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## DEVELOPMENT OF A BAIT AND BAITING SYSTEM FOR DELIVERY OF ORAL RABIES VACCINE TO FREE-RANGING AFRICAN WILD DOGS (*LYCAON PICTUS*)

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**ABSTRACT:** The objective of the study was to develop a bait and baiting system capable of delivering one effective dose of oral rabies vaccine to each member of a free-ranging African wild dog (*Lycaon pictus*) pack. Trials were conducted between June and October 2000. The results of cafeteria-style bait preference trials testing seven candidate baits in captive wild dogs revealed a significant preference for chicken heads (June trials:  $P = 0.023$ , September trials:  $P = 0.021$ ). Trials using a topical biomarker (rhodamine B) showed that chicken head baits were sufficiently chewed on most occasions to rupture the vaccine container. Free-ranging wild dogs and young pups ingested chicken head baits. Significant dominance of bait intake by a single individual was seen in four of six study packs and in the three packs in which an alpha pair could be distinguished, the dominant feeder was an alpha animal. Pattern of bait distribution and degree of satiation had no effect on pack coverage (proportion of pack ingesting at least one bait). Pack coverage was significantly related to trial number ( $r = 0.71$ ,  $P < 0.001$ ), with pack coverage increasing with increased exposure of the pack to the baits. During 46 hr of diurnal observations of free-ranging wild dogs only two baits were lost to non-target species. A baiting system for the oral vaccination of captive and free-ranging wild dogs is proposed.

### INTRODUCTION

African wild dog (*Lycaon pictus*) populations have declined throughout their range in the past century, with this trend accelerating in the past three decades (Woodroffe et al., 1997). The species is currently listed as endangered by the International Union For Conservation of Nature and Natural Resources (1996), with an estimated 3,000–5,500 individuals remaining (Woodroffe et al., 1997). In addition to persecution, habitat loss, snaring, and road accidents, infectious disease has emerged as a major cause of deaths among wild dogs (Woodroffe et al., 1997; Woodroffe and Ginsberg, 1999). Rabies in particular causes high mortality and has been diagnosed in wild dog populations that suffered dramatic declines or local extinctions in Madikwe Game Reserve in South Africa (Hofmeyr et al., 2000), the Serengeti region of Tanzania (Gascoyne et al., 1993), Masai Mara National Reserve in Kenya (Kat et al., 1995), and Etosha National Park in Namibia (Scheepers and Venzke, 1995). It also has been implicated in wild

dog deaths in the Central African Republic, as well as in Zambia and Zimbabwe (reports cited in Woodroffe et al., 1997). Being a lethal viral disease transmitted through saliva (Swanepoel, 1994), and due to the highly social nature of wild dogs, rabies is transmitted rapidly within a pack (Mills, 1993). Even if some pack members survive, it appears that a critical minimum number of helpers is needed for a pack to persist (Carbone et al., 1997; Woodroffe et al., 1997; Courchamp et al., 1999). This has important conservation implications for wild dogs as the pack, not the individual, is considered the basic unit of a wild dog population (Woodroffe et al., 1997).

The failure of parenteral vaccination to adequately protect free-ranging wild dogs against rabies (Kat et al., 1995; Scheepers and Venzke, 1995; Hofmeyr et al., 2000) and the logistic difficulties, costs, and potential for injury to the dogs associated with this method, have prompted research into developing an effective oral vaccination technique. Oral vaccination has been successfully used to control rabies in red

foxes (*Vulpes vulpes*) in Europe (Aubert et al., 1994) and North America (Campbell et al., 1994) and is presently used in the field for coyotes (*Canis latrans*; Farry et al., 1998), gray foxes (*Urocyon cinereoargenteus*; Steelman et al., 1998) and raccoons (*Procyon lotor*; Roscoe et al., 1998). Oral vaccination has also been considered for jackals (*Canis adustus* and *C. mesomelas*) in southern Africa (Bingham et al., 1999) and domestic dogs (Fekadu et al., 1996). Wild dogs differ, however, from the other species targeted in oral rabies vaccination programs in that they are highly social animals. They breed and hunt cooperatively in packs of up to 20 adults plus dependent young (Creel and Creel, 1995); there are separate rank hierarchies among male and female pack members, and one breeding (alpha) pair maintains social and reproductive dominance (Estes, 1991). Wild dogs also utilize large home ranges (mean = 650 km<sup>2</sup>, range 150–2,460 km<sup>2</sup>; Woodroffe and Ginsberg, 1999), but become confined to a relatively small area during the denning season. These unusual characteristics require a specifically designed oral vaccination system.

Successful oral vaccination depends on two integrated components that must both be effective for each target species: an oral vaccine and a bait. The oral vaccine is contained in liquid form within a capsule that is inserted into a suitable bait, so when the bait is chewed the capsule ruptures and the live vaccine infects the oral and pharyngeal mucous membranes, inducing an immune response. For wild dogs an effective oral rabies vaccine, SAG-2 (Virbac, Carros, France), has passed preliminary efficacy trials and is currently undergoing further testing by the authors. The objective of this study was to develop the next stage, which is the selection of a bait and baiting system capable of delivering at least one effective (2 ml) dose of oral vaccine to each member of a free-ranging wild dog pack, with minimal exposure of non-target species. We set the criteria for an effective bait to include properties that

protect the vaccine against microorganism contamination, temperature extremes, and ultraviolet radiation. Additionally the bait substrate should be highly palatable and safe for ingestion by all pack members including pups; it should stimulate chewing before swallowing to rupture the capsule and deliver a sufficient amount of the vaccine to the oropharyngeal mucosa; and should be cheap and readily available in adequate quantities across the wild dog range. The overall baiting system should be suitable for deployment around the den, as this is the time of the year when wild dogs are most accessible, and should ensure the delivery of at least one bait to each pack member including pups.

## MATERIALS AND METHODS

### Study areas

Trials were conducted on captive and free-ranging wild dog packs in South Africa. Captive animals were at the Rhino and Lion Nature Reserve (RLNR; 26°00'S, 27°45'E) and the De Wildt Cheetah Research Centre (DCRC; 25°35'S, 27°55'E), while free-ranging animals were in the Kruger National Park (KNP; 25°00'S, 31°35'E) and Madikwe Game Reserve (MGR; 24°45'S, 26°20'E). The size, composition, and diet of the packs were recorded for each of the various trials (Table 1).

### Bait preference trials

The aim of these trials was to determine the most preferred bait type from a range of seven candidates (Table 2) selected for practicality in the field, being either commercially available ready-made baits or baits that could be prepared quickly and cheaply from locally available material. In total 29 dogs from six captive packs were used in the bait preference trials. In addition to the pack trials, three individually enclosed adult females (RAF<sub>1</sub>, RAF<sub>2</sub>, and RAF<sub>3</sub>) were also tested at RLNR. These females were pregnant and were separated in late gestation to protect the pups. RAF<sub>3</sub> lost her litter, but the other two were approximately 4 wk into lactation when they were tested.

Two sets of preference tests were conducted in June and September 2000. Due to a delay in the manufacture of the liver- and fish-flavored baits only the first five baits were evaluated in the June trials. The remaining two baits were then compared in September against the most preferred bait from the June trials. Each

TABLE 1. Size, composition, and dietary history of the African wild dog packs used in baiting trials conducted between June and November 2000.

Pack names and location	Total number of dogs	Adults		Yearlings		Pups	Diet	Trials <sup>a</sup>
		M	F	M	F			
<b>RLNR<sup>b</sup></b>								
RA	8	2		5	1		Meat	1, 4
RB	4			2	2		Meat	4
RAF <sub>1</sub>	1		1				Meat	1
RAF <sub>2</sub>	1		1				Meat	1
RAF <sub>3</sub>	1		1				Meat	1
<b>DCRC<sup>c</sup></b>								
DA	2	1	1				Meat and chicken	2
DC	3	1	2				Meat and chicken	2, 3, 4
DM	3			3			Meat	1, 3, 4
DP	3					3	Dog food	3, 5
DS	7	1	1	2	3		Dog food	1, 2, 3, 4
DT	2	1	1				Meat and chicken	2, 3, 4
DX	6			3	3		Dog food	1
DY	3			2	1		Meat and dog food	1
DZ	2			1	1		Meat and dog food	1
<b>KNP<sup>d</sup></b>								
KR	4	2	2	0	0	?	Meat	4
KB	21	3	1	0	0	17	Meat	4, 5
<b>MGR<sup>e</sup></b>								
MD	17	3	2	1	1	10	Meat	3, 4

<sup>a</sup> Numbers indicate the specific trials in which the pack was utilized: 1 = bait preference trials 2 = attractant preference trials 3 = dominance trials 4 = pack coverage trials 5 = pup intake trials.

<sup>b</sup> Rhino and Lion Nature Reserve.

<sup>c</sup> De Wildt Cheetah Research Centre.

<sup>d</sup> Kruger National Park.

<sup>e</sup> Madikwe Game Reserve.

pack/individual was only tested once. Preference tests took the form of a cafeteria trial, in which an array of food types are presented to a subject in equal abundance so that availability does not directly affect the measurement of preference (Krebs, 1989). Baits of each type were placed in a large Perspex tray (45 × 28 × 12 cm), with the same number of baits of each type being offered in a single trial. The number of all baits offered was, however, adjusted between trials depending on the number of dogs in the pack. This was so that the total bait mass per dog fell within the range of reported daily consumption rates (Fuller and Kat, 1990; Fuller et al., 1995) but was well below maximum gut capacity (4.4 kg; Reich, 1981). The trays (one for each bait) were put out randomly on the ground about 0.5 m apart in the dogs' enclosure, close to the normal feeding area. Tests were all conducted around the animals' normal feeding time, prior to them having eaten. The rate at which each bait type was eaten was determined by recording the number of baits that

remained in each tray at 2 min intervals during the 10 min observation period. A bait was considered 'eaten' if it was deemed to have been sufficiently chewed to potentially rupture a concealed vaccine container. The cumulative proportion of each bait type eaten was plotted against elapsed time, and Rodger's index (Krebs, 1989) was used as a measure of preference. The index is determined by calculating the area under each graph and standardized by dividing each result by the largest area obtained, so that the most preferred bait receives an index score of one. The Rodger's index of a bait is thus a composite score of the amount of the bait that is eaten and the rate at which it is eaten. The bait selected in these trials was then utilized in subsequent field tests.

#### Attractant preference trials

Two attractants were tested, based on known feeding behavior of wild dogs and on field observations. The first was a liver-offal slurry of

TABLE 2. Characteristics of the seven baits evaluated in preference trials conducted on captive African wild dogs in June and September 2000.

Bait type	Description	Dimensions (mm)	Weight (g)
Dog food <sup>a</sup>	Hollowed-out cube of dog food/ polymer additive	34 × 34 × 19	14
Lard/wax (meat) <sup>b</sup>	60% (by weight) pork lard, <sup>d</sup> 30% paraffin wax, <sup>e</sup> 10% meat-flavored attractant <sup>f</sup>	45 × 30 × 20	25
Lard/wax (chicken) <sup>b</sup>	As above, but with chicken-flavored attractant <sup>g</sup>	45 × 30 × 20	25
Mince/chicken foot <sup>b</sup>	Ball of beef mince packed around a chicken foot	40 × 40 × 40	50
Chicken head <sup>b</sup>		45 × 39 × 39	49
Liver flavored <sup>c</sup>	Proprietary formulation	50 × 40 × 14	25
Fish flavored <sup>c</sup>	Proprietary formulation	50 × 40 × 14	25

<sup>a</sup> Bait-Tech, Orange, Texas, USA.

<sup>b</sup> Homemade.

<sup>c</sup> Virbac, Carros, France.

<sup>d</sup> Eskort, Heidelberg, South Africa.

<sup>e</sup> Merck Laboratory Supplies, Halfway House, South Africa.

<sup>f</sup> Meat boost, Nutritional foods, Industria, South Africa.

<sup>g</sup> Chickon, Nutritional foods, Industria, South Africa.

chopped beef liver, minced offal, and blood. The second was cheetah scats, selected on the basis of anecdotal evidence that large felid scats, which are pungently odiferous, are highly palatable to wild canids. Attractant preference trials were conducted on four packs at DCRC (Table 1). Placebo baits were rolled-up sheets of paper toweling, dipped in attractant and placed in the Perspex trays described above. A control tray had rolled-up toweling only. The experiments were conducted in the same way as the bait preference trials, using Rodger's index as a measure of preference. The time that the alpha female in the pack spent with each attractant (alpha female contact time, or AFCT) was recorded as an additional indication of preference.

Values of Rodger's index were arcsine-square root transformed and tested for normality using the Kolmogorov-Smirnov goodness of fit test (Zar, 1996). A one-way analysis of variance (ANOVA) was used to test the effect of bait type on the Rodger's index and of attractant type on AFCT. The Student-Newman-Keuls (SNK, Zar, 1996) method was used for multiple comparison procedures when a significant F-test was obtained. All tests were considered statistically significant at  $P \leq 0.05$ . The data derived from the attractant preference trials were found to be non-normally distributed and withstood all attempts at transformation. The Kruskal-Wallis one-way ANOVA on ranks was therefore applied. Dunn's method (Howell, 1987) was applied for multiple comparisons.

#### Biomarker trials

For effective vaccine delivery, a bait has to be chewed sufficiently to rupture the vaccine capsule and we tested this using rhodamine B (Sigma Chemical Company, St Louis, Missouri, USA) in the vaccine capsules (Virbac). Rhodamine B is a topical tissue marker that discolors fur, skin, and mucous membranes. It has been successfully used to simulate and evaluate oral vaccine/bait combinations in several species (Farry et al., 1998; Steelman et al., 1998; Bingham, 1999). A 2 ml dose of 375 mg rhodamine B was placed in each capsule, equivalent to 15 mg/kg body mass based on a mean mass of 25 kg for wild dogs (Estes, 1991). The vaccine containers were then stapled under the skin of chicken heads (which were found to be the most effective baits, see Results). One such bait was then fed to each of eleven adult wild dogs at DCRC that were to be immobilized for an unrelated management procedure. Dogs were immobilized 100–140 min after the baits were administered. During this time dogs were observed from a distance for any signs of visible staining of the oral cavity and during the immobilization they were observed for any signs of regurgitation. The oral cavity was examined under ambient light and the presence of staining on the tongue, buccal mucosa, palate, tonsils, and oropharynx recorded. The fate of the vaccine capsules was also noted.

#### Bait uptake by wild dog pups

Five trials were conducted on a pack of three captive 12 wk old pups (DP) to ascertain if they

could successfully ingest chicken heads. An additional six trials were conducted on a free-ranging pack in the KNP that contained 17 10 wk old pups (KB). The mean proportion of baits eaten per trial and the mean number of chews per bait were calculated for both packs.

#### Bait uptake by non-target species

Observations on uptake of chicken head baits by diurnal non-target species were made during 46 hr of trials involving free-ranging packs around dens. To determine potential vaccine exposure to nocturnal non-target species, sites were selected away from the wild dog dens to avoid disturbing the dogs. At each of four sites, located at least 10 m away from any dirt roads, a sandy area was cleared and swept. Twenty chicken head baits were placed in two rows, 1 m apart. Baits were placed in the late afternoon and plots were examined 12 hr later for remaining baits and all mammalian tracks were recorded.

#### Dominance trials

To test if one or a few high-ranking individuals in a pack dominate bait intake, a total of 25 dominance trials were conducted on five captive packs, with a further six trials on one free-ranging pack in MGR (MD pack). During the captive trials the equivalent of one chicken head bait per pack member was placed by hand in the pack's enclosure. Baits were placed approximately 1 m apart in a single line. Trials were conducted in the early morning, prior to the normal feeding time. In the MD pack, testing was done when the pack did not go out on a hunting session, either in the early morning or late afternoon. In this way it was ensured that the dogs had last eaten at least 10 hr previously. Baits were placed from a vehicle, 1 m apart on the edge of a dirt road running very near the den. Packs were observed for 3 hr, or until all baits had been ingested. The proportion of baits eaten by each pack member was recorded. Individuals were identified by their unique coat patterns. The number of antagonistic interactions between pack members and the individuals involved was also noted. Proportions were arcsine-square root transformed. Data from the captive packs were found to be normally distributed and a one-way ANOVA was used to test for significant differences in mean proportion of baits eaten by each pack member. The SNK method was used as a multiple comparison procedure if a significant difference was found. Data derived from the MD pack failed the normality test and resisted transformation. The Kruskal-Wallis one-way ANOVA on ranks was therefore applied and

Dunn's method was used for multiple comparisons.

#### Pack coverage tests

Two factors, pattern of bait distribution and degree of satiation, were investigated in relation to pack coverage (the number of dogs in a pack ingesting at least one bait). Three patterns of bait distribution were examined; clumped (all baits thrown out in a small area, approximately 1 m<sup>2</sup>), spaced (baits placed out in a single row and spaced 1 m apart), and targeted (bait thrown directly at a selected animal in a pack). A total of 30 trials were conducted in six captive packs and three free-ranging packs. Only one targeted delivery trial was conducted in the free-ranging packs as it was found to disturb the dogs excessively. The proportion of pack members ingesting at least one bait in the 3 hr observation period was recorded. The dataset was arcsine-square root transformed and subjected to a one-way ANOVA to test for the effect of distribution pattern on pack coverage.

The free-ranging MD pack was used to investigate the relationship between degree of satiation and pack coverage, assuming the higher-ranking animals might relax their dominance of food intake when satiated. A 'belly fullness score' (BFS) was used as an index of satiation. Scores were assessed visually and ranged from 1 (empty) to 4 (markedly distended). Baits were placed in one of two patterns (spaced or clumped) when the dogs left on a hunt in the morning and afternoon and a BFS was assigned to each animal on their return. It was found that all animals in a pack returned from hunting with the same BFS, so scores were pooled into a common pack BFS prior to each trial. A total of 22 trials were conducted. Again the proportion of the pack ingesting more than one bait in the 3 hr after their return was recorded for each trial. A two-way ANOVA was performed using distribution pattern and BFS as treatments.

## RESULTS

#### Bait preference trials

In the pack preference trials (Table 3) chicken head baits were most preferred in all six packs tested (June trials:  $F = 4.621$ ,  $df = 14$ ,  $P = 0.023$ ; September trials:  $F = 7.810$ ,  $df = 8$ ,  $P = 0.021$ ). In the trials on individual dogs, chicken heads scored a Rodger's index of 1 with both RAF<sub>1</sub> and RAF<sub>2</sub>, but RAF<sub>3</sub> did not ingest any baits during the 10 min observation period.

Only one antagonistic encounter was

TABLE 3. Results of bait preference trials conducted on packs of captive African wild dogs during June and September 2000.

Trial date	n <sup>a</sup>	Bait type	Preference index <sup>b</sup>	
			x <sup>c</sup>	SE
June	3	Chicken head	1.000 <sup>†</sup>	0.000
		Mince/chicken foot	0.453 <sup>†††</sup>	0.269
		Lard/wax (chicken)	0.223 <sup>†††</sup>	0.118
		Dog food cube	0.200 <sup>†††</sup>	0.195
		Lard/wax (meat)	0.123 <sup>†††</sup>	0.118
September	3	Chicken head	1.000 <sup>†</sup>	0.000
		Liver flavored block	0.537 <sup>†††</sup>	0.167
		Fish flavored block	0.473 <sup>†††</sup>	0.384

<sup>a</sup> Number of packs of wild dogs used in a trial.

<sup>b</sup> Rodger's index of preference, expressed in terms of  $x \pm SE$ . See text for details of calculation.

<sup>c</sup> Means with the same number of <sup>†</sup> are not significantly different ( $\alpha = 0.05$ ) within a trial.

observed during the trials, in which the alpha female in a pack showed aggression towards a subordinate feeding from the same tray. She ignored other dogs also feeding from the tray and the subordinate animal returned to it almost immediately.

#### Attractant preference trials

Medians of Rodger's index values differed significantly among the three attractants (liver-offal, cheetah scats, and control;  $H = 9.494$ ,  $df = 2$ ,  $P = 0.005$ ). In pair-wise comparisons the liver-offal and cheetah scats differed significantly ( $P < 0.05$ ), with the liver/offal attractant being preferred (liver-offal median = 1; cheetah scats median = 0), but in a multiple comparison of the two attractants against the control group no significant preference was observed. There was no significant variation among attractants in terms of alpha female contact time.

#### Biomarker trials

All eleven wild dogs readily ate the chicken head baits offered. No regurgitation occurred after immobilization and upon examination eight of eleven (73%) wild dogs showed oral staining. All eight dogs showed staining of the tongue and soft and hard palates, while five showed staining of the tonsils and four and three dogs showed staining of the buccal mucosa and oropharynx, respectively. No vaccine

containers were recovered, indicating that all were swallowed.

#### Bait uptake by wild dog pups

Captive 3 mo old pups successfully consumed chicken heads, ingesting  $97\% \pm 3\%$  (mean  $\pm$  SE) of baits over five trials. Younger, free-ranging pups also ingested baits, although uptake was lower ( $51\% \pm 11\%$ ). Pups were observed chewing the baits more thoroughly (mean number of chews =  $16.6 \pm 2.11$ ,  $n = 14$ ) than the adults (mean =  $11.1 \pm 0.545$ ,  $n = 154$ , Student's  $t$ -test:  $t = -2.890$ ,  $df = 166$ ,  $P = 0.004$ ).

#### Bait uptake by non-target species

During the 46 hr of daytime observations of baits around the wild dog dens, only two chicken heads were seen to be taken by other species. One was taken by a juvenile bateleur eagle (*Terathopius ecaudatus*) and the other by a black-backed jackal. Both incidents occurred while the adult dogs were at the den, although not in the vicinity of the baits. In the nocturnal trials, no baits remained on any of the four plots in the morning. The tracks of five species were identified: spotted hyena (*Crocuta crocuta*) and genet (*Genetta* spp.) on three plots, dwarf mongoose (*Helogale parvula*) on two plots, and slender mongoose (*Galerella sanguinea*)

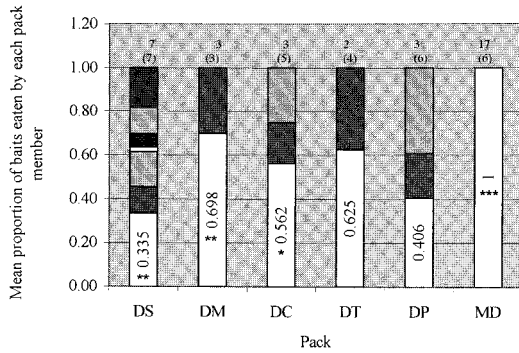


FIGURE 1. Mean proportion of baits eaten by each pack member in six packs during the bait dominance trials. Figures in columns are mean number of baits eaten by the individual ingesting the most baits. Asterisks indicate if this number was significantly more than any other animal in the pack. \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*  $P \leq 0.001$ . Figures above columns show pack size (top) and number of trials (in parentheses).

and warthog (*Phacochoerus aethiopicus*) on one plot.

#### Dominance trials

A significant difference in the proportion of baits ingested by each pack member was observed in four of the six study packs (Fig. 1). In all four of these packs a single individual dominated intake. In the three packs in which an alpha pair could be distinguished (DS, DC, and MD), the dominant feeder was an alpha animal. The fourth pack, DM, consisted of three young males and no clear rank hierarchy could be identified. Of the two packs in which no dominance of bait intake occurred, one (DT) consisted of an opposite sex pair and the other (DP) of 3 mo old pups. Only two aggressive interactions occurred during the 31 trials, involving the DS alpha female and a subordinate yearling.

#### Pack coverage tests

The pattern of bait distribution had no significant effect on pack coverage (one-way ANOVA:  $F_{2,27} = 0.429$ ,  $P = 0.654$ ). However, an interesting difference in the success of the targeted delivery method was noted between captive and free-ranging packs. In this method, an animal in a

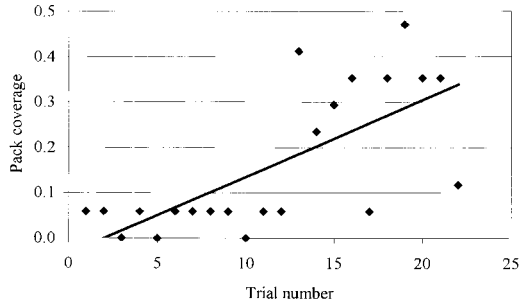


FIGURE 2. Linear regression plot showing the relationship between trial number and pack coverage (proportion of pack members ingesting at least one bait in a trial) in the MD pack ( $r = 0.71$ ,  $P \leq 0.001$ ).

pack was selected at random and the bait thrown directly at it. If the selected animal ate the bait, the throw was deemed a success. The throw was recorded as a failure if another animal ate the bait, or if the bait was not ingested at all. A significant difference in the number of successful throws between captive and free-ranging packs was seen ( $\chi^2 = 13.9$ ,  $df = 1$ ,  $P < 0.001$ ), with 85.7% of throws to captive dogs being successful, compared to none in free-ranging dogs.

No difference in pack coverage was detected with either of the two distribution patterns or across the four BFS in the MD pack trials (two-way ANOVA:  $F_{3,17} = 1.747$ ,  $P = 0.195$ ). During these trials it was noted that pack coverage increased as further trials were conducted with pack coverage being significantly related to trial number (Fig. 2,  $r = 0.71$ ,  $P < 0.001$ ). A maximum pack coverage of 47% was obtained in the free-ranging MD pack after 19 trials, equivalent to 8 days of exposure. Increased exposure also resulted in a dilution of the initial dominance effect, with other animals in the pack starting to ingest a larger proportion of the baits.

## DISCUSSION

#### Bait preference

The intensely social nature of wild dogs precluded the possibility of conducting the majority of the bait preference tests on individual animals. It was recognized that



using the pack as the experimental unit in the preference trials could bias results if the alpha pair dominated intake of a preferred bait, because they might force the remainder of the pack onto a less preferred choice. The subordinate members, being numerically superior, would then spuriously inflate the consumption rate of the less favored bait. In all trials dominant animal(s) were, however, observed feeding on the bait for which the largest area under the curve was ultimately obtained. In attractant preference trials the alpha female spent the most amount of time with the attractant that obtained the highest Rodger's index in the trial. The low number of antagonistic interactions observed at the bait trays supports the notion that dominant animals did not aggressively defend preferred baits from subordinate animals. In addition, results from the group preference trials concurred with the results obtained from those tests that were conducted on individual animals.

The trials therefore support the conclusion that, of the seven candidate baits tested, chicken heads are most preferred by captive-bred wild dogs. Chicken heads have been successfully used as baits for the delivery of oral rabies vaccine in mass immunization campaigns in Europe (Steck et al., 1982). They were superseded by machine-made baits mainly as a result of the manual labor required to prepare large numbers of chicken head baits (inserting and securing the vaccine capsule under the skin). Given the small number of remaining African wild dogs this factor is unlikely to prove an obstacle, and chicken heads are cheap and readily available even in remote areas. Thermostability trials in Zimbabwe using chicken head/SAG-2 combinations showed that baits lying in shade during the cooler months of the year (July) insulated the vaccine adequately to prevent significant loss of virus titer for up to 48 hr (Bingham et al., 1999). This is adequate for the purposes of wild dog vaccination, considering that the denning pe-

riod in southern Africa (April–July) is in the cool season.

The fact that no significant differences in intake between two attractant types and the control were detected indicates that addition of an attractant is unlikely to significantly increase bait uptake by wild dogs. In other species odor attractants are usually added to enhance bait discovery by target species (Bachmann et al., 1990; Linhart, 1993; Farry et al., 1998). In contrast to the proposed directed oral vaccination of wild dogs, however, these campaigns have typically involved mass vaccination of rabies vectors in which baits are broadcast at relatively low densities (5–20 baits/km<sup>2</sup> in Europe; Barrat and Aubert, 1993).

#### **Pack coverage**

The biomarker trials showed that chicken heads are sufficiently well chewed to result in perforation of the vaccine capsule in the majority of cases. The proportion of wild dogs with stained mucous membranes was nevertheless lower than that obtained in a similar study done on jackals, in which 96% of animals showed staining after consumption of chicken heads loaded with rhodamine B-filled capsules (Bingham, 1999). The lower biomarker success among wild dogs is probably due to size-related differences in chewing frequency and ingestion rate per bait.

Young wild dog pups readily accepted chicken head baits. Although no biomarker trials were conducted to test for perforation of the vaccine capsule, the more thorough chewing response exhibited by pups suggests the perforation rate should be at least as high as that seen in adults. Results of these trials offer the potential to vaccinate free-ranging wild dogs as young as 10 wk old. Additional research is, however, required on vaccination schedules and potential interference of maternal antibodies from immunized females.

Bait delivery to pack members was hampered by the initial dominance of intake by a single individual, usually a member of the alpha pair. Variations in the pattern of

delivery and the degree of satiation of the pack prior to delivery did not influence this effect. The low number of overt aggressive interactions observed during dominance trials suggests that dominance of bait intake is not a result of active bait defense by high-ranking animals, but rather due to initial lack of interest by subordinates. Subordinates' behavior was suggestive of a neophobic response to a novel food item (Launchbaugh et al., 1997); they were often seen investigating a bait but not ingesting it. However, once a dog ingested a bait it would seldom ignore further baits and would indeed actively search them out. This behavior explains why pack coverage increased after repeated exposure to baits, providing a potential management tool to maximize vaccine coverage within wild dog packs. A pack of dogs could be primed to chicken head baits by throwing out baits without oral vaccine until the necessary level of exposure was attained. At this stage bait and vaccine combinations could be delivered. Although a maximum pack coverage of only 47% was obtained in this trial, it may be possible to increase this figure through such a priming method.

The high pack coverage (86%) obtained with the 'targeted' bait delivery method in captive packs is encouraging as it provides a means of circumventing the dominance effect. This technique could potentially be utilized in captive breeding institutions as a component of the rabies control program. In contrast to traditionally used methods of pole-syringing or dart-vaccinating, stress to the dogs is minimal and the risk of injury is lower, particularly in younger animals.

During bait uptake trials 76% of baits were ingested during the 3 hr observation period, and in no case did any baits remain by nightfall. The rapidity with which baits were contacted and ingested also increased with increasing exposure to baits. Loss of baits to non-target animals, as indicated by the low incidence of uptake by diurnal species, is therefore unlikely to be a major factor in wild dog oral vaccination

campaigns. Potential human exposure could cause some concern because chicken heads form part of the diet of many rural people in southern Africa, although it is highly unlikely that wild dogs will den near human settlements.

#### **A proposed baiting system for oral vaccination of African wild dogs**

For free-ranging animals we propose that oral vaccination should be scheduled for the latter part of the denning season (June/July), when pups are almost ready to leave the den. An effective baiting system would use chicken heads as baits, and the pack should be primed to accept chicken heads for about 1 wk prior to introducing the vaccine. For vaccine delivery a blister pack of vaccine can be inserted under the skin of each chicken head and stapled in place. These loaded baits should then be placed out around the den in shade and replenished until observations indicate that all dogs have probably ingested at least one bait each. For captive animals, individual animals can be targeted after priming by throwing baits directly to them and uptake can be observed. Ongoing research is now required to test the efficacy of oral vaccination, optimal periods for boosting, and effects of maternal rabies antibodies on vaccination of pups.

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#### **LITERATURE CITED**

- AUBERT, M. F. A., E. MASSON, M. ARTOIS, AND J. BAR-RAT. 1994. Oral wildlife rabies vaccination field trials in Europe, with recent emphasis on France. *In* Lyssaviruses, C. E. Rupprecht, B. Dietzschold and H. Koprowski (eds.). Springer-Verlag, Berlin, Germany, pp. 219-243.

- BACHMANN, P., R. N. BRAMWELL, S. J. FRASER, D. A. GILMORE, D. H. JOHNSTON, K. F. LAWSON, C. D. MACINNES, F. O. MATEJKA, H. E. MILES, M. A. PEDDE, AND D. R. VOIGHT. 1990. Wild carnivore acceptance of baits for delivery of liquid rabies vaccine. *Journal of Wildlife Diseases* 26: 486–501.
- BARRAT, J., AND M. F. A. AUBERT. 1993. Current status of fox rabies in Europe. *Onderstepoort Journal of Veterinary Research* 60: 357–364.
- BINGHAM, J. 1999. The control of rabies in jackals in Zimbabwe. Ph.D. Thesis, University of Zimbabwe, Harare, Zimbabwe, 292 pp.
- , C. L. SCHUMACHER, F. W. G. HILL, AND A. AUBERT. 1999. Efficacy of SAG-2 oral rabies vaccine in two species of jackal (*Canis adustus* and *Canis mesomelas*). *Vaccine* 17: 551–558.
- CAMPBELL, J. B. 1994. Oral rabies immunization of wildlife and dogs: Challenges to the Americas. In *Lyssaviruses*, C. E. Rupprecht, B. Dietzschold and H. Koprowski (eds.). Springer-Verlag, Berlin, Germany, pp. 245–266.
- CARBONE, C., J. T. DU TOIT, AND I. J. GORDON. 1997. Feeding success in African wild dogs: Does kleptoparasitism by spotted hyenas influence hunting group size? *Journal of Animal Ecology* 66: 318–326.
- COURCHAMP F., T. CLUTTON-BROCK, AND B. GRENFELL. 1999. Inverse density dependence and the Allee effect. *Trends in Ecology and Evolution* 14: 405–410.
- CREEL, S., AND N. M. CREEL. 1995. Communal hunting and pack size in African wild dogs *Lycaon pictus*. *Animal Behaviour* 50: 1325–1339.
- ESTES, R. D. 1991. The behavior guide to African mammals. Russel Friedman Books, Halfway House, South Africa, 611 pp.
- FARRY, S. C., S. E. HENKE, S. L. BEASOM, AND M. G. FEARNEYHOUGH. 1998. Efficacy of bait distributional strategies to deliver canine rabies vaccines to coyotes in southern Texas. *Journal of Wildlife Diseases* 34: 23–32.
- FEKADU, M., S. L. NESBY, J. H. SHADDOCK, C. L. SCHUMACHER, S. B. LINHART, AND D. W. SANDERLIN. 1996. Immunogenicity, efficacy and safety of an oral rabies vaccine (SAG-2) in dogs. *Vaccine* 14: 465–468.
- FULLER, T. K., AND P. W. KAT. 1990. Movements, activity and prey relationships of African wild dogs (*Lycaon pictus*) near Aitong, south-western Kenya. *African Journal of Ecology* 28: 330–350.
- , T. H. NICHOLLS, AND P. W. KAT. 1995. Prey and estimated food consumption of African wild dogs in Kenya. *South African Journal of Wildlife Research* 25: 106–110.
- GASCOYNE, S. C., A. A. KING, M. K. LAURENSEN, M. BORNER, B. SCHILDGER, AND J. BARRAT. 1993. Aspects of rabies infection and control in the conservation of the African wild dog (*Lycaon pictus*) in the Serengeti region, Tanzania. *Onderstepoort Journal of Veterinary Research* 60: 415–420.
- HOFMEYR, M., J. BINGHAM, E. P. LANE, A. IDE, AND L. NEL. 2000. Rabies in African wild dogs (*Lycaon pictus*) in the Madikwe Game Reserve, South Africa. *The Veterinary Record* 146: 50–52.
- HOWELL, D. C. 1987. *Statistical methods for psychology*, 2nd Edition. Duxbury Press, Boston, Massachusetts, 636 pp.
- INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES. 1996. IUCN red list of threatened animals. IUCN, Gland, Switzerland, 105 pp.
- KAT, P. W., K. A. ALEXANDER, J. S. SMITH, AND L. MUNSON. 1995. Rabies and African wild dogs in Kenya. *Proceedings of the Royal Society of London Series B* 262: 229–233.
- KREBS, C. J. 1989. *Ecological methodology*. HarperCollins, New York, 654 pp.
- LAUNCHBAUGH, K. L., F. D. PROVENZA, AND M. J. WERKMEISTER. 1997. Overcoming food neophobia in domestic ruminants through addition of a familiar flavor and repeated exposure to novel foods. *Applied Animal Behaviour Science* 54: 327–334.
- LINHART, S. B. 1993. Bait formulation and distribution for oral rabies vaccination of domestic dogs: An overview. *Onderstepoort Journal of Veterinary Research* 60: 479–490.
- MILLS, M. G. L. 1993. Social systems and behaviour of the African wild dog *Lycaon pictus* and the spotted hyaena *Crocuta crocuta* with special reference to rabies. *Onderstepoort Journal of Veterinary Research* 60: 405–409.
- REICH, A. 1981. The behavior and ecology of the African wild dog *Lycaon pictus* in the Kruger National Park. Ph.D. Dissertation, Yale University, New Haven, Connecticut, 425 pp.
- ROSCOE, D. E., W. C. HOLSTE, F. E. SORHAGE, C. CAMPBELL, M. NIEZGODA, R. BUCHANNAN, D. DIEHL, C. E. RUPPRECHT, AND H. S. NIU. 1998. Efficacy of an oral vaccinia-rabies glycoprotein recombinant vaccine in controlling epidemic raccoon rabies in New Jersey. *Journal of Wildlife Diseases* 34: 752–763.
- SCHEEPERS, J. L., AND K. A. E. VENZKE. 1995. Attempts to reintroduce African wild dogs *Lycaon pictus* into Etosha National Park, Namibia. *South African Journal of Wildlife Research* 25: 138–140.
- STECK, F., A. WANDELER, P. BICHEL, S. CAPT, AND L. SCHNEIDER. 1982. Oral immunization of foxes against rabies. A field study. *Zentralblatt Veterinärmedizin B* 29: 372–396.
- STELMAN, H. G., S. E. HENKE, AND G. M. MOORE. 1998. Gray fox response to baits and attractants for oral rabies vaccination. *Journal of Wildlife Diseases* 34: 764–770.
- SWANEPOEL, R. 1994. Rabies. In *Infectious diseases of livestock with special reference to southern Africa*, J. A. W. Coetzer, G. R. Thomson and R.

- C. Tustin (eds.). Oxford University Press, Oxford, UK, pp. 493–552.
- WOODROFFE, R., AND J. R. GINSBERG. 1999. Conserving the African wild dog *Lycan pictus*. I. Diagnosing and treating the causes of decline. *Oryx* 33: 132–142.
- , ———, AND D. W. MACDONALD. 1997. The African wild dog: Status survey and conservation action plan. International Union For Conservation of Nature And Natural Resources, Gland, Switzerland, 166 pp.
- ZAR, J. H. 1996. Biostatistical analysis, 3rd Edition. Prentice-Hall, Engelwood Cliffs, New Jersey, 718 pp.

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