-11-one-17-CMO ) were added and the plates incubated overnight at 4 °C. Following incubation, the plates were washed four times after which 250 μl (4.2 mU) of a streptavidin horseradish-peroxidase conjugate was added to each well. Following incubation in the dark for 45 min at 4 °C, plates were washed again, before 250 μl (69.4 nmol) tetramethylbenzidine was added and the plates were incubated for a further 45 min at 4 °C. The reaction was terminated by adding 50 μl of 2M H2SO4; the absorbance was then measured at 450 nm (reference filter: 620 nm) using an automated plate reader.

The sensitivity of the assays at 90 % binding was 3.0 pg/well and the intra- and interassay coefficients of variation, determined by repeated measurements of high and low value quality controls, were 3.0 % and 12.5 %, respectively (Ganswindt A., 2003).

*Analysis of fecal progesterone metabolite concentrations*

The time lag time between IV injection of 14C progesterone and the peak excretion in the faeces is approximately 48 hours (Wasser et al 1996). Moreover, the majority of progesterone metabolites are excreted in the faeces (55%). It is therefore accepted that analysis of faecal progestins is an effective non-invasive means of characterizing ovarian activity in free ranging African elephants.

Faecal extracts were examined for progesterone metabolite concentrations using a competitive double antibody-enzyme immunoassay (EIA) for immunoreactive 5α-pregnane-3-ol-20-one *(5α-P-3-OH)*, which has previously been shown to provide reliable information on ovarian function and pregnancy in the African elephant (Fieβ et al., 1999; Wasser et al. 1996). The antibody for the EIA was raised in a rabbit against 5 β-pregnane-3*α*-ol-20-one. 5 *α*-pregnane-3β-ol-20-one-3HS:DADOO-B was used as label. Progesterone *(4-pregnene-3,20-dione)* was used as a standard, and serial dilutions of faecal extracts gave a displacement curve parallel to that of the standard curve (Schwarzenberger 1996). For the assay, duplicate 50 μl aliquots of the 5α-P-3-OH standards (range 9.8–2500 pg), quality controls, and diluted faecal extracts were pipetted into coated microtiter plate wells, 50 μl of label (DADOO-B) and antiserum were added, and the plates were incubated overnight at 4°C. Following incubation, the plates were emptied, washed four times and blotted dry before 150 μl (20 ng) of streptavidin–peroxidase was added to each well, followed by incubation for 45 min. Next the plates were emptied, washed and dried again and substrate (tetramethylbenzidine) was added, followed by incubation in the dark for 30-60 min. at 4°C, with the exact duration determined by the rate of colour change. The reaction was terminated by adding 50 μl of 4M H2SO4, and the absorbance was measured at 450 nm.

Sensitivity of the assay at 90% binding was 0.3 pg/well. Inter- and intra-assay coefficients of variation, determined by repeated measurements of high and low value quality controls were 6.4% and 10.5%.

*Statistics*

To test the correlation between the behaviours thought to indicate stress (temporal gland secretion, trumpeting, dust kicking, headshaking, ear-flapping and mock charging) and the presence of a bull, the chi squared test was used.

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Using the assumption that no more than 20 % of the observed and expected frequencies may have a count smaller than 5 and that the absolute minimum count per frequency is 1, the chi squared test could not be used to examine the correlation between the presence of a bull and the frequency of mock charging because the number of mock charges in the presence of a bull was zero.

The degrees of freedom used for each chi squared test was 1, and statistical significance was assumed at p< 0.05.

To test the correlation between the cortisol concentration in the faeces and external factors, independent two-sample t-tests were used.

T-test:

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Where  was the mean cortisol level in the presence of a specified external factor,  was the mean cortisol level in the absence of that factor, S was the pooled standard deviation, n was the number of observations in the presence of a given factor and m was the number of observations in the absence of the factor.

The external factors examined were:

* a bull with the herd
* consort behaviour
* bulls testing the scent of cows

Because cortisol has a 30 hour excretion lag time, the cortisol level detected in the faeces was coupled to external factors recorded approximately 30 hours before the dung sample was collected.

The significance level was again set at p< 0.05.

To test the correlation between progesterone concentration and cortisol concentrations in the faeces ‘Pearson’s R test’ was used.

Pearson’s test:

r = \frac{\sum ^n _{i=1}(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum ^n _{i=1}(X_i - \bar{X})^2} \sqrt{\sum ^n _{i=1}(Y_i - \bar{Y})^2}}.  

The Pearson correlation coefficient is a measure of the correlation between two variables (X and Y). In this case Xi was used for the cortisol concentration, Yi for the progesterone concentration,  for the mean cortisol concentration and  for the mean progesterone concentration. The correlation coefficient ranges from -1 to 1. A value of -1 implies a linear equation that describes the relation between X and Y perfectly, with all data points lying on a line for which Y increases as X increases. A value of 0 implies that there is no linear correlation between the variables.

**Results:**

*Correlation between behaviuoral observations and the presence of a bull*

*Figure 1. Frequencies of recorded behavioural patterns in the presence or absence of bulls (TGS=Temporal Gland Secretion).*

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Bulls present** | **No bulls present** |  |
| **TGS** | 8 | 2 | 10 |
| **No TGS** | 36 | 27 | 63 |
|  | 44 | 29 | 73 |

*Table 2. Frequency of temporal gland secretion (TGS) sightings in the presence and absence of bulls.*

The presence of bulls was correlated with temporal gland secretions (p<0.05).

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Bulls present** | **No bulls present** |  |
| **Trumpeting** | 8 | 1 | 9 |
| **No trumpeting** | 36 | 28 | 64 |
|  | 44 | 29 | 73 |

*Table 3 . Frequencies of trumpeting in the presence and absence of bulls.*

The presence of bulls was correlated with trumpeting (p<0.05).

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Bulls present** | **No bulls present** |  |
| **Headshaking** | 5 | 3 | 8 |
| **No headshaking** | 39 | 26 | 65 |
|  | 44 | 29 | 73 |

*Table 4. Frequencies of headshaking in the presence and absence of bulls.*

The presence of bulls was not correlated with headshaking.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Bulls present** | **No bulls present** |  |
| **Ear flapping** | 6 | 3 | 9 |
| **No ear flapping** | 38 | 26 | 64 |
|  | 44 | 29 | 73 |

*Table 5. Frequencies of ear-flapping in the presence and absence of bulls.*

The presence of bulls was correlated with ear-flapping (p<0.05).

*Correlation between cortisol measurements and the presence of bulls.*

*Figure 2. Mean Cortisol concentration ((Ugr/gr) in the presence and absence of bulls, of consort behaviour and of a bull testing scent.*

When the herd was accompanied by a bull displaying consort behaviour, the mean faecal cortisol concentration was 0.2 µg/g, i.e. 0.1 µg/g lower than in the absence of a bull. When there was a bull testing the scent of a cow, the cortisol level was 0.23 µg/g higher than when no scent testing was recorded. In the absence of a bull, the cortisol level was 0.05 µg/g higher than in the presence of a bull. However, none of the differences were statistically significant.

*The interrelationship between cortisol and progesterone measurements*

The calculated r had a positive value of 0.34, i.e. there was a marginal tendency for higher cortisol concentrations to be associated with higher progesterone concentrations.

R squared is the proportion of the variability that can be predicted from, or explained by X. In this case r2 was 0.12, i.e. only 12% of the variance in the progesterone concentration could be accounted for by changes in cortisol concentrations.

*Figure 3. Correlation between mean fecal cortisol and progesterone concentrations.*

**Discussion**

*Fecal sample collection*

To draw any statistical conclusions it was necessary to collect at least 5 samples per individual. In most cases this was not achieved.

Seven reasons why it was difficult to spend more time with the elephants:

1. Elephants are hard to track. There are days on which no elephants were found.
2. Not every drive had the priority of finding elephants, during the elephant drives other species also needed to be inventarised. We estimate that around 14 hours a week was specifically dedicated to tracking elephants.
3. Even after localization of the elephants, it is hard to identify them because of impaired visibility due to dense vegetation, other elephants or because the ID sheets were not present (only the major cows had their own sheets) or were outdated.
4. Sometimes, after finding the elephants, we were unable to stay with them the whole time either because we had to leave for the other vehicles on game drives, which we tried to solve by letting them go to the sighting first so we would be the last ones in, or because the elephants seek shelter in thick bush when it is getting hot. Also if the animals seemed to be too stressed we chose to leave them. The mean obervation time was 34.4 mins.
5. If the elephants disappear out of sight it is difficult to follow them in the dense vegetation and in Pidwa, a big area in the north of the reserve, it is prohibited to go off-road.
6. After identification of the elephants it is not always possible to collect a faecal sample, simply because the elephant does not always defecate or because it is not possible to find the corresponding dung sample. This could be because the area is too dangerous to leave the vehicle to collect or because there is more than one dung sample and it is hard to find out which one belongs to the identified animal. In 43% of the elephant sightings it was possible to collect a dung sample.
7. The behavioural observations were very difficult because there are a lot of external factors that play a role, like the weather conditions and the presence of cars for example, including our own. These factors were recorded so that they could be taken into account during interpretation of the data.

*Correlation between presence of bulls, behavioural observations and cortisol levels.*

There was a significant correlation between the presence of bulls and temporal gland secretion and trumpeting by cows (p<0.05). Temporal gland secretion and trumpeting could be indicators of stress. Conversely there was no significant correlation found between the presence of a bull near a herd and cortisol levels. With the small set of data obtained however, it is important to be careful in making any conclusions.

The time spent with the herd to observe their behaviour (Appendix 3.) was only a small portion of their total activity. Of all our game drive hours (624) only about 41 hours (6%) were actuallly spent observing elephants. Also the game drives only took 8 hours a day maximum, meaning 16 hours of daily activity was inevitably missed. In this 16 hours, environmental factors could have influenced the stress level of the cows without being recorded.

Seasonal factors are also of influence on stress levels. In a study by Viljoen et al. (2008) the mean 3α,11oxo-CM concentrations in faeces samples collected from African elephants (*Loxodonta Africane*) from the Kruger National parkin the dry season (n=196) were significantly higher than in the wet season (n=178) (u=15206.06; p= 0.032). A study by Foley et al. (2001) confirmed this finding. Feacal cortisol metabolite concentrations showed significantly higher levels in the dry season. Foley also found an inverse relationship with rainfall across seasons. The samples in the GMPGR were taken in the months March--June, just after the rainy season, but it’s not clear how this influenced cortisol metabolite levels.

*Faecal progesterone and cortisol measurements*

One of aims of the study was todetermine whether there was a correlation between the progesterone concentration (i.e. phase of the cycle) of pZP vaccinated elephants and their stress levels. Only 12% of the variance in the progesterone concentration could be accounted for by changes in cortisol concentrations. However, all of the progesterone measurements were far below 3 μg/g (a level previously reported to be consistent with luteal activity) and it is not, therefore, possible to draw conclusions about an affect of cycle stage based these on results. Possibly the test results somehow underestimated the progesterone values.

**Conclusion**

In the current study, fecal 11-oxo-cortisol didn’t indicate a significant stress response to the presence of a bull near a matriarchal herd. However, the approach described provides a useful basis for further studies on the influence of pZP vaccination, and resulting increased frequency of bull presence, on stress in elephant cows.

**Acknowledgements**

First of all I would like to thank my supervisors Prof. Tom Stout, Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands and Prof. H.J. Bertschinger, Dept. of Production Animal Studies, Section of reproduction, University of Pretoria, South Africa.

I would also like to thank:

The staff at the Department of Production Animal Studies, Section of Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria and, in particular, Dr. Andre Ganswindt.

The Department of Production Animal Studies, Section of Reproduction, Faculty of Veterinary Science, University of Pretoria, Veterinary Hormone laboratory; in particular Marissa Grant for assisting with the hormone analysis.

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|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Apendix 1.** |  |  |  |  |  |  |  |
| **ELEPHANT HERD ID AND BEHAVIOR SHEET** | | | | | | | |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Date:** |  | **Time:** |  | **Position: (road name)** | | **Position Precision\*: A B C D E** | |
| **Observer (initials):** |  | **Map:** |  | **GPS East (30)** |  | **GPS South (-24.)** |  |
| **Herd/s Identified :** | Queeny | Holey Ear | Yvonne's | Kwatile |  |  |  |
| **Bulls Present:** |  |  |  | **Total No. of animals:** |  | **Total No. of infants:** |  |
| **Herd Mood:** | Relaxed | Skittish | Aggressive | **Bull interaction with females:** | Testing scent | Chasing | Consort behaviour |
| **Herd Activity:** | Feeding | Drinking | Bathing |  |  |  |  |
|  | Mobile (Specify) | Resting | Other (Specify) |  |  |  |  |
| **Female Behaviour:** | Urinating | Oestrous walk | Running | **Bull Mood:** | Relaxed | Skittish | Aggressive |
| **Female Mockcharge** | Head chaking | Ear flapping | Charging |  | **Towards** | Vehicle | Other Animal\* |
|  | Trompetting | Temporal gland secretion | Dust kicking |  |  |  |  |
| **Habitat:** | Closed Woodland | Dam | Open Grassland | Open Woodland | River |  |  |
| **Weather:** | Hot/Sunny | Windy | Overcast | Raining |  | **Elapsed time of sighting:** |  |
| **\*Comments: (Reaction to Vehicle or Helicopter, New Ear markings,Granuloma's, Mobile direction etc.)** | | | | | | | |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **\*Position Precision: A = 0-50m B = 50-100m C = 100-200m D = >200m E = Cartalinx** | | | | | | | |

**Apendix 2.** Vaccination schedule elephants in GMPGR

**Queeny’s Herd**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Status** | **Age** | **Vaccinated since** | **Last vaccination** | **Calves born since 2000 vaccinations** |
| 1. Queeny | AF Matriarch | >40 yr | 2000 | 2007 | - |
| 2. Friendly | AF Sub Matriarch | > 40 yr | 2000 | 2007 | April 2002 |
| 3. Stripper | AF | > 40 yr | 2000 | 2007 | Sept 2000 |
| 4. Waves | AF | > 40 yr | 2000 | 2007 | March 2001 |
| 5. Plain Jane | AF | > 20 yr | 2000 | 2007 | - |
| 6. Smelly | AF | > 20 yr | 2000 | 2004 | Dec 2007 |
| 7. Pokerhontas | AF | > 20 yr | 2000 | 2007 | - |
| 8. Cheeky | AF | > 20 yr | 2001 | 2004  (accidentally vaccinated in 2006) | Oct 2001 |
| 9. Tiny | AF | > 12 yr | 2000 | 2004 | - |
| 10. Cindy | AF | > 12 yr | 2002 | 2007 | June 2002 |
| 11. Madame M | SAF | > 12 yr | 2001 | 2007 | August 2002 |
| 12. Bubbles | SAF | > 12 yr | 2005 | 2007 | Nov 2005 |
| 13. Tinkerbell | SAF | > 12 yr | 2005 | 2007 | Dec 2004 |

**Kwatile’s herd**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Status** | **Age** | **Vaccinated since** | **Last Vaccination** | **Calves born since 2000 vaccinations** |
| 1. Kwatile | AF Matriarch | > 55 yrs | 2000 | 2007 | - |
| 2. Anna | AF- collar | >30 yrs | 2000 | 2007 | Sept 2000 |
| 3. Dracula | AF | > 40 yrs | 2000 | 2007 | Oct 2000 |
| 4. Connie | SAF | 9-12 yr | 2002 | 2004 | Dec 2001 |
| 5. Wanda | SAF | 9-12 yr | 2006 | 2007 | Jan 2004 |

**Yvonne’s herd**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Status** | **Age** | **Vaccinated since** | **Last Vaccination** | **Calves born since 2000 vaccinations** |
| 1. Yvonne | AF Matriarch | > 40 yrs | 2000 | 2004 | - |
| 2. Markina | AF- collar | > 40 yrs | 2000 | 2007 | Sept 2001 |
| 3. Enigma | AF | > 12 yrs | 2000 | 2007 | Aug 2002 |
| 4. Raton | SAM | 9-12 yr | 2002 | 2004 | - |

**Holey Ear’s herd**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Status** | **Age** | **Vaccinated since** | **Last Vaccination** | **Calves born since 2000 vaccinations** |
| 1. Holey Ear | AF Matriarch | > 40 yrs | 2000 | 2007 | Aug 2000 |
| 2. Toni | AF- collar | > 40 yrs | 2000 | 2007 | Dec 2001 |
| 3. #2 | AF | > 20 yrs | 2000 | 2007 | Nov 2001 |
| 4. U-Boat | AF | > 20 yrs | 2000 | 2007 | Feb 2001 |
| 5. Knop-kop | AF | 12-15 yrs | 2000 | 2007 | Oct 2001 |
| 6. Quizzy Lizzy | AF | 9-12 yrs | 2005 | 2007 | Jan 2005 |
| 7. Fin | AF | 9-12 yrs | 2005 | 2007 | Jan 2004 |

(Delsink et al 2007)

**Apendix 3.** Time schedule

|  |  |
| --- | --- |
| Total elephant sightings nr. | 72 |
| Total nr sightings with dung collection | 31 |
| Percentage of elephant sightings with dung collected | 43 |
| Mean sighting time (hours) | 34.4 |
| Total sighting time incl. no time noted (min) | 2476 |
| Tot. elephant sighting (hours) | 41.3 |
| Tot. Game drive (hours) | 624 |
| Drives spent on elephant sighting (%) | 6.6 |
| Mean hours of elephant drives per week | 14 |
| Mean elephant drives per day (hours) | 2.8 |
| Total elephant drive (hours) | 218.4 |
| Total elephant sighting (hours) | 41.3 |
| Elephant drives spent on sightings (%) | 18.9 |
| Days spent on game drive | 78 |
| Estimated total number of elephant drives | 55 |