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Field applications of immunocontraception in African elephants (*Loxodonta africana*)

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The primary aim of the Makalali elephant immunocontraception programme is to test the efficacy of porcine zona pellucida (PZP) vaccine for practical population control of elephants in small, enclosed reserves, with the goal of stabilizing the current growth rate and reducing it to the 5–10% per annum displayed currently in the Kruger National Park. A secondary aim is to test the hypothesis that PZP treatment does not affect patterns of elephant social behaviour. Eighteen sexually mature cows (age > 12 years) were vaccinated in May 2000 using remote darts. Behavioural observations before, during and after vaccination included noting the activity of individual animals every minute for 15 min. No changes in general behaviour patterns have been noted to date although the animals' spatial use of the reserve was erratic during the period of vaccination, indicating irregular or disturbed patterns associated with vaccination. Normality was resumed on completion of the vaccinations. No aggressive or indifferent behaviour related to nursing, calf proximity or bull–cow interactions have been noted. Ten of the females were in various stages of pregnancy when treated. Subsequently, seven of them gave birth to healthy calves and the other three females are expected to calve shortly. It is too early in the study to draw conclusions about stabilization of growth rates.

Introduction

Poaching and drought have reduced Africa's elephant population by two-thirds over the past 25 years. Anti-poaching efforts have stabilized the numbers in some sections of Africa, but large areas of former habitat have been converted to agricultural use, thereby trapping elephants in parks and reserves, where the opportunity for them to have negative interactions with humans is minimized. At the same time, high rates of fertility have led to population increases within these reserves, which have necessitated population control measures in the form of either culling or translocation. These forms of population control are often not publicly acceptable and, from a conservation viewpoint, both methods rob populations of genetic diversity (Whyte *et al.*, 1998).

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One form of population control that has proved publicly acceptable is immunocontraception. A vaccine that stimulates antibodies, which can block fertilization, has been applied to a variety of wildlife, including feral horses (Kirkpatrick *et al.*, 1990), white-tailed deer (Kirkpatrick *et al.*, 1996), captive exotic species (Kirkpatrick *et al.*, 1995) and, more recently, African elephants (Fayrer-Hosken *et al.*, 2001). Despite high degrees of efficacy and the ability to deliver the vaccine remotely to free-roaming wildlife, some issues still remain regarding the safety of the vaccine, particularly in species valued by the public. With regard to elephants, one of these issues is whether the vaccine will alter the complex behaviour patterns of this highly social species. In a preliminary study of immunocontraception of Kruger Park elephants, no changes in family group integrity or cow–calf bonds have been noted (Fayrer-Hosken *et al.*, 2001), but long-term behavioural studies are necessary. In addition, the ability to deliver this form of contraception to free-roaming elephants by means other than from a helicopter has not been investigated.

This study was undertaken to test five hypotheses. Firstly, that the porcine zona pellucida (PZP) vaccine could be delivered successfully to free-roaming elephants in a small game park. Secondly, that the use of a new adjuvant, Freund's modified adjuvant, could increase the efficacy of the PZP vaccine beyond that achieved in the Kruger Park elephant project. Thirdly, that PZP treatment would not alter selected social behaviours. Fourthly, that treatment of pregnant females with the PZP vaccine would not harm pregnancies in progress or affect the health of the offspring. A fifth hypothesis, not yet tested and not reported in the present study, is that PZP immunocontraception could reduce the rates of population increase and stabilize elephant populations in a small game reserve.

Materials and Methods

Study area

Makalali private game reserve, Northern Province, South Africa is located between 30.49974°S and –24.00517°E and is approximately 14 500 hectares in size. The non-perennial Makhutswi river, originating from the Drakensberg Mountains and a tributary of the Olifants River, flows in a west to east direction through the middle of the park. The Kameelsloot (Stomane) river forms the main tributary of the Makhutswi. It has been dry since 1996 apart from a short flow period in January 1999.

Elephant identification

All the elephants, except for infants and some juveniles, have complete identification comprising frontal, left and right-sketched templates with corresponding photographs. The animals are identified according to their unique ear patterns, comprising nicks, tears and holes, as well as their tusk shape, length and absence or presence. When no distinguishing ear or tusk features were visible, ear venation patterns were completed. Other distinguishing features such as growths, lumps, scars and tail hairs were also recorded as identification criteria. The herds are monitored on a daily basis where the Global Positioning System (GPS) co-ordinates, date, time, herd matriarch, mood, weather and presence of bulls are noted. Identification templates and photographs are updated on a regular basis and whenever new identification features are observed.

Presently, the population consists of 57 animals comprising four breeding herds headed by the matriarchs 'Holey Ear', 'Queeny', 'Kwatile' and 'Yvonne', respectively and 12 adult bulls (aged > 12 years).

The Makalali elephant population was translocated from Kruger National Park in 1994 and

Table 1. Makalali elephant population demographics 1996–2001

	1996	1997	1998	1999	2000	2001	Present population (June 2001)
Total 1 January	37	41	44	46	48	55	57
Calves born during the year	4	3	2	2	5	2 (after January)	–
New introductions	–	–	–	–	3	–	–
Fatalities	–	–	–	–	1	–	–
Finite rate of increase[a]	–	1.108	1.073	1.045	1.043	1.083	1.038

[a]Excludes new introductions and fatalities.
Average population growth rate 1996–2001: (57 – 37)/37 × 100 = 54.05%.
54.05/6 = 9.01% per annum.

1996 and consisted of 13 and 24 animals, respectively. Since the last introduction in 1996, the elephant population has shown an average annual growth rate of 9.2%. (Table 1).

Criteria for selection

Pregnancy status was not a criterion for selection and, therefore, all the sexually mature cows (aged > 12 years) were selected for vaccination. Eighteen cows were vaccinated in May 2000. Pregnancy status was estimated according to the age of calves at foot or retrospectively, based on the dates of parturition, to determine the efficacy of the vaccine as a contraceptive (whether the cows conceived before or after vaccination).

Vaccination regimen

The PZP antigen was produced by a modification of the methods described by Dunbar *et al.* (1980). All the targeted animals received an initial vaccination of 600 µg PZP plus 0.5 ml Freund's modified adjuvant (Sigma Chemical Co., St Louis, MO) followed by two booster vaccinations of the same antigen emulsified in Freund's incomplete adjuvant, each 2–3 weeks apart. For minimal impact and stress on the herds, the selected elephants were darted from the ground using the Dan-Inject darting system (Dan Inject International, Borkop). Two millimetre darts and smooth 60 mm needles that were modified by increasing the bore of the side parts and which would fall out a short time after impact and therefore allow them to be recovered, were used. After each vaccination, the date, dart site, the animal's longitudinal and latitudinal position as recorded with a Garmin Global Positioning System, the animal's reaction and whether darted from foot or a vehicle were recorded. An attempt was made to recover all darts to document the success of vaccine delivery. On average, the darts were fired at ranges of 10–45 m.

Behavioural observation methods

Behavioural observations were based on 15 min time budgets, in which an animal's activity was recorded every minute for 15 min. The method was derived from the models described by Pulliam *et al.* (1981), in which time is used as a currency of the animals' behaviour. The time budget is also based on the method used by Moss (1988), known as 'focal samples', in which the observer focuses on one animal for a given amount of time.

Activities were divided into active (feeding, drinking, bathing (dust, mud or sand), moving),

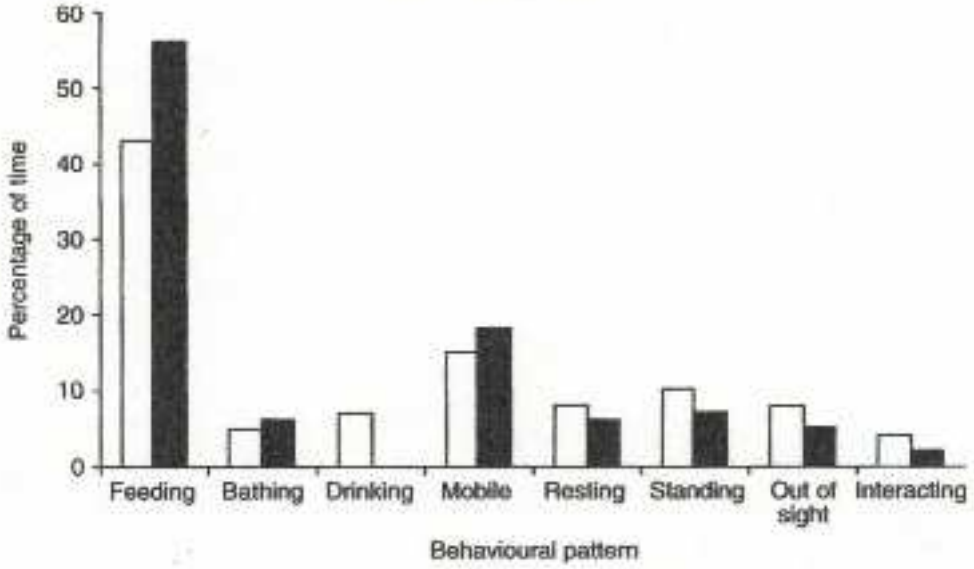

Fig. 1. General behaviour patterns of 30 Makalali elephants for 14 months before (□) and 9 months after (■) porcine zona pellucida (PZP) treatment.

inactive (resting, standing, lying down) and interactive (displacement behaviour, sparring) categories as determined originally by Poole (1996). A range of animals from different age groups was monitored, irrespective of whether they were vaccinated. These observations were made for 14 months before and 9 months after treatment (Fig. 1). Although individual animals were studied, in most instances, family groups or herds are synchronized in their activities (all the members feed, walk, rest and drink simultaneously), as described by Moss (1988). Thus, an individual's actions are normally reflective of the entire group. The presence and behaviour of bulls were also recorded. Through daily monitoring, other details such as calf births, displays of oestrous behaviour, bulls in musth, herd associations, general mood, weather conditions, time of day and latitudinal and longitudinal co-ordinates were recorded. A visual database was also compiled using footage gathered on a Sony digital camera. These data recorded the animals before, during and after the vaccinations.

Spatial use

Spatial use of the 14 500 ha reserve was determined from systematic sightings of the animals. At each sighting, the animal's or the herd's geographic locations were recorded using a GPS. These latitudinal and longitudinal points before, during and after the vaccinations were plotted on to a map of the park using Cartalinx and Arcview Version 2 Geographic Information System (GIS) software. Kernel home range patterns were determined using Arcview's Animal Movement Analysis (United States Geographical Service) extension.

Results

Dart retrieval and success rate

The darting period was from 30 May 2000 to 21 July 2000. Of the 62 darts fired, 49 (79%) were retrieved and found to have discharged their contents correctly. Four (6%) darts did not

Table 2.

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Not retrieved
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Only half disch
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Table 2. Dart retrieval and success rate for year 2000 vaccinations of Makalali elephant population

Darts	Initial vaccine	First booster	Second booster	Total	Percentage
Fired	22	18	22	62	–
Retrieved	19	14	16	49	79
Not retrieved	3	4	6	13	21
Unsuccessful	4	0	4	8	13
Not discharged	2	0	2	4	6
Only half discharged	2	0	1	3	5
Misfired	0	0	1	1	2

Table 3. Darting from vehicle versus darting on foot of Makalali elephant population

Method	Initial vaccine	First booster	Second booster	Total	Percentage
From vehicle	21	17	10	48	77
From foot	1	1	12	14	23
Total	22	18	22	62	100

discharge at all; two (5%) discharged only a portion of the contents and one (2%) dart misfired due to gas leakage (Table 2).

Development of granulomas

Of the 18 cows vaccinated and given booster vaccinations between May and June 2000, 9 (50%) displayed granulomas (fluid-filled sterile abscesses). Two months later, granulomas were present in an additional seven (38%) cows. Thus, in total, 89% of the targeted cows displayed granulomas within 3 months after vaccination. The presence of a granuloma indicated a good immunological response. The granulomas ranged from 2 cm to 10 cm in diameter and were all resorbed eventually.

Behavioural disadvantages

Of the 62 darts fired, 47 (77%) were fired from the vehicle and 15 (23%) were fired on foot, away from the vehicle. As time progressed, the animals associated the vehicle with darting and became increasingly wary of it. Accordingly, the necessity to dart from on foot increased (Table 3).

At the completion of the second booster injection, the animals' spatial use patterns became more haphazard and unpredictable. The animals' Kernel home range patterns (their fixed kernel home range use distribution) before (100 sightings), during (66 sightings) and after (100 sightings) the vaccinations are shown (Fig. 2a–c). Kernel home range patterns before the vaccination programme indicated a 75% occupancy of the western portion of the Makhutswi river. However, by the second booster injection the elephants' movements became more haphazard and irregular with a noticeable change in their Kernel home range pattern. The 75% area of occupancy increased and was more pronounced.

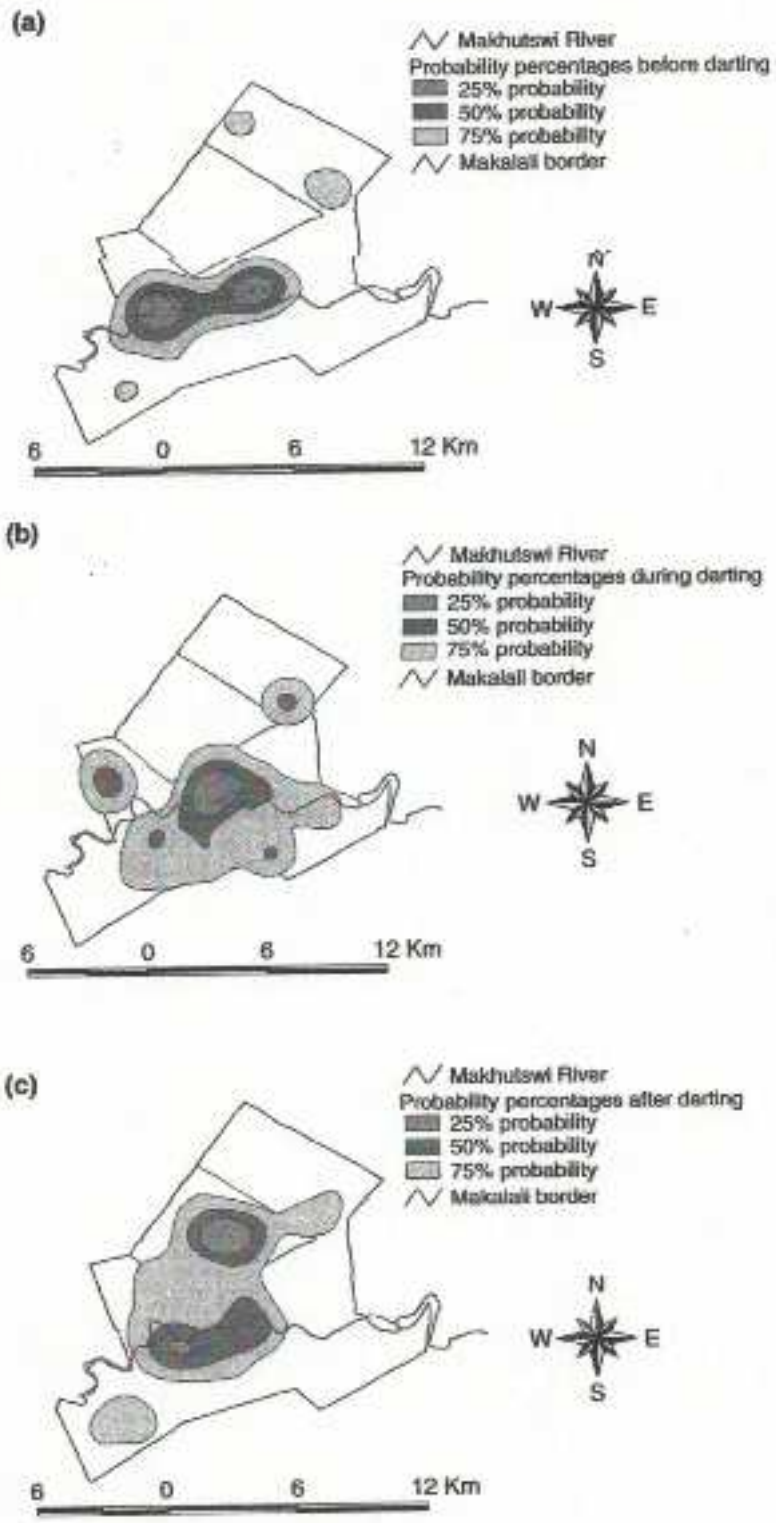

Fig. 2. For legend see facing page.

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Herd integrity

To date, no change in the social hierarchy among the cows of the various breeding herds has been noted. Daily observations have indicated that the matriarchs have remained in rank position and the other cows that received anti-fertility treatment have not changed social rank.

Since the vaccination programme in 2000, seven calves have been born to cows that received anti-fertility treatment. (These cows were all pregnant before vaccination.) Monitoring of these youngsters has shown normal cow–calf relationships in all cases and there is no evidence of aggressive or indifferent behaviour by the mother with regard to nursing time and nursing behaviour.

Bull interactions

After the vaccinations were completed, there appeared to be no differences in the interactions of bulls among themselves or with the cows, and bulls remained in close proximity to the family groups.

Discussion

Using the Dan-Inject darting system and provided that the target females can be identified easily so that the additional booster injections can be administered to the correct females, the PZP vaccine can be delivered successfully to free-roaming elephants in a small game park. With technology moving forward towards a single-injection immunization procedure (Turner and Kirkpatrick, 1996), the time required for delivery of the vaccine and the impact on the animals will be reduced further. The use of a helicopter would further increase the efficacy of immunocontraception by eliminating the time spent tracking animals on foot and allowing a larger number of animals to be vaccinated in a shorter period.

As some of the vaccinated females are pregnant and are only due to receive their first annual booster injection in June 2001, it is too early to determine whether the use of Freund's modified adjuvant will increase the efficacy of the PZP vaccine beyond that achieved in Kruger National Park.

The third aim of the present study was to determine whether the PZP treatment protocol would alter selected social behaviour patterns. Given the fact that elephants lead extremely complex social lives and that this network of relationships is the fibre that makes up elephant society (Moss, 1988), the answer will be difficult to obtain. Despite the numerous accounts of fertility parameters associated with contraceptive studies in captive and free-roaming mammals, there are few data on the social and behavioural effects of the treatments (Asa, 1996). However, on the basis of the immunocontraceptive trials conducted on 21 elephants in Kruger National Park during 1996, the use of GPS-collar location indicated that there was no separation of vaccinated females from family groups. This finding, together with field observations made on the vaccinated females, indicates that the immunocontraceptive protocol causes no behavioural abnormalities. As the vaccine is non-hormonal and non-steroidal, fertilization is prevented without the side effects normally associated with hormonal contraceptives (Fayrer-Hosken *et al.*, 2001).

The only change noted was the increasing wariness of the animals toward the vehicle used for darting. However, soon after completion of the initial vaccination programme, the animals once again became relaxed enough to allow the open vehicles to approach them without

Fig. 2. Kernel home range pattern of Makalali elephants (a) before, (b) during and (c) after vaccination with porcine zona pellucida (PZP) vaccine in 2000.

running away. As a result, all subsequent booster vaccinations were carried out on foot to avoid any further association of game viewing vehicles with the darting procedure.

Ten cows in various stages of pregnancy were vaccinated in May 2000 and subsequent to these treatments, seven calves were born alive and three more calves are expected within the next few months based on the ages of their calves at foot. Six of the new calves remain healthy and display no abnormalities and the seventh calf was killed by an unexplained attack by a young bull elephant. A healthy calf was born from a cow vaccinated in the last trimester of gestation in Kruger Park, indicating clearly that the vaccination of a pregnant elephant with PZP in late gestation has no effect on gestation, fetal development or parturition (Fayrer-Hosken *et al.*, 2001).

As the Makalali immunocontraception programme has only been underway for 1 year and the target animals will only receive their first annual booster injection in June 2001, it is too early for conclusive findings in relation to stabilization of the rate of population growth. Some target cows were pregnant before contraception and some should calve within the next few months. As a result, full reproductive control will only be achieved in the third year of the vaccination programme.

In conclusion, no significant changes in the target animals' general behaviour were noted and feeding remained their foremost activity. Owing to the high impact of the initial darting phase that involved three vaccinations at intervals of 2–3 weeks, the animals became wary of vehicles and remained skittish for approximately 3 weeks thereafter. Their spatial use increased during the 6 week darting period and their movements were more sporadic and widespread. A further complication after the darting period was the removal of adjoining fences to add a further 2000 hectares to the reserve. Once the darting had been completed and the fences were removed fully, the elephants used this area extensively.

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