Oral rabies vaccination of red foxes and golden jackals in Israel: preliminary bait evaluation

S.B. Linhart (1), R. King (2), S. Zamir (3), U. Naveh (2), M. Davidson (3) & S. Perl (3)

(1) Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, United States of America
(2) Nature Reserves Authority, 78 Yirmeyanu Street, Jerusalem 94467, Israel
(3) Veterinary Field Services and Animal Health, Ministry of Agriculture, Beit Dagan, P.O. Box 12 50250, Israel

Summary
Field trials were conducted in late April to early May of 1995 and 1996 in central Israel to assess the potential for controlling rabies in red foxes (Vulpes vulpes) and golden jackals (Canis aureus) by using vaccine-laden baits. Of the bait types which were field tested, polymer fish meal baits were selected as the most suitable for both species. Fish meal baits containing tetracycline hydrochloride, an oral biomarker, were distributed by four-wheel-drive vehicle at a density of ~30 baits/km² in two test areas of 35 km². Of the animals which were trapped and euthanased seven to ten days after treatment, 65% of foxes and 56% of jackals gave positive results when tested for the biomarker. These results indicate the potential effectiveness of oral rabies vaccination of these species in Israel and possibly elsewhere in the Middle East, where rabies is a problem in wild canids.

Keywords

Introduction
The use of vaccine-laden baits to immunise wildlife orally against rabies is an emerging technology which has been successfully employed to control the disease in red foxes (Vulpes vulpes) in Europe (1) and Canada (C. MacInnes, personal communication). The efficacy of the technique depends, in part, upon the use of vaccine baits which are accepted well by target species and which also meet other criteria, as set forth by the World Health Organisation and other bodies (4, 7).

Rabies in red foxes and golden jackals (Canis aureus) is a problem in Israel (A. Shimshony, personal communication) and elsewhere in the Middle East, although, for some countries in this region, surveillance and reporting of the disease is marginal, especially in wildlife species (8). Until recently, the potential of oral rabies vaccination (ORV) for use in wild canids has received little attention in this part of the world. Efforts have been made by the Ministry of Agriculture of Israel to obtain international funding for regional development of the technique. Pending such support, the authors undertook initial field trials to determine canid bait preferences and acceptance, as well as the percentages of local red fox and golden jackal populations which took placebo vaccine baits on two test sites.

Materials and methods

Types of bait
In 1995, four different bait types were tested in the field to determine which was most preferred by red foxes. All four baits were identical in shape (cylindrical, 2 x 5 cm) (Bait Tek, Beaumont, Texas), and were composed of either a fish meal/fish oil or dog food meal matrix. Ethylene vinyl acetate, a patented co-polymer product (Elvax®, E.I. Du Pont de Nemours, Wilmington, Delaware), was used by the bait manufacturer to bind and waterproof the bait material. The polymer fish meal bait was identical in composition to that previously described for various wildlife species, especially raccoons (Procyon lotor) and coyotes (Canis latrans) in the United States of America (USA) and red foxes in western Europe (2). The polymer dog meal bait, as obtained from the manufacturer, had minimal odour. This bait was therefore modified by adding a coating of liquid and dried animal food.
supplements to enhance both odour and taste. The composition of the test baits was as follows:

- bait A: polymer dog food meal bait coated with 3% (by weight) poultry oil and 3% Poultry Biodigest (Bioproducts, Louisville, Kentucky)
- bait B: polymer fish meal (proprietary ingredients) (Bait Tek, Beaumont, Texas)
- bait C: polymer dog food meal bait coated with 3% melted rendered beef tallow and a dry mixture of 1.5% Cheese Plus (International Ingredients, St Louis, Missouri) and 1.5% MFD (Mondovi Foods Corporation, Mondovi, Wisconsin)
- bait D: polymer dog food meal bait coated with a mixture of 32% gelatin, 42% H₂O, 5% roast beef flavour concentrate (Flavors of North America, Northfield, Illinois), 20% sucrose and 0.15% potassium sorbate as a preservative (Eastman Chemical, Kingsport, Tennessee).

In 1996, the polymer fish meal bait (bait B) was used in a different configuration (rectangular, 2 x 3.5 x 5.5 cm). This bait also contained ~150 mg of the biomarker tetracycline hydrochloride (THCl).

Test areas

Three test areas, all located in central Israel, were used for these bait trials.

The Palmahim area (2.5 x 14 km = 35 km²; Fig. 1) is located approximately 20 km south of Tel Aviv, adjacent to the Mediterranean. The area consists of low hills of semi-stabilised sand dunes, with a plant community based principally upon Artemisia monosperma, in association with a large shrub, Retama raetam. The climate is moderate, due to close proximity to the sea, with an annual precipitation of 400 mm to 500 mm and temperatures which fluctuate between 12°C and 26°C. The area is estimated to have a high density of red foxes. It was for this reason that the area was selected to evaluate fox baits and baiting procedures both in 1995 and 1996. Non-target species fauna is diverse and includes gerbils (Gerbillus sp.), rats (Ratus ratus), house mice (Mus musculus), jerboas (Jaculus jaculus), brown hares (Lepus capensis), long-eared hedgehogs (Hemiechinus auritus), Indian crested porcupines (Hystrix indica), mountain gazelles (Gazella gazella), wild boar (Sus scrofa), golden jackals (Canis aureus), black rats, brown hares, porcupines, hedgehogs and mountain gazelles. The Modiin area also supports jerboas, wild boar and mongooses.

The Latrun test area (~2 x 5 km = 10 km²) is located approximately 20 km south-east of Tel Aviv and was also selected because of the high jackal density. Jackal activity in the area is concentrated about a large, centrally located refuse dump. The jackals disperse throughout the surrounding hills during daylight hours and move to the dump site at dusk. The area consists of chalky hills dissected by dry stream beds at an elevation of 150 m to 200 m. Temperatures in this area fluctuate between 7°C and 19°C in winter and from 20°C to 32°C in summer. The annual precipitation is 500 mm to 600 mm. Overgrazing and fire have resulted in a plant community dominated by the shrub Hyparrhenia hirta and the tree species Ziziphus spinachristi. In addition to the various mammal species mentioned above, red foxes are present in

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low numbers, as well as the striped hyena (*Hyaena hyaena*) and marbled polecat (*Vormela peregrina*).

**Bait acceptance trials**

In May 1995, 80 tracking stations (circles of smoothed sand, ~ 1 m in diameter) were placed in the Palmahim test area at intervals of approximately 200 m. These stations were located along sand trails and roads and placed in series of four, with a single bait type (A, B, C or D) placed in random sequence in the centre of each of the four stations. This procedure was continued until all 80 stations (20 per bait type) had been established. The stations were checked daily for animal tracks and missing baits. Tracks were identified by their characteristic shape and size, after which the stations were smoothed and the missing baits replaced. The stations were established and checked daily over two time periods (1 to 3 May and 18 to 19 May), and the numbers of stations visited and of baits removed were used to calculate bait discovery and preference rates for each bait type in both red foxes and non-target species.

A second bait acceptance trial was conducted in the Latrun test area in early May 1996, to determine golden jackal acceptance of polymer fish meal baits (bait B). Fifty tracking stations, 1 m in diameter, were placed along a 6 km segment of road, leading into a valley where jackals scavenged at the refuse disposal site of a nearby dairy community. The distance between stations was reduced from 200 m to 50 m as the route approached the refuse disposal site. One polymer fish meal bait was placed at each station. All stations were checked daily for three days to determine bait disappearance rates and the species which visited the stations. Baits which had been removed were replaced daily, as required.

**Placebo vaccine bait trials**

Field trials were conducted in two areas in late April to early May 1996, to determine what percentages of the local fox and jackal populations would find and consume THCl-laden polymer fish meal baits. The Palmahim area was used for the red fox test. Baits were placed at a density of 30 baits/km². This bait density was about 1.5 to 2 times that which is normally used for red foxes in western Europe and Ontario, Canada (2). The authors decided to bait the Palmahim area more heavily because of the high fox visitation rate to tracking stations which had occurred during a bait acceptance trial the previous spring. A high rate of success in trapping after bait placement confirmed the observation of the authors that very high numbers of foxes were present in the area. A total of 1,050 polymer fish meal baits were distributed by dividing the test area into five zones (Fig. 1) and apportioning the bait density in each (range = 25 to 50 baits/km²) according to zone size, the number of fox tracks observed and prior subjective assessment of fox population densities. Baits were distributed along the sand roads and trails, and especially around the periphery of and within the large refuse dump at the northern end of the test site. Three four-wheel-drive vehicles were aligned abreast, approximately 0.5 km to 1 km apart, to distribute baits throughout the larger 3 km to 4 km wide zones. Tracking stations were used to sample the rates of discovery and removal of baits for target and non-target species. One hundred stations (one bait per station) were located at ~ 300 m intervals along roads oriented in a north-south direction and checked daily for two days.

The golden jackal placebo vaccine (i.e., THCl-laden) bait trial was conducted in the Modlin area. The 35 km² test site was divided into four zones with a higher bait density used in the immediate vicinity of the refuse site (Fig. 1; Zone 1). Existing paved and gravel roads were used to distribute baits adjacent to the refuse site. Thus, in Zone 1, baits were placed approximately every 20 m along the roadsides. Some baits were also placed at the intersections of vehicular roads and the well-defined trails made by the jackals, which follow regular routes from their daytime resting areas in the surrounding hills to the refuse site in the evening hours. A total of 1,000 baits were placed in the area (~ 30 baits/km²), with 400 being placed in Zone 1 and the remainder (600) at ~ 50 m intervals along outlying roads. An extensive portion of the test area is composed of outlying hills which are generally inaccessible except by foot or by aircraft. As these hills were not baited, the probability is high that some jackals were never exposed to baits. As a result of time constraints and the extremely rocky terrain (and lack of soil), the authors did not place tracking stations in the Modlin test area to determine bait removal rates.

Both the Palmahim and Modlin areas were sampled by capturing animals with Victor® padded foothold traps (number 1.5 size for foxes and number 3 size for jackals) (Woodstream Corporation, Lititz, Pennsylvania). Trapping was initiated seven days after bait placement and continued for three days in both areas. Captured foxes and jackals were tranquillised with ketamine hydrochloride intra-muscularly (~ 10 mg/kg) and euthanised with pentobarbitals intravenously (~ 100 mg/kg). The lower mandible, a mid-section of femur, blood serum and the female reproductive tract were removed and frozen or preserved in 10% formalin for subsequent THCl analysis, and to determine age and reproductive status. Mandible and femur samples were later sectioned (100 μ to 150 μ) with an Isomet® double-bladed saw (Buehler Ltd, Lake Bluff, Illinois). These sections were mounted in glycerine and examined with an ultraviolet light-equipped microscope at 80 x or 160 x. Tetracycline hydrochloride deposition was detected in growing bones and teeth by the characteristic yellow fluorescence. Lower canine teeth from both jackals and foxes were sent to a commercial laboratory (Mason's Laboratory, Milltown, Montana) for sectioning, staining and age analysis, based upon standard methodology using counts of the number of annuli present in the tooth cementum. Female reproductive tracts and ovaries were examined in the laboratory and the number of corpora lutea, foetuses and placental scars were determined and later correlated with the age of the animal.
The data collected during the 1995 and 1996 field trials were limited and thus the results of these studies are preliminary in nature. Such limitations were the result both of time constraints and of the restricted funds made available for this research.

Results and discussion

Bait acceptance trials
Tracking station data from the 1995 Palmahim fox bait preference trial revealed that all baits, regardless of type, were rapidly removed from the stations. Excluding replacement baits from the analysis, 49% (39/80) of the original 80 baits which had been laid were taken the first night; 61.3% (49/80) had been removed by the second night and 74.7% (59/79) were gone by the third night of exposure. An analysis of the bait acceptance data over the entire five nights of exposure (5 x 80 = 400 exposure nights minus 4 inoperable stations) indicated that 57.3% (227/396) of the baits were taken by all species combined. Of the removed baits, red foxes took 78.9% (179/227). Bait acceptance by foxes was excellent; they took nearly all the baits encountered at stations (94.7% or 179/189), regardless of bait type. The fish meal polymer bait (bait B) had the highest rate of discovery and ‘take-up’ by red foxes (58% or 58/100). A chi-square test revealed that baits C and D were taken by foxes at a significantly lower rate than baits A and B. The tallow, cheese and MFD-coated polymer dog meal bait (bait C) was discovered at the lowest rate (25.3% or 25/99), but, once discovered, was taken by foxes at about the same frequency (89.3%) as the other three bait types. Only 12.1% (48/396) of all four bait types combined were removed by non-target species. Non-target animals which visited and took baits were gerbils (10 baits taken/of 49 visits), crows (20/37), insects (0/13), jackals (6/11), domestic dogs (0/9), unidentified birds (0/8), jerboas (2/8) and unidentified species (10/10).

The 1996 field trial in the Latrun area to determine jackal acceptance of polymer fish meal baits revealed that this species discovered and removed baits in an exceptionally short time, at least under the field trial protocol used by the authors. A total of 143 jackal visits to stations was recorded during the 147 operable station nights (a discovery rate of 97.3%). Of the 143 baits visited by jackals, all were removed by this species (i.e., an acceptance rate of 100%; Table I). Visits by non-target species were insignificant; one or possibly two baits were taken by red foxes, one by a crow and one by an unidentified animal.

Placebo vaccine bait trials
Results of the 1996 placebo vaccine trial for red foxes in the Palmahim area were encouraging and are summarised in Table I. A total of 90% (90/100) of the baits at tracking stations had been taken (by all species combined), following 48 hours of exposure; 76% (76/100) had been removed by red foxes. Foxes visited 78% (78/100) of the stations and took 97.4% (76/78) of the baits. The frequency with which non-target species visited stations and removed baits was low; domestic dogs (3/3), jackals (2/2), crows (1/4) and unidentified rodents or other species (4/11) took baits. Assuming that the visitation and bait removal rates observed at the tracking stations were indicative of the animal activity associated with all 1,050 baits distributed in the Palmahim area, the bait uptake by red foxes was very high.

Fifteen adult and five juvenile (15 to 16 weeks old) foxes were trapped and euthanased. The success of this trapping (Table I)

<table>
<thead>
<tr>
<th>Findings</th>
<th>Palmahim</th>
<th>Test area</th>
<th>Modin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary species</td>
<td>Red fox</td>
<td>Golden jackal</td>
<td>Golden jackal</td>
</tr>
<tr>
<td>Bait acceptance (percentage taken)</td>
<td>97</td>
<td>100</td>
<td>NP</td>
</tr>
<tr>
<td>Number of animals captured</td>
<td>20</td>
<td>NP</td>
<td>23&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Trap nights/animals captured</td>
<td>4.1</td>
<td>NP</td>
<td>2</td>
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<tr>
<td>Percentage of animals giving positive results for THCI</td>
<td>65</td>
<td>NP</td>
<td>56.5</td>
</tr>
<tr>
<td>Total number of adult females captured</td>
<td>5</td>
<td>NP</td>
<td>12</td>
</tr>
<tr>
<td>Number with corpora lutea</td>
<td>4&lt;sup&gt;b&lt;/sup&gt; (2.75; 2 to 4)</td>
<td>NP</td>
<td>7&lt;sup&gt;c&lt;/sup&gt; (4.6; 3 to 8)</td>
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<tr>
<td>Number with foetuses</td>
<td>0</td>
<td>NP</td>
<td>1</td>
</tr>
<tr>
<td>Number with placental scars</td>
<td>4&lt;sup&gt;b&lt;/sup&gt; (2.5; 2 to 3)</td>
<td>NP</td>
<td>5 (4.8; 3 to 6)</td>
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<tr>
<td>Age and sex</td>
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<td></td>
<td></td>
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<tr>
<td>Number of juveniles</td>
<td>5 (3♂, 2♀)</td>
<td>NP</td>
<td>0</td>
</tr>
<tr>
<td>Number of adults</td>
<td>15 (10♂, 5♀)</td>
<td>NP</td>
<td>23 (11♂, 12♀)</td>
</tr>
<tr>
<td>Mean age in years</td>
<td>2.15 (0.3 to 7)</td>
<td>NP</td>
<td>2.91 (1 to 9)</td>
</tr>
</tbody>
</table>

NP: not performed
♂: male
♀: female
THCI: tetracycline hydrochloride

<sup>a</sup> In all, 21 jackals were trapped and 2 were shot
<sup>b</sup> Values in parentheses are means, ranges or data concerning sex ratios
<sup>c</sup> An ovary was missing from the reproductive tract of one necropsied female
indicated that an extremely large fox population was present in the Palmahim area. An unsuccessful effort was made to take more foxes by night lighting and shooting. Examination of the lower mandible sections revealed that 65% (13/20) gave positive results for THCl, indicating consumption of one or more baits. As previously reported, juvenile foxes display more intense THCl-induced fluorescence than adults because young animals have more rapid bone growth. THCl-induced fluorescence occurred equally in both mandibular and femoral sections from individual foxes, but was observed only infrequently in the roots of premolars which had been left intact within mandibular sections. It was suggested that the low occurrence of THCl in premolars was due to cellular replacement in the root of this tooth being much slower than that in the canine, the latter generally being the tooth of choice for THCl analysis (D. Johnston, personal communication). The percentage of THCl-marked foxes in this study sample was comparable to results obtained from earlier red fox vaccination efforts conducted in western Europe and Canada, in areas where baits were distributed by ground crew (i.e., mean = 64% giving positive results for THCl, range = 34% to 90%) (2).

A total of 21 adult jackals were trapped in the Modiin test area and two more were shot. The high success rate achieved for trapping (2 trap nights/jackal) indicated an extremely high density of jackals. In all, 56.5% (13/23) of the jackals were found to give positive results for THCl (Table I). Mandibles from four additional jackals were suggestive of the presence of THCl, but were not used to calculate the percentage of marked animals. Two different observers agreed that, for unknown reasons, the detection of THCl in golden jackal mandibles was considerably more difficult than in red fox mandibles.

Although the results from the Palmahim and Modiin test areas were satisfactory, higher levels of bait uptake may be possible in future if long, narrow areas such as the Palmahim site are avoided because of the likely high rate of interchange (i.e., movement) between THCl-marked and unmarked foxes along the periphery (Fig. 1). Moreover, although jackals in the Modiin area were concentrated about the central dumpsite to feed on refuse, only a small percentage of the total area could be baited by ground vehicle. Aerial baiting of the surrounding hills would most likely have resulted in a higher percentage of THCl-marked jackals.

**Reproductive and age data**

Demographic data from vector populations which are susceptible to rabies infection, specifically information concerning their productivity and age structure, help to assess the potential of ORV. For example, a high percentage of juvenile animals in the population, a short average life span and a high rate of reproduction indicate that the numbers of new susceptible animals entering the population each year will be high. Thus, revaccination efforts must be planned accordingly to control the disease successfully.

Reproductive and age data for trapped foxes are given in Table I. While sample sizes are limited, they do suggest that, although a high percentage of female foxes were reproducitively active and had bred and borne young, the mean number of placental scars (and thus pups weaned) was considerably below that reported for this species in the literature (means = 3.3 to 5.5) (3, 5, 6). This low productivity is most likely a result of the high fox population density in the Palmahim area. High population densities, at or exceeding the carrying capacity of the habitat, normally result in the suppression of productivity (6).

Data on jackal reproduction and age are also given in Table I. Except for one of the reproducitively active females, these data indicate that all or nearly all the ova were fertilised, had been implanted and had resulted in viable foetuses and subsequent placental scars. The mean number of placental scars (4.8), indicative of the number of young produced, was higher than that reported for this species (range 2 to 4) in the literature (3). Thus, although only 7 out of 12 (58.3%) of the female jackals in the study area were reproducitively active, those that were active produced, on average, larger than normal litters of pups.

In summary, the results of the 1995 to 1996 field trials described in this study indicate the potentially effective use of ORV in Israel and possibly elsewhere in the region. However, these trials were conducted at sites where both red fox and golden jackal populations were very dense and there were such small populations of non-target species that their uptake of baits was not a problem. Moreover, baits were distributed by ground crew, whereas aerial application is probably the only feasible technique for widespread application in the future. Thus, although the initial results showed promise, aerial bait drops, conducted under more representative field conditions, determination of the minimum effective bait densities required and cost-benefit analyses will all be needed to further ascertain the potential for controlling rabies in wild canids by using vaccine-laden baits.

**Acknowledgements**

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Vaccination orale du renard roux et du chacal doré contre la rage en Israël : évaluation préliminaire des appâts

S.B. Linhart, R. King, S. Zamir, U. Naveh, M. Davidson & S. Perl

Résumé
Des essais sur le terrain ont été menés de fin avril à début mai 1995 et 1996 dans le centre d’Israël pour évaluer les possibilités de prévention de la rage chez le renard roux (Vulpes vulpes) et le chacal doré (Canis aureus) à l’aide d’appâts dissimulant le vaccin. Parmi les différents types d’appâts testés sur le terrain, la farine de poisson polymère s’est avérée la plus adaptée aux deux espèces. Des appâts de farine de poisson contenant du chlorhydrate de tétracycline, un marqueur biologique oral, ont ainsi été distribués par des véhicules tout terrain à raison d’environ 30 appâts/km² dans deux zones d’essai de 35 km². Sur l’ensemble des animaux capturés et euthanasiés sept à dix jours après le traitement, le marqueur biologique a été retrouvé chez 65 % des renards et 56 % des chacals. Ces chiffres montrent l’efficacité potentielle de la vaccination orale de ces espèces contre la rage en Israël et peut-être ailleurs au Moyen-Orient, où cette maladie constitue un véritable problème chez les canidés sauvages.

Mots-clés

Vacunación antirrábica por vía oral de zorros y chacales en Israel: evaluación preliminar de los cebos

S.B. Linhart, R. King, S. Zamir, U. Naveh, M. Davidson & S. Perl

Resumen
Entre finales de abril y principios de mayo de 1995 y 1996, se llevaron a cabo en el centro de Israel varias pruebas de campo destinadas a evaluar el potencial que presentaba el uso de cebos impregnados de vacuna para la profilaxis de la rabia en el zorro común (Vulpes vulpes) y el chacal común (Canis aureus). De los tipos de carnaza ensayados sobre el terreno, el compuesto por polímeros de harina de pescado fue juzgado el más conveniente para ambas especies. Con ayuda de vehículos todo terreno se distribuyeron en dos áreas de prueba de 35 km² cebos de harina de pescado que contenían hidrocloruro de tetraciclina, un biomarcador oral, a una densidad aproximada de 30 cebos/km². De todos los animales que fueron capturados y sacrificados (de forma indolora) entre siete y diez días después del tratamiento, un 65% de los zorros y un 56% de los chacales arrojaron resultados positivos a las pruebas de detección del marcador. Ello es indicativo de la posible efectividad de la vacunación antirrábica por vía oral de esas especies en Israel, y posiblemente en el resto de Oriente Medio, donde la rabia representa un serio problema entre las poblaciones de cánidos salvajes.

Palabras clave
References


