PORCINE ZONA PELLUCIDA IMMUNOCONTRACEPTION OF AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*): BEYOND THE EXPERIMENTAL STAGE

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ABSTRACT: In southern Africa there is a need for elephant (*Loxodonta africana*) 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 7 private game reserves in South Africa. A total of 108 individually-identified cows were treated and monitored for 6 years. 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 2 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, 35 (32.4%) and 22 (20.4%) 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. One hundred percent of cows passed the 4-year, 67.6% the 5-year, and 47.2% the 6-year inter-calving 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.

KEY WORDS: African elephants, game reserves, efficacy, immunocontraception, porcine zona pellucida, vaccine.

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According to Kerley et al. (2008) the impact of African elephants (*Loxodonta africana*) on ecosystems and biodiversity is difficult to assess. Elephants 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 km^2 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—7% (Hanks and McIntosh 1973, Calef 1988) whereas recently they have been found to be >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 (Caughley 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 to avoid stress of family members left alive (Slotow et al. 2008). In practice this probably seldom happens. Also, in small populations this could mean removing all, half or a third of the breeding animals, depend-

Table 1. African elephant (*Loxodonta africana*) populations on the seven private game reserves where cows were treated with pZP vaccine.

	Game Reserves						
	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
Provincial location	Limpopo	Limpopo	KwaZulu Natal	Limpopo	Limpopo	Limpopo	Limpopo
Broad vegetation type[a]	Granite lowveld	Central sandy bushveld	Zululand lowveld/West Maputaland clay bushveld	Central sandy bushveld	Granite lowveld	Waterberg mountain bushveld	Central sandy bushveld/western sandy bushveld
Population size (*n*) year 1	53	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[b]	4	19	4[c]	19	35	4[c]
Age of cows year 1 (years)	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)[d]	21.7%[e]	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]Classifications as per Mucina and Rutherford (2010)
[b]18 cows were treated in 2000 (Delsink *et al.* 2000), 2 added in 2001 and 3 in 2002
[c]Only vaccinated twice during year 1 (2004) and boosted for another 3 years. Moved to another game reserve in 2008 with no bulls
[d]Per number of cows judged to be of breeding age
[e]Adapted from Delsink *et al.* 2006

ing 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, 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 domestic and wild 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 1 medium and 6 small reserves over periods of 6 years. Makalali is included in this study as additional information is included.

STUDY AREA

The game reserves, their sizes, provincial locations in South Africa and the broad vegetation types of each are shown in Table 1.

METHODS

The protocols for this project were approved by the University of Pretoria's Animal Care and Use Committee, Project number: VO49/11. The elephants on each of the 7 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 (Delsink et al. 2006, 2007); Mabula, 2002, 4 cows; Phinda, 2004, 19

Table 2. Number and percentage calves born to treated African elephant (*Loxodonta africana*) cows 1—6 years after the start of pZP vaccination on 7 private game reserves in South Africa.

	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Number of reserves	7	7	7	7	6	6
Cows treated	108	108	108	107[a]	98[b]	98
Calves born	35	22	0	0	0	0
Calving %	32.4%	20.4%	0%	0%	0%	0%

[a]One cow removed to allow reversal (Mabula)
[b]5 cows removed to allow reversal at Makalali and 4 Shambala cows moved to Entabeni Game Reserve with no bulls of breeding age

cows; Shambala, 2004, 4 cows; Thornybush, 2005, 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) (Delsink et al. 2006, 2007), and in years 4 (Mabula, $n = 1$) and 5 (Makalali, $n = 5$) 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. At the beginning of year 5, after 4 years of contraception, the Shambala population was captured and translocated to Entabeni Private Game Reserve where no bulls of reproductive age were present. The vegetation type (Waterberg) is similar in the 2 reserves. Monitoring of the cows continued on the new reserve.

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, MT for the 2000—2003 vaccinations. Thereafter, it was produced and supplied by the pZP Laboratory of the Department of Production Animal Studies, University of Pretoria. During year 1 each cow of reproductive age was given 3 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); 2 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 4 cows each in Shambala and Kaingo only received 1 booster during year 1. This was followed by annual boosters with 200 µg (400 µg at Makalali and Mabula during 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 2 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 3 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, PA) darts with 50 mm 13 gauge needles with gel collars were used. Elephants were either darted from the ground or a helicopter. 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, South Africa).

Monitoring of Cows Post Vaccination

Cows on all game reserves were mostly seen 1—3 times a week but during wet periods spotting intervals were sometimes longer and as much as 2 weeks between sightings. Birth dates of new calves were taken as the date of first sighting. Mothers were identified with their calves that were either in close proximity or being nursed (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. To simplify reporting, gestation was divided into trimesters as follows: first trimester, 0—8 months; second trimester, 9—15 months and third trimester, 16—22 months.

Data Analysis

The total number of calves born per annum for years 1 through to 6 was expressed as a percentage of the total number of cows treated each year. Expressing the an-

Table 3. Calves born after the primary vaccination showing the stage of gestation and conception in relation to primary vaccination presuming a gestation period of 22 months for African elephant cows (*Loxodonta africana*) (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
18	20	19	55	1	1

nual 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—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 numbers of cows treated each year varied from 98—108 as a result of some individuals being removed from the program for reversal and with the translocation of 4 cows from Shambala to Entabeni Game Reserve (Table 2). With a gestation period of 22 months pregnancies that would have been initiated during the first 4 years at the old reserve would have given rise to the birth of calves during the first 2 years at Entabeni.

RESULTS

Approximate calving data was available for 5 of the 7 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 26.4% for 108 cows. This was equivalent to 1.06 calves per cow per cycle of 4 years and a mean inter-calving interval of 3.8 years.

Following primary vaccination 35 calves were born during year 1 and 22 during year 2 providing calving percentages of 32.4% and 20.4.3%, respectively (Table 2). No calves were born during years 3, 4, 5 and 6 ($P < 0.001$). With the exception of 2, all calves ($n = 57$) 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, 100% passed the 4-year, 67.6% (73 of 108) the 5-year and 47.2% (51 of 108) the 6-year intercalving interval. From the calving dates it was apparent that 57 cows were 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 embryos or foetuses were exposed to the primary vaccination. There was an even spread of gestation stages in terms of pregnancy trimesters. About one third ($n = 18$) were in the first trimester and were thus exposed to possible effects of the vaccine as early as the embryonic stage.

Calculated according to their calving dates, 2 of the 5 cows that were allowed to reverse during year 5 at Makalali, (last vaccination in June 2004) conceived 23 and 34 months after the last treatment, respectively. The remaining 3 cows at Makalali and 1 cow at Mabula have yet to calve 7 years after the cessation of treatment.

DISCUSSION

The mean calving percentage of 26.4% for all 7 reserves during years 1 and 2 of the trial was higher than those recorded prior to inception of contraception in the 5 reserves that had historical data. There are 2 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 prior to inception of the program should only be regarded as estimates. The mean calving percentages 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 2 years shows population growth rates of 10.8%, 6.1% and 8.5%, respectively. The fact that fewer calves were born during year 2 than year 1 is likely to be due to chance.

As reported previously (Delsink et al. 2006) no more calves were born from the third year onwards in this study. The mean inter-calving interval of <4 years (3.8 years) has been passed in 100% of females while 67.6% and 47.2% of cows passed 5- and 6- year intervals, respectively. For Makalali and Mabula, excluding the cows taken off contraception, no calves born to the original 21 treated cows after 10 years. These data once again demonstrate the efficacy of the vaccine to control fertility in African elephant.

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 1 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 55 cows that calved after inception of the program had conceived prior to the primary vaccination. The elephants that were treated with a lower dose of pZP (400 μg, primary and 200 μg for boosters vs 600 μg, primary and 400 μg for boosters) gave equivalent results from year 3 onwards. They have, however, not been treated for as long as the cows in Makalali and Mabula. Based on these results 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 (100 μg) if one adjusts for body mass. Similarly the dose of GnRH vaccine used to immuno-regulate androgen 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 than that 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 (*Bison bison*), mountain goats (*Oreamnos americanus*), wapiti (*Cervus canadensis*), fallow deer (*Dama dama*) and moose (*Alces alces*; Frank et al. 2005). Efficacies within the ungulate species varied from 60—83% in 6 species and 91.6—100% in the remaining 18 species. All animals reported by Frank et al. (2005) were held and treated in zoos. The 1 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 2 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). Ahlers et al. (2012) in a 1-year study found that of 9 adult and 5 subadult cows treated with pZP and monitored by means of faecal progestagen metabolite concentrations, 6 showed regular and 2 irregular luteal cycles. Three cows that showed no proper luteal cycles had calved a mean of 9.3 months and 21.3 prior to the start and end of the study, respectively. This would indicate that the cows were acyclic or in anoestrus throughout the study period. The remaining 3 acyclic cows were subadults indicating that they had not reached puberty yet. Furthermore, at any 1 time, one can expect approximately 50% of African elephant 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 2 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%) 2 booster were administered at 2-weekly intervals.

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 2 out of 57 calves was accidental and unrelated to the use of the vaccine. Irrespective of the stage of pregnancy during vaccination, the 55 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.

Previously short-term reversibility of pZP-immunocontraception could be demonstrated in 3 free-ranging African elephant cows after only year 1 treatment (primary and 2 booster vaccinations). Twenty-two months after the primary vaccination all 3 cows were found to be pregnant on transrectal ultrasound examination (Fayrer-Hosken et al. 2000). The present study investigated the reversal potential in 6 cows that had been treated for 3 (n = 1) and 4 (n = 5) years, respectively. Two cows treated for 4 years conceived 25 and 36 months after the last treatment with pZP. The remaining 4 cows have yet to produce a calf. It seems thus that the interval from last treatment to reversal is quite variable. Two studies have investigated ovarian function using faecal progestagen metabolite concentrations in free-ranging elephant cows treated for 2 to 3 (n = 14; Ahlers et al. 2012) and 4 years (n = 4; Benavides et al. 2012), respectively. With exception of cows that had calved a mean of 9.3 months before the start of the study (Ahlers et al. 2012), all adult females treated with pZP showed evidence of luteal activity. Although the studies were 12 and 14 months long, respectively, neither one could demonstrate negative effects of pZP treatment on luteal ovarian function. In both studies, faecal progestagen concentrations were significantly lower during the dry than the wet season and, in pZP-treated and untreated cows at Entabeni (Benavides et al. 2012), seasonal anoestrus was common. Importantly the latter study showed that, in the absence of conception, free-ranging elephant cows do not necessarily cycle continuously as was previously believed (Bertschinger et al. 2008).

MANAGEMENT IMPLICATIONS

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 cows of reproductive age were targeted. Despite the mountainous terrain of the reserve, a 100% efficacy was achieved meaning that the treatment of larger populations is feasible. 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. Finally, pZP-immunocontraception provides managers of small to medium-sized reserves with a viable and ethically acceptable means of controlling reproduction in African elephants

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