Chapter 6

Immunocontrol of reproductive rate of African elephant cows using porcine zona pellucida vaccine on seven private game reserves in South Africa

HJ Bertschinger[1], A Delsink[2], JF Kirkpatrick[3], JJ van Altena[4], M Bates[5], T Dickerson[2], D Powrie[6]

[1]Section of Reproduction, Department of Production Animal Studies, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa henkbert@tiscali.co.za; [2] School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville Campus, Durban, South Africa; [3]ZooMontana, Science and Conservation Centre, 2100 South Shiloh Road, Billings, MT 59106, USA; [4] Catchco Africa, P O Box 1148, Highlands North 2037, South Africa; [5]Thornybush Private Game Reserve, PO Hoedspruit, South Africa; [6]Private Game Reserve, Po Box 433, Vaalwater 0530, South Africa

Abstract

In southern Africa there is a need for elephant population control, especially in small to medium-sized, fenced reserves. The objectives of this study were to investigate the effects of porcine zona pellucida-immunocontraception on the reproductive rate as well as the safety during pregnancy of elephant cows in seven private game reserves in South Africa. A total of 108 individually identified cows were treated and monitored for 4 to 9 years, depending on when treatment commenced in each reserve. Primary vaccinations consisted of 400 or 600 μg porcine zona proteins with 0.5 ml Freund's modified complete adjuvant and boosters of 400 or 200 μg zona proteins with 0.5 ml Freund's incomplete adjuvant. Vaccine was delivered remotely: Year 1, primary plus two boosters 3-6 weeks apart; Year 2 onwards, annual boosters. Birth of calves was monitored continually and the result expressed as a percentage of cows treated on an annual basis. During Years 1 and 2, 38 (35.2%) and 24 (22.2%) calves were born, respectively. No more calves were born from Year 3 onwards. One cow conceived around the time of primary vaccination and a second between the primary vaccination and first booster. Two calves died soon after birth from unrelated causes. The remainder survived and were normal healthy calves. Sixty two cows (62.0%) have passed the 4-year and 24 (22.4%) the 6-year intercalving interval. The results show that it is possible to achieve a contraceptive efficacy of 100% in small to medium-sized free-ranging populations of African elephants.

Introduction

According to Kerley *et al.* (2008) the impact of African elephants (*Loxodonta Africana*) on ecosystems and biodiversity is difficult to assess. They improve conditions for other herbivores while negatively affecting a number of other animals. They decrease the diversity of plant species on the one hand while improving the landscape on the other. Be that as it may, the general consensus amongst reserve managers is that elephant populations, left uncontrolled in small to medium-sized reserves, will have a negative impact on habitat and thus biodiversity of the reserve concerned. Small reserves are defined as around 100 and medium-sized reserves around 500 km^2 (Mackey *et al.*, 2006). In South Africa many elephant populations were introduced into smaller fenced parks during the 1980s and 1990s. Previously maximum annual population growth rates were estimated at 4 to 7 % (Hanks and McIntosh, 1973; Calef, 1988) whereas recently they have been found to exceed 10 % (Mackey *et al.*, 2006). The rapid population increase known as irruptive growth (Mackey *et al.*, 2009) from density-independent population increase, may eventually lead to die-offs from starvation (Caughly, 1970).

The need to manage elephants, while controversial in the Kruger National Park (KNP), is well accepted in small to medium-sized fenced reserves (Mackey *et al.*, 2006). Traditionally culling has been regarded as the method of choice for controlling large populations (Slotow *et al.*, 2008). However, besides the opposition from many quarters, culling is hardly applicable to smaller populations. The practice is to cull entire breeding herds in order to avoid stress of family members left alive (Slotow *et al.*, 2008). In practice this probably seldom happens (Moss, 1992). In small populations this could mean removing all, half or a third of the breeding animals, depending on the size of the population. Despite being very costly, translocation is regarded as an ideal solution, however, in South Africa, habitat availability is limited (Delsink *et al.*, 2006).

Besides enlargement of parks the only other option to manage elephants is to decrease reproductive success by means of contraception. In selecting a contraceptive method for free-ranging mammals such as African elephants, the following requirements should be met. It must be efficient, reversible, safe, remotely deliverable, which largely determines the cost and have a minimal impact on the social behaviour of the target species (Kirkpatrick and Turner, 1991). Immunocontraception using porcine zona pellucida (pZP) vaccine satisfies all these requirements as has been shown in intensive studies in wild and domestic horses (Liu *et al*. 1989; Kirkpatrick and Turner, 2008) white tailed deer (Turner et al. 1992; McShea *et al.,* 1997; Rutberg and Naugle, 2008) and a number of other free-ranging and captive-held herbivores (Deigert *et al*. 2003; Frank *et al*. 2005; Kirkpatrick and Frank 2005; Kirkpatrick *et al.,* 2009). The putative mechanism for the success of pZP immunocontraception is the

production of antibodies that bind to ZP proteins of target animals' oocytes to prevent sperm binding (specifically to ZP3; Clarke and Dell, 2006), fertilisation and thus pregnancy. Fortunately zona proteins have been well conserved across mammal species and antibodies to pZP have been shown to recognise the African elephant ZP proteins (Fayrer-Hosken *et al.*, 1999).

Earlier immunocontraception trials on African elephants in the KNP showed that the porcine pZP vaccine is safe and effective as a contraceptive in African elephant cows and, in the short term, reversible (Fayrer-Hosken *et al.*, 1997; 1999; 2000). The final efficacy rate achieved was 80 % of vaccinated cows. This initial work was followed by an extensive study in the Greater Makalali Private Game Reserve (Makalali). The vaccine was shown to be 100% effective and, once all cows pregnant at inception of the program had calved, no more calves were born from the third year of the project (Delsink *et al.*, 2006; 2007).

This paper describes the effect of pZP vaccine on reproductive rate of free-ranging African elephant cows in one medium and six small reserves over periods of 4 to 6 years. Makalali is included in this study as previously reported data only covered the first 5 years after inception of the pZP contraception program (Delsink *et al.*, 2006; 2007). Another 5 cows were added to the program (2 in Year 2 and 3 in Year 3) that was not included in the previous papers. Also, dose rates of pZP antigen used during the first three years (2000-2002) were higher than used later on.

Materials and Methods

This is project, *Non-lethal control of African elephant (Loxodonta africana) Game reserves and respective elephant populations,* has been approved by the University of Pretoria's Animal Care and Use Committee, Project number: 36-5-251.

Game Reserves and elephants

The game reserves, their sizes, locations in South Africa and details of elephant populations are shown in Table 1.The elephants on each of the seven reserves were introduced by means of translocation and adult bulls were present on each reserve. Game Reserve, year of inception of the contraception program and number of cows of reproductive age (Laws, 1966; Lee *et al.,*1995) vaccinated during Year 1 were: Makalali, 2000, 18 cows (Deslsink *et al.,* 200); Mabula, 2002, 4 cows; Phinda, 2004, 19 cows; Shambala, 2004, 4 cows; Thornybush, 2004, 19 cows; Welgevonden, 2005, 35 cows and Kaingo, 2005, 4 cows. Additional cows were added during Years 2 (2 cows, Makalali) and 3 (3 cows Makalali), and in Years 4 (Mabula , 1 cow) and 5 (Makalali, 3 cows) were removed from the program so that they could be allowed to reverse. Either before or during the course of Year 1 each target animal was

individually identified (Delsink *et al.*, 2002). This allowed vaccination to take place on an individual cow basis. Prior to treatment, the populations typically had an inter-calving interval of 4.5-5 years and at inception of each program cows were at various unknown stages of reproduction. The Shambala population was captured and translocated to Entabeni Private Game Reserve at the beginning of Year 5 where no bulls of reproductive age were present.

Vaccine and vaccine delivery

The pZP antigen was produced by a modification of the methods described by Dunbar *et al.* (1980). The vaccine was manufactured at the Science and Conservation Centre, ZooMontana, Billings, Montana for the 2000/03 vaccinations. Thereafter, it was produced and supplied by the pZP Laboratory of the Department of Production Animal Studies, University of Pretoria. The During Year 1 each cow of reproductive age was given three pZP vaccinations: primary of 400 µg (600 µg at Makalali and Mabula) pZP in 1 ml phosphate buffered saline (PBS) with 0.5 ml Freund's complete modified adjuvant (Sigma Chemicals Co., St Louis, MO); two boosters of 200 µg (400 µg at Makalali and Mabula) pZP each in 1 ml PBS with 0.5 ml Freund's incomplete adjuvant (Sigma Chemicals Co., St Louis, MO). The intervals between vaccinations were 3-6 weeks. The four cows each in Shambala and Kaingo only received one booster during Year 1. This was followed by annual boosters with 200 µg (400 µg at Makalali and Mabula from 2000-2003; thereafter 200 µg) pZP in 1 ml PBS with 0.5 ml Freund's incomplete adjuvant. Shortly before use, the pZP antigen and adjuvant were mixed using two syringes joined by means of a connector. The fluid was pushed forwards and backwards between the syringes approximately 60 times creating a stable emulsion. Darts were then loaded with the emulsion. During the first three years at Makalali Dan-Inject® (DAN-INJECT ApS, Børkop, Denmark) darts with 60 mm needles were used (Delsink *et al.*, 2007). Thereafter and on the other reserves, Pneu-Dart® (Pneu-dart, Williamsport, USA) darts with 50 mm 13 gauge needles with gel collars were used. Elephants were either darted from the ground or a helicopter (Table 1). To facilitate the identification of cows within a group already darted during helicopter work, most cows were vaccinated with Pneu-Dart® mark and inject darts containing a pink dye (Wonder Mark®, Mafuta Products, Ventersdorp, RSA; Fig.1).

Monitoring of cows post vaccination

Cows on all game reserves were mostly seen one to three times a week but during wet periods spotting intervals were sometimes longer and as much as two weeks between sightings. Birth dates of new calves were taken as the date of first sighting. Mothers were identified with their calves from the close proximity and nursing of the claves (Delsink *et al*., 2002). Duration of gestation was taken as 22 months (Laws, 1966; Hodges *et al.,* 1994). Using this period, stage of gestation could be calculated in cows

pregnant at the time of inception of contraception or shortly thereafter. In order to simplify reporting, gestation was divided into trimesters as follows: 1st trimester, 0-8 months; 2nd trimester, 9-15 months and 3rd trimester, 16-22 months.

Data analysis

The total number of calves born per annum for Years 1 through to 6 were expressed as a percentage of the total number of cows treated each year. Expressing the annual reproductive rate as a calving percentage (calves born/annum/100 cows) was preferred to population growth rate because of the varying circumstances of each population. The χ^2 test was used to analyse annual differences in calving percentage. As the year of commencement of contraception differed between reserves (2000 to 2005) they were normalised so that the date of primary vaccination was the first day and 365 days later the last day of Year 1. The cows added to the trial during Years 2 and 3 at Makalali were also normalised to fit the data. Day 366 was then the start of Year 2 and so on. The staggered commencement also means that by Year 5 the total cow number decreased from 108 to 49 and in Year 6 to 23.

Results

Approximate calving data was available for five of the seven reserves prior to inception of contraception and varied from 16.7% to 25.0% in terms of annual calving percentage per cow of breeding age (Table 1). The mean calving percentages for Years 1 and 2 of the trial varied from 12.5% to 39.5% between reserves with an overall annual mean of 28.7% for 108 cows. This translates to 1.15 calves/cow per cycle of four years or an intercalving interval of less than 4 years and is more accurate than the estimates made prior to the start of contraception.

Following primary vaccination 38 calves were born during Year 1 and 24 during Year 2 providing calving percentages of 35.2% and 22.2%, respectively (Table 2). The difference between the years was significant ($\chi^2 = 4.81$; $p < 0.05$). No calves were born during years 3, 4, 5 and 6 ($p < 0.001$). With the exception of two, all calves (n=60) were conceived prior to the primary vaccination (Table 3). One calf was conceived around the time of primary vaccination and the other between the primary vaccination and the first booster. Of the 108 cows vaccinated during Year 1, 67 (62.0%) have passed the 4 year and 24 (22.4%) the 6 year intercalving interval.

Table 1: Elephant populations on the seven reserves where cows were treated with pZP vaccine

	Makalali	Mabula	Phinda	Shambala	Thornybush	Welgevonden	Kaingo
Size	24 500 ha	8 000 ha	22 800 ha	8 000 ha	11 548 ha	35 000 ha	8 461 ha
Population size (n) Year 1	47	11	92	10	35	117	9
Start of treatment	June 2000	May 2002	July 2004	July 2004	May 2005	Sept 2005	Oct 2005
Cows treated (n) Year 1	23[a]	4	19	4[b]	19	35	4[b]
Age range of cows (years) Year 1	12-50	13-16	10-35	19-25	6-31	9-44	10-40
Cows (n) calved before treatment	No data	3	18	No data	11	25	No data
Estimated mean calving% before treatment (number of years)[c]	21.7%[d]	25.0% (3)	21.0% (6)	No data	16.7% (6)	20.6% (6)	No data
Mean annual calving% during Years 1 and 2 of the study	32.6%	12.5%	39.5%	25.0%	15.8%	30.0%	25%

[a]18 cows were treated in 2000 (Delsink *et al.,* 2000); 2 added in 2001 and 3 in 2002
[b]Only vaccinated twice during Year 1 – primary vaccination was hand-injected
[c]Per number of cows judged to be of breeding age
[d]Adapted from Delsink *et al.* (2006)

Table 2: Number and percentage calves born to treated cows 1-6 years after the start of pZP vaccination

	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Number of reserves	7	7	7	7	4	2
Cows treated	108	108	108	107[a]	49[b]	23
Calves born	38[c]	24[d]	0	0	0	0
Calving %	35.2%	22.2%	0%	0%	0%	0%

[a]One cow removed to allow reversal (Mabula)
[b]Another 3 cows removed to allow reversal (Makalali)
c and d significantly different ($\chi^2 = 4.81$; $p < 0.05$)

Table 3: Calves born after the primary vaccination showing the stage of gestation during primary vaccination and conception in relation to primary vaccination presuming a gestation period of 22 months (Hodges *et al.,* 1994).

Trimester of gestation at time of primary vaccination Number of calves			Conception in relation to primary vaccination Number of calves		
First trimester	Second trimester	Third trimester	Before	Around the primary	Between primary and 1st booster
22	19	21	60	1	1

From the calving dates it was apparent that 62 cows were pregnant at various stages of pregnancy. One calf died as a result of a physical injury soon after birth and another as a result of haemorrhage from the umbilicus at birth. The remaining calves were healthy and survived. Table 3 indicates the stage of pregnancy when the calves as foetuses were exposed to the primary vaccination. About one third (n=22) were in the first trimester and were thus exposed to possible effects of the vaccine as early as the embryonic stage and then at intervals for the remainder of pregnancy.

Discussion

The mean calving percentages of 28.7% during Years 1 and 2 of the trial was higher than those recorded in prior to inception of contraception in 5 of the 7 reserves. There are two possible reasons for these differences. Firstly, contrary to post-inception, birth dates of calves were not available in most reserves during the previous years and ages of calves were estimated according to shoulder height (Laws, 1966; Jachmann, 1988; Lee and Moss, 1995). Secondly, a number of cows in the trial only reached reproductive age around Year 1 of the trial and some were even younger. Although we compensated cow numbers to correct for this, figures quoted should only be regarded as estimates. The mean calving percentage for Years 1 and 2, on the other hand, are in agreement with recently published data for introduced populations (Mackey *et al.*, 2006) which quotes population growth rates of up to and even exceeding 10%. Our data for Years 1, 2 and the mean for the two years shows population growth rates of 12.7%, 7.1% and 9.4%, respectively. The fact that fewer calves were born during Year 2 than Year 1 is likely to be due to chance although the difference between the two years was statistically different.

As reported previously (Delsink *et al.,* 2006) no more calves were born from the third year onwards in this study. Although the number of cows decreased from after Year 4 as a result of the staggered dates of inception, no births were recorded by the end of Year 6. Expressed differently, 62% (n=67) of treated cows have passed the 4-year intercalving interval and 22.4% (n=24) the 6-year intercalving interval by the end of Year 6. For Makalali (excluding the cows taken off contraception) there were still no calves born to treated cows after 7 and 8 years.

The question that surely must be asked is, from when onwards in terms of the initial vaccinations are elephant cows infertile. Our data reflect that one cow conceived around the time of the primary vaccination when the antibody titre was either baseline or just starting to increase. A second cow conceived between the primary vaccination and first booster indicating that at least one booster is necessary to provide sufficient antibodies to block sperm-zona binding and thus a pregnancy from taking place. All remaining 60 cows that calved after inception of the program

conceived prior to the primary vaccination. Although, elephants in the reserves that were treated with the lower dose of pZP (400 µg, primary and 200 µg for boosters vs 600 µg, primary and 400 µg for boosters) have not been running as long as those in Makalali and Mabula, the protocol gave equivalent results from Year 3 onwards. Based on this we have routinely used the lower dosage regimen since the beginning of 2004. The doses required to achieve immunocontraception with pZP in the elephant are considerably smaller than is required for horses if one adjusts for body mass. Similarly the dose of GnRH used to immuno-regulate testosterone secretion in the pig (400 µg) is relatively much larger than is used for the same purpose in African elephant bulls (600 µg; DeNys *et al.*, 2010).

Curiously, the 95% efficacy of pZP immunocontraception achieved over a period of 17 years in wild horses (Kirkpatrick and Turner, 2008) was lower the 100% achieved in African elephant cows. The collective efficacy of pZP immunocontraception in 24 ungulate species 25 bears and 11 sea lions was 93.3% and ranged from 60% (nyala; *Taurotragus angasi*) to 100% in 16 other species such as Bison, Mountain goats, Wapiti, Fallow deer and moose (Frank *et al.,* 2005). Efficacies within the ungulate species varied from 60 to 83% in 6 species and 91.6-100% in the remaining 18 species. All animals reported in the above paper were held and treated in zoos. The one major advantage that possibly contributes to the success rate in elephants is the long interval of approximately 4 years between calves. This means that, with a gestation period of 22 months, the elephant cow takes approximately two years to conceive again. The precise physiology of the latter period is unknown but thought to be similar to lactation anoestrus seen in some domestic species like the sheep and the pig (Bertschinger *et al.*, 2008). Horses on the other hand can conceive within two weeks of giving birth during the so-called foal heat. Furthermore, at any one time, one can expect approximately 50% of cows to be pregnant (Bertschinger *et al.*, 2008). Thus in the elephant there is ample time during the presumed anoestrus and pregnancy periods to achieve good pZP antibody titres capable of preventing fertilisation and pregnancy later on. The very first two pZP-immunocontraception field trials in elephants recorded contraceptive success rates of only 56% and 80%, respectively (Fayrer-Hosken *et al.*, 2000). In both trials 400 µg and 200 µg pZP was used for the primary and booster vaccinations, respectively, but instead of Freund's adjuvants synthetic trehalose dicorynomycolatei (5 mg per vaccinations) was used as adjuvant. During the first trial (n=18; efficacy 56%) the boosters were administered 6 weeks and 6 months after the primary vaccination. In the second trial (n=10; efficacy 80%) two booster were administered at 2-weekly intervals. Another difference which may have been important is the selection of the target elephants. The only selection criterion in our trial was age and all cows estimated to be of reproductive age were vaccinated irrespective of age of calf at foot or being possibly pregnant. The selection of the cows in the Kruger National Park (Fayrer-Hosken *et al.*, 2000) was initially

based on the fact that they had a small calf (< 2 years old) at foot and later, after immobilisation and transrectal ultrasound examination, diagnosed as non-pregnant. The cows selected for treatment may have been in anoestrus or already have resumed ovarian cyclicity. Vaccination of cows that were about to or had already resumed ovarian cyclicity may have been too late to prevent a pregnancy. pZP antibody titres of these cows were never determined meaning that the precise reasons for differences between efficacies of the earlier trials and ours cannot be elucidated.

Just like as in the previous study in elephants (Delsink *et al.*, 2006), we clearly demonstrated the safety of pZP-immunocontraception during pregnancy. The loss of two out of 62 calves was accidental and unrelated to the use of the vaccine. Irrespective of the stage of pregnancy during vaccination, the 60 other calves were born healthy and viable and have survived until today. This means that no developmental abnormalities during pregnancy could be attributed to the use of the vaccine in elephants.

Conclusions

Immunocontraception using the pZP vaccine is highly effective as a method of birth control in African elephants. Calving in treated animals ceases two years after inception of the program. It is 100% safe for conceptuses at any stage of development. The delivery of the vaccine is remote and at no stage requires target animals to be caught or immobilized. The largest population treated so far is Welgevonden with 117 elephants of which 35 are cows of reproductive age. Despite the mountainous terrain of the reserve, a 100% efficacy was achieved meaning that the treatment of larger populations is feasible. According to population modelling of Mackey *et al.,* (2009) contraception of 75% of breeding-age females taking an annual mortality rate of 2-3% is sufficient to achieve an annual population growth of 0%. pZP-immunocontraception presents a proactive means of population control in elephants whereas culling is reactive, and once implemented, must continue indefinitely if it is to succeed. Reproductive rate in African elephants is density dependent (Laws, 1969; Laws *et al.,* 1975) and the response to culling will be an increase in this rate. To improve the practicality of immunocontraception and make the treatment of large populations possible, a slow or sequential release formulation is needed. This will mean only a single vaccination during the first year and maybe greater intervals later on.

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