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WILD CARNIVORE ACCEPTANCE OF BAITS FOR DELIVERY OF LIQUID RABIES VACCINE

Peter Bachmann,^{1,8} Richard N. Bramwell,^{1,4} Sarah J. Fraser,² Douglas A. Gilmore,^{1,5} David H. Johnston,¹ Kenneth F. Lawson,³ Charles D. MacInnes,¹ Frank O. Matejka^{1,6} Heather E. Miles,^{1,7} Michael A. Pedde,¹ and Dennis R. Voigt¹

¹ Ontario Ministry of Natural Resources, Wildlife Research Section,
P.O. Box 5000, Maple, Ontario, Canada L6A 1S9

² Department of Microbiology and Immunology, University of Toronto,
Toronto, Ontario, Canada M5S 1A1

³ Connaught Laboratories Limited, 1775 Steeles Ave. West, Willowdale, Ontario, Canada M2R 3T4

⁴ Present address: R.R. 1, Cheltenham, Ontario, Canada L0P 1C0

⁵ Present address: Box 1132, Red Lake, Ontario, Canada P0V 2M0

⁶ Present address: Aviation, Fire and Forest Resources Branch, 747 Queen Street, Sault Ste. Marie, Ontario, Canada P6A 5M7

⁷ Present address: 20 Preston Place, Toronto, Ontario, Canada M4N 2S9

⁸ The authors are listed in alphabetic order, in recognition of varied contributions from a team with diverse talents

ABSTRACT: A series of experiments are described on the acceptance, by red foxes (*Vulpes vulpes*) and other species, of two types of vaccine-baits intended to deliver liquid rabies vaccine. The baits consisted of a cube of sponge coated in a mixture of tallow and wax, or a plastic blister-pack embedded in tallow. All baits contained tetracycline as a biological marking agent: examination of thin sections of carnivore canines under an ultraviolet microscope revealed a fluorescent line of tetracycline if an individual had eaten baits. Baits were dropped from fixed-wing aircraft flying about 100 m above ground at approximately 130 km/h. Flight lines followed the edges of woodlots midway between parallel roads. Baits were dropped at one/sec, resulting in one bait/36 m on the ground, or 17 to 25 baits per km². Crows (*Corvus brachyrhynchos*) removed many baits, but did not appear to lower the percent of the fox population which took bait. Dropping baits only into corn and woodland to conceal baits, to reduce depredation by crows, reduced acceptance by foxes. Acceptance by foxes ranged between 37 and 68%. Meat added as an attractant did not raise acceptance. Presence, absence, color and perforations of plastic bags did not alter bait acceptance. Dispersal by juvenile foxes probably lowered the estimates of bait acceptance. It took 7 to 17 days for 80% ($n = 330$) of foxes to eat their first bait. The rapidity with which foxes picked up their first bait appeared more affected by unknown characteristics of years or study areas than by experimental variables. Skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*) also ate these baits, but acceptance was lower. Small mammals contacted baits, but rarely contacted the vaccine, which had the potential for vaccine-induced rabies in some species. Aerial distribution of baits was more cost-effective than ground distribution as practiced in Europe. This system has potential for field control of rabies, although higher acceptance will be desirable.

Key words: Rabies vaccine-baits, rabies virus, aerial distribution, acceptance of baits, red fox, striped skunk, raccoon, *Vulpes vulpes*, *Mephitis mephitis*, *Procyon lotor*, field study.

INTRODUCTION

Red foxes (*Vulpes vulpes*) and striped skunks (*Mephitis mephitis*) are the important rabies vectors in Ontario, Canada. During 1984 to 1987 those two species accounted for 95% of all diagnosed wild animal cases of rabies in Ontario ($n = 6,628$), or 70% of all animal cases ($n = 9,005$; Agriculture Canada, 1984, 1985, 1986, 1987). This paper describes experiments conducted from 1984 to 1987 to evaluate factors affecting acceptance by foxes, skunks and raccoons (*Procyon lotor*) of baits de-

signed to deliver modified live virus (MLV) rabies vaccine, in liquid form, into the mouths of foxes. These experiments were part of research to develop an effective and economical method to immunize wildlife vectors against rabies.

Modified live virus (MLV) rabies vaccine was shown to be effective for immunizing foxes by mouth in the early 1970's (Black and Lawson, 1970, 1973, 1980; Baer et al., 1971; Debbie et al., 1972; Mayr et al., 1972; Winkler et al., 1975), but use in the field in North America was delayed by concerns about safety (Lawson

et al., 1982) and problems with duration of effectiveness under field conditions (Campbell et al., 1985). Early experiments showed that freeze-dried vaccine was much more stable than liquid for long periods (World Health Organization, 1975), but acceptance by wild foxes of baits containing simulated freeze-dried vaccine was poor. Liquid preparations were effective when absorbed through the mucosa of the mouth and pharynx, but not in the stomach (Black and Lawson, 1973; Baer et al., 1975).

The first use of MLV rabies vaccine in the field occurred in Switzerland (Steck et al., 1982), followed by large scale use in West Germany (Schneider, 1985; Schneider et al., 1988), and recently in several other European countries (Artois et al., 1987; Pastoret et al., 1987). European programs initially used chicken-heads as bait, with 1.8 ml of vaccine in a small plastic blister-pack inserted under the skin by hand (Steck et al., 1982). When that proved too labor-intensive, a mixture of fish meal and fat was developed, which could be poured into molds, allowing large-scale manufacture (Schneider, 1985). All the European programs used liquid vaccine to be absorbed through the oropharyngeal mucosa.

In Ontario, we sought a bait, suitable for mass-production, which could be distributed over large areas from aircraft. This paper describes experiments with two synthetic baits: a sponge bait, and a fat bait containing vaccine in a blister-pack. The aim of these experiments was to test some characteristics of baits to see which were essential for (i) stimulating acceptance by foxes, and (ii) reducing depredation by crows (*Corvus brachyrhynchos*). We also studied bait acceptance by skunks and raccoons, although we did not design specific experiments to optimize acceptance by those species.

The primary experimental objectives were to (1) determine the acceptance by foxes of the two synthetic baits, (2) test whether a plastic bag surrounding the bait

changed acceptance by foxes and crows, (3) determine if habitats into which baits were dropped affect acceptance by foxes, (4) discover whether color of the bag affected acceptance, and (5) determine whether meat attractants were essential.

METHODS

Study area

The experiments were conducted in Huron and Grey counties, in southern Ontario, Canada (43°28' to 44°12'N, 80°37' to 81°43'W). That area was chosen because it had several successful fox trappers and the highest sustained fox harvest in Ontario (Voigt and Tinline, 1982). Specimens from trappers were used to evaluate bait acceptance.

The area has gently rolling topography overlain with glacial till. Sizes of study plots are given in Table 1. Observations of land use in 1987 indicated that over 60% of the land area was farmed, with cropland averaging 50 to 60%, and pasture an additional 5 to 18%. The major crops were corn and beans (soya, white, red kidney). About 10% of the area was abandoned pasture in various stages of succession, and woodlots (deciduous and mixed) occupied an additional eight to 27%. Towns, farm buildings, roads and high-use recreational areas such as golf courses and sports fields constituted one to six % of the area.

The first rural land surveys in Ontario established straight and parallel roads, legally designated as concessions, from which the farm lots extended perpendicularly. Orientation and spacing of the concession roads varied from township to township; the normal interval between concessions was 1.8 km. One result of this pattern of settlement was that there was often an irregular strip of woodlots midway between the concession roads, because farms were cleared starting at the roads. Additional woodland was located along streams and rivers. The flight lines for air-dropping baits followed the edges of the woodland strips, parallel to the roads.

The 1984 to 1986 and 1987 areas A and B were all in the same location. The five areas used in 1987 were established to provide samples of about 90 foxes each, based on previous years' trapping records. Unfortunately, a rabies outbreak across the entire region during the summer of 1987 resulted in smaller than expected samples.

Baits

The sponge bait used in 1984 to 1986 had three components, the bait, an attractant, and

TABLE 1. Characteristics of experiments conducted with rabies vaccine-baits in Huron and Grey Counties, Ontario, Canada.

Year	Date (Sept)	Area size (km ²)	Bait density		Bait type	Bag type	Attractant	Habitats into which baits were dropped ^a
			Num-ber/km ²	Num-ber/flight km				
1984	26	542	18.4	17.2	Sponge	Clear	Liver	All except woods and corn ^b
1985	24	542	19.8	18.7	Sponge	Clear	Liver	All
1986	22	760	19.7	18.9	Sponge	Clear	Liver + beef ^c	All
1987A	21	340	20.7	19.5	BP ^e	Clear	Chicken ^d	All
1987B	21	290	22.8	24.6	BP ^e	None	Chicken ^d	All
1987C	22	332	21.8	22.6	BP ^{e,f}	Green, perf.	Chicken ^d	All
1987D	22	321	24.9	25.1	BP ^{e,f}	Green	Chicken ^d	All
1987E	23	350	17.2	14.8	BP ^{e,f}	None	Chicken ^d	Woods and corn only

^a Baits were never dropped on towns, roads, near farmhouses, over water, or on recreation areas.

^b In 1984, baits were dropped in standing corn only within 30 m of the edge of the field.

^c 15-g ball of ground beef.

^d Chicken essence was added to the wax mixture, see methods.

^e BP = plastic blister-pack.

^f Tallow mixture only, no BP.

a plastic bag (Fig. 1) (Johnston and Lawson, 1987). First, a 4 × 3 × 3-cm cube of polyurethane sponge (Engineered Foam Products Ltd., Weston, Ontario, Canada M9L 1N1) was dipped

in a mixture of histological paraffin (Fisher Scientific Division, Allied Canada Ltd., Don Mills, Ontario, Canada M3A 1A9) and tallow (Minor Meats Ltd, Lowbanks, Ontario, Canada N0A

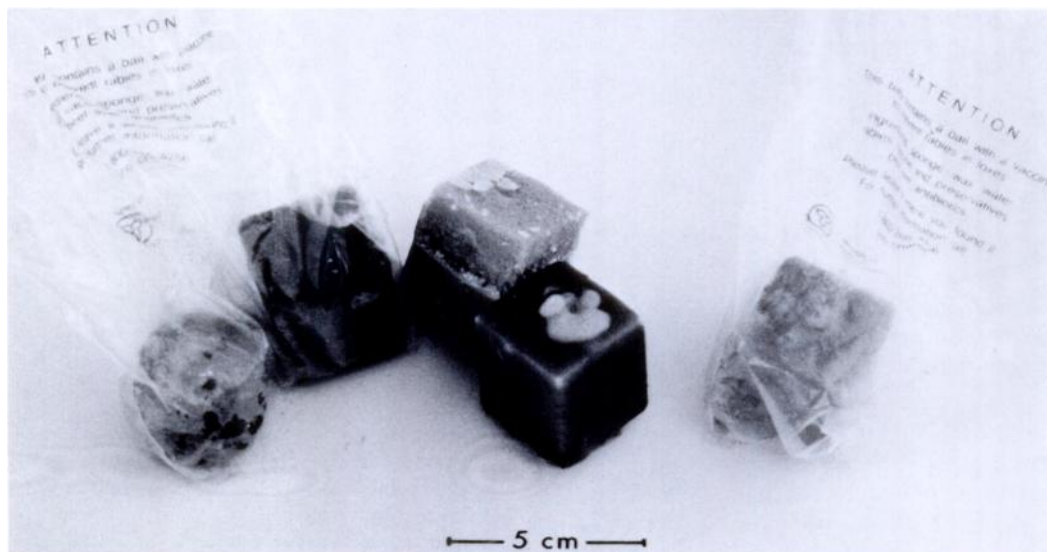


FIGURE 1. The sponge bait used in 1984 to 1986 was a cube of polyurethane sponge (center rear) coated with two to three layers of a paraffin and tallow mixture (center front). The tetracycline biomarker was incorporated into the outer wax layer, giving a caramel color. In 1985 to 1986 a wax strengthener was added to the mixture. The 14 ml of rabies vaccine (1985 to 1986) or placebo (1984) were injected into the sponge matrix. The coated cube was placed into a labelled plastic bag (right) for aerial distribution; 10 to 15 ml of a beef liver and water mixture were added into the bag as a fox attractant. In 1986 a 15-g ball of fresh Grade A ground beef was also added (left).



FIGURE 2. In 1987 the blister pack (BP) bait was a labelled cube (left) consisting of tallow, mineral oil, wax strengthener, commercial chicken essence, green food color and tetracycline. The rabies vaccine (2 ml) was carried in a BP placed in the center of the cube (center). The BP was a polystyrene container with a labelled plastic and aluminum foil cover (right).

1K0). In 1985 and 1986, Microbond (International Wax Ltd., Agincourt, Ontario, Canada M1S 2A8), a wax strengthener, was added to the mixture to decrease impact damage to air-dropped baits. Three separate immersions in the molten mixture resulted in a 2- to 3-mm thick coating, providing firmness and moisture resistance. Tetracycline-HCl (Novopharm Ltd., Toronto, Ontario, Canada M1P 2Y1), the bio-marker used to evaluate bait acceptance, was suspended in the wax bath for the outermost coat, sufficient to give 400 to 475 mg tetracycline per bait in 1984, and 75 to 140 mg in subsequent years. The bait was then sterilized by irradiation (1.5 megarad; Sterirand Ltd., Mississauga, Ontario, Canada L4W 1V7).

After the wax layers had hardened, 14 ml of vaccine (or placebo) were injected into the sponge, and the hole sealed with hot wax. In 1984, a placebo was used, consisting of the cell-culture medium in which the vaccine was normally grown (for ingredients and sources, see Lawson et al. 1989). In 1985, the vaccine was ERA* (Connaught Laboratories Ltd., Willowdale, Ontario, Canada M2R 3T4, as described by Ableseth [1964]), a MLV rabies vaccine derived from the SAD strain of rabies virus (Rhodes, 1981). In 1986 and 1987, a higher-titred ERA preparation was used (details given in Lawson et al., 1989). Results of vaccination will be presented in a future paper.

The second component of sponge baits was attractant. In 1984 and 1985 this attractant was a slurry made by mixing raw beef liver (Adams Meat and Abattoir, Minesing, Ontario, Canada L0L 1Y0) (1 kg) and water (1 l) in a blender. About 10 to 15 ml were added to each bait. In

1986, a 15 g ball of fresh (Grade A) ground beef (Corpack Ltd., Concord, Ontario, Canada L4K 1A8) was added with the liver (Minesing Meats, Minesing, Ontario, Canada L0L 1Y0).

The third component of the sponge bait was a clear polyethylene bag (C.W. Packaging Ltd., Markham, Ontario, Canada L3R 2V4), 17 × 23 × 0.0025 cm, stamped with an identification label (Fig. 1). The cube and attractant were placed together in an unsealed bag. Each bait weighed about 41 g, not including ground beef. The bag weighed <1 g. Baits were stored at -20 C in larger, sealed bags containing 48 baits each.

The 1987 baits were a much modified derivative of the sponge bait. Vaccine (2 ml) (made as described by Lawson et al. 1989) was enclosed in a 2 × 2 × 1 cm polystyrene blister-pack (BP) with a plastic and aluminum foil cover (Fig. 2) (Norpack Ltd., Brampton, Ontario, Canada L6W 3J6). That was cast into a 3.5 × 3.5 × 2 cm block of tallow (59%) (Fig. 2) which also contained microbond (32%), mineral oil (8%) (Daminco Inc., Mississauga, Ontario, Canada L4W 2R9), a commercial chicken flavor essence (1%, International Flavors and Fragrances (Canada) Ltd., Concord, Ontario, Canada L4K 1Y2), 100 mg of tetracycline, and a custom-blended dye (0.3%, Dyeco Ltd., Kingston, Ontario, Canada K7L 4X6) to give baits a dirty greenish color. Some baits contained the same volume of tallow mixture but no BP (Table 1). Each bait had a label (Connaught Laboratories Ltd., Willowdale, Ontario, Canada M2R 3T4) affixed to the surface, and a printed warning on the BP cover (Fig. 2). Completed BP baits weighed about 20 g. Baits were stored at -20 C.

Experimental design

Features of the experimental design are listed in Table 1. Before 1987, a plastic bag was essential to keep the attractants and the sponge bait together, but that was no longer necessary with the BP bait. There was some question as to whether the bag was a visual attractant for foxes. Therefore, in 1987 some baits (experiments 1987B and 1987E, Table 1) were dropped without bags. Croze (1970) suggested that the color green was less attractive to crows, so bags of an opaque light forest green (J.M.C. Plastics Ltd., Markham, Ontario, Canada L3R 2Z8) were used for experiments 1987C and 1987D. There was concern in 1984 to 1986 that many baits were found with the mouth of the bag held closed by the weight of the sponge cube, as a result of rolling after impact. Therefore, the green bags in one area (C) were perforated, to allow diffusion of scent regardless of how the bait landed. Perforation was achieved by pulling the bags between two cylindrical brushes made of stiff wire 0.9 mm in diameter.

Bait distribution

Frozen baits were dropped in late September (Table 1), from Cessna 172 aircraft (Cessna Aircraft Co., Wichita, Kansas 67277, USA chartered from Western Air Services, Goderich, Ontario, Canada N7A 3Y4) flying approximately 100 m above ground level, at about 130 km/h. The flight crew included a pilot, navigator and baiter. Flight lines were marked in advance on 1:50,000 topographic maps. The navigator directed the pilot along flight lines, marked deviations from the planned routes, and recorded where each set of 48 baits started and ended. An electronic metronome (Metrina, purchased from Cosmo Music Warehouse Ltd., Thornhill, Ontario, Canada L4J 1X5) was used to standardize the dropping rate at 60 baits/min (one bait/36 m on the ground). The actual rate was varied from 50 to 72 baits/min, to compensate for variations in ground speed due to wind. Baits were held in a 50 × 30 × 10-cm tray connected to a 1.25-m length of 15-cm diameter PVC pipe (Canadian Tire Corporation, Richmond Hill, Ontario, Canada L4C 5T2) which protruded from the plane's baggage hatch. A single bait was dropped down the pipe at each click of the metronome. The navigator signalled the baiter when not to drop baits.

Flight lines were planned to pass over fields about 30 m from the edges of woodlots. There were two flight lines between each pair of concession roads, one on each side of the strip of woodlots. Baits were not dropped over water, towns, farmyards, houses or within 50 m of roads. In 1984 baits were dropped in standing corn only within 30 m of field edges, and no baits

were dropped into woodland. In subsequent years baits were dropped into corn and woodland whenever a flight line passed over those habitats. In 1987, baits were dropped into only corn and woodland in experiment 1987E. That was done to provide maximum concealment from crows.

The intent was to achieve the same density of baits in all experiments. The drop rate was kept constant, but variations in the number and size of places where baits were not dropped, and differences between areas in the spacing of roads, resulted in some variations in overall density of baits (Table 1).

Trappers caught most foxes between mid-October and mid-November. Because vaccine was used in some of these experiments, we dropped baits 3 wk before trapping began to allow time for the foxes to develop antibody to the vaccine prior to being caught.

Field evaluation

Ground surveys were conducted along flight lines during the first week after baiting to evaluate the condition and rate of disappearance of baits. Tracks near the bait, and bite marks on the bag were used to identify the species which had contacted the bait. Biting, chewing, and moving the cube was defined as disturbance of the bait.

In 1987, additional experimental sets of baits were placed by hand on 11 plots located outside the air-drop areas. These were designed to allow direct comparisons of animals' choices between different baits. We tested baits without bags, and baits in clear, opaque white and opaque green plastic bags. Each bag color was tested in perforated and unperforated forms. Nine of 11 plots were established on open, sandy sites where tracks would be easily seen; the remaining two were on pasture. Baits were placed in parallel rows 35 m apart on the first five plots, with baits at 35 m intervals within the row. Each row contained only one bait type. Ideally each row within a single habitat type would have had ten baits, but in some cases only eight or nine baits could be placed before a woodlot was encountered. In the second group (six plots), 35 m spacing between rows and baits was retained, but bait types were placed in random sequence. Baits were checked on days 1, 2, 3 and 7.

Bait acceptance

Fox, skunk and raccoon carcasses, taken both inside and outside the baited areas, were collected from local trappers. The trappers provided date of death and location of capture. Carcasses were collected from early October until late November each year, although 90% of animals were taken between 15 October and 15

November. Thin, undecalcified sections of the roots of canine teeth were examined for evidence of tetracycline, seen as fluorescent lines in the dentin and cementum of juveniles' teeth, or the cementum of adults' teeth (Johnston et al., 1987). Bait acceptance was defined as the percentage of all animals examined which revealed tetracycline deposits in their teeth when examined under an ultraviolet (UV) microscope.

Dentin in sections of teeth from juvenile animals was examined to determine the minimum number of baits eaten. In juvenile foxes only, the dates on which baits were eaten were determined by locating individual tetracycline deposits with reference to the incremental lines of von Ebner (Johnston et al., 1987). These dates are accurate to ± 1 day (Johnston et al. 1987). Although at least 46 foxes in this study were estimated to have eaten a bait on day 0 (Fig. 1), there were only three cases where examination of a tooth gave an estimate of a bait eaten on day -1 .

Small mammal experiment

An experiment was conducted in 1984 outside the baited area to evaluate the rate of small mammal contacts with the baits. Two 140×140 -m grids were placed at opposite ends of an abandoned pasture. Sponge baits were placed at 10-m intervals along the grids, resulting in 196 baits/grid. Those baits were examined for animal contact after four days. Animals were grouped as: small mammals, carnivores or birds. On the seventh day after placement, traps (Victor snap, Ekco Canada Ltd., Scarborough, Ontario, Canada M1K 1M5) were baited with a mixture of peanut butter and oatmeal (both purchased from Loblaw's Supermarkets Ltd., Richmond Hill, Ontario, Canada L4C 3C7), and one set within 1 m of each bait site. Traps were emptied and rebaited daily, and removed after four nights, giving 784 trap-nights/grid. Mandibles of all trapped animals were examined for tetracycline by UV-microscopic examination of thin sections cut directly from the jaw (Johnston et al., 1987).

Statistics

The chi-square statistic was used for all comparisons of bait acceptance unless a sample contained < 25 individuals, in which case Fisher's exact test was used. Values are reported as $\bar{x} \pm \text{SE} (n)$. Alpha was set as 0.05 unless otherwise noted (Tacha et al., 1982). Statistical methods generally followed Sokal and Rohlf (1981).

The pattern of bait acceptance was examined as follows. The number of baits (B) remaining at the beginning of time interval i can be expressed as

$$B_i = B_0 r^i \quad (1)$$

where r is the proportion of baits remaining at the end of each period. This assumes that r did not vary over time. If we assume that the number of foxes (F) picking up baits during the interval is proportional to the number of baits present, then

$$F_i = F_0 r^i \quad (2)$$

This can be analyzed by linear regression after logarithmic transformation:

$$\ln(f_i) = \ln(F_0) + i \cdot \ln(r) \quad (3)$$

The exact number of baits picked up by foxes during a time interval could not be determined, but the number of days on which juvenile foxes picked up baits was estimated by locating tetracycline marks within growth lines in the dentin (Johnston et al., 1987). We performed two analyses, one on the number of foxes picking up their first bait in each time period, the other including all the days on which foxes picked up baits.

It proved necessary to combine data for consecutive days. While it would be ideal to use a one-day interval, none of our fox samples was large enough, with the result that variance about the regression was too large. Experiments indicated that the most useful interval was four days. Combining data for more than four days resulted in too few degrees of freedom in many regressions. Where there were < 40 juvenile foxes in a sample, results were variable even with this adjustment.

Counts of foxes which ate their first bait in each 4-day period were standardized by multiplying by the reciprocals of (1) the size of the study area, (2) the density of baits, and (3) the total foxes (adult and juvenile) taken per km^2 on that area. If we assume that such adjustments minimize effects due to specific study areas, then the quantity F_0 in equation (2) may be an index to the initial attractiveness of a particular bait to foxes, and the estimate of r should express the rate of depletion by all species.

RESULTS

Bait dispersion

Dispersion statistics of each experiment are listed in Table 1. Distance between baits on the ground varied between 38.2 ± 1.33 m ($n = 282$, 1984) and 45.0 ± 2.03 m ($n = 45$, 1987C). Those values are minimum estimates, because gaps where no bait was found were not included in the

TABLE 2. The condition of air-dropped rabies vaccine-baits found 1 to 8 days after distribution in Huron County, Ontario.

	1984	1985	1986	1987 clear bag	1987 green ^a bag
Search days	8	8	2	2	2
Number found	325	279	389	58	157
Location					
% in open ^b	95	84	74	90	86
% in cover ^c	5	15	26	10	14
% Baits not disturbed	53	47	58	64	89
% with cube damaged	16	9	3	3	0
% with bag open	0	32	54	89	86
% Disturbed by animal	47	53	42	36	11
% with species identified	66	68	76	86	94
% with vaccine or placebo still present	35	18	25	29	—

^a Results from C and D experiments pooled.^b Plowed fields, open stubble, grazed pasture.^c Hay, corn, beans, woodland, idle pasture.

measurements. It was impossible to tell whether gaps resulted from a bait having been removed by some animal, or having drifted out of line during descent, or not having been dropped at the precise 1 second interval. Baits were difficult to find in some habitats, so a gap might represent a bait present but not found.

There appeared to be a weak positive relation between density of baits (Table 1) and fox acceptance (Table 6), but it was not significant (linear regression of arcsine

transformed acceptance on density, $R^2 = 0.32$ $P = 0.142$).

Fate of baits

The fates of baits found during field surveys are summarized in Table 2. Crows were seen carrying sponge baits by the bag, and in one case several empty bags were found under a row of trees about 50 m from a line of baits in a plowed field. We were unable to find BP baits dropped without bags in 1987.

TABLE 3. Numbers of air-dropped rabies vaccine-baits contacted by various animals, Huron County, Ontario, Canada.

Species	Fate of cube										Percent removed by each species		
	Disturbed, liquid present					Removed							
	1987					1987							
	1984	1985	1986	C ^a	G ^a	1984	1985	1986	C ^a	G ^a	1984	1985	1986
Crow	15	11	15	6	2	36	69	69	8	0	63	87	72
Fox	0	0	2	0	0	16	8	19	1	0	28	10	20
Skunk	0	0	0	0	0	0	0	0	2	0	0	0	0
Raccoon	0	3	6	0	1	0	1	1	0	0	0	1	1
Coyote	0	0	0	0	0	0	0	0	1	0	0	0	0
Small mammal	24	7	2	0	13	0	0	0	0	0	0	0	0
Dog	0	0	0	0	0	4	1	7	0	0	7	1	7
Cow	3	0	0	0	0	0	0	0	0	0	0	0	0
Insect	1	0	3	0	0	1	0	0	0	0	2	0	0

^a C = clear bag, G = green bag (C + D results pooled).

TABLE 4. The fate of 405 hand-placed rabies vaccine-baits on 11 different plots in Huron County, Ontario, in 1987.

	Clear bag		White bag		Green bag		No bag
	Unperforated	Perforated	Unperforated	Perforated	Unperforated	Perforated	
Total baits observed	64	49	69	44	63	53	63
% disturbed	53	78	25	53	44	45	87
Species contacts							
Crow	2	8	3	3	5	2	0
Fox	2	3	3	3	1	0	3
Skunk	0	3	2	0	2	0	0
Raccoon	0	0	1	0	0	0	4
Small mammal	4	14	2	10	4	10	23
Insect	3	1	1	1	1	1	0
Unidentified	23	9	5	6	15	11	25

Crow contact with baits was higher in 1985, 1986 and 1987 than in 1984 ($P < 0.05$, Table 3). Since ground beef was added to the liver attractant only in 1986, that result suggested that ground beef did not increase crow depredation on the baits.

Results of the 1987 ground experiment are shown in Table 4. Baits without bags were contacted more than bagged baits ($P < 0.001$). Bag color had no significant effect on contact ($P > 0.20$). Small mammals encountered perforated bags more than unperforated ($P < 0.05$). Target species

and crows tested individually showed no preferences ($P > 0.20$) for perforated or entire bags, but samples were small.

Bait acceptance

The data on acceptance of baits appear in Tables 5, 6, 7 and 8. The achieved density of baits was lower in experiments 1984 and 1987E, because some habitats received no baits (Table 1). Statistical comparisons involving the 1984 and 1986 experiments included only foxes trapped within 28 days after baits were dropped (Table 6), to reduce the effects of fox dispersal.

TABLE 5. Bait acceptance by red foxes (*Vulpes vulpes*) in Huron and Grey Counties, Ontario, Canada. The data are divided into animals trapped more or less than 28 days after the baits were dropped. Acceptance is the percent of animals which had tetracycline marks in their teeth.

Experiment	Days after baits dropped				<i>P</i> Difference between ≤28 and >28
	≤28		>28		
	%	<i>n</i>	%	<i>n</i>	
1984	64 <i>a</i> [*]	89	37 <i>a</i> [*]	60	0.001
1985	63 <i>a</i>	75	65 <i>b</i>	71	0.789
1986	61 <i>a</i>	111	44 <i>a</i>	72	0.026
1987A	67 <i>a</i>	48	71 <i>b</i>	21	0.130
1987B	50 <i>a</i>	8	71 <i>b</i>	21	0.729
1987C	54 <i>a</i>	13	20 <i>ab</i>	10	0.680
1987D	50 <i>a</i>	8	74 <i>b</i>	27	0.351
1987E	50 <i>a</i>	16	23 <i>a</i>	22	0.104

^{*} Values in a column which are followed by the same letter did not differ at $\alpha = 0.05$.

TABLE 6. Total acceptance of air-dropped rabies vaccine-baits by red foxes (*Vulpes vulpes*), striped skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*), Huron and Grey Counties, Ontario, Canada.

	Fox		Skunk		Raccoon	
	%	n	%	n	%	n
1984	53 a ^{*,b}	149	38 a [*]	88	29 a [*]	83
1985	64 a	146	33 a	63	43 b	108
1986	55 a ^b	183	25 a	44	5 c	89
1987A	68 a	69	24 a	51	47 b	157
1987B	66 a	29	23 a	30	24 a	41
1987C	39 ab	23	14 a	7	44 b	43
1987D	69 a	35	24 a	34	29 a	68
1987E	37 b	38	20 a	5	—	0 ^c

^{*} Values in a column which are followed by the same letter did not differ at $\alpha = 0.06$.

^b Testing done with only foxes trapped within 28 days of bait placement, because of dispersal; see Table 5.

^c No sample collected.

TABLE 7. Numbers of days on which target species took rabies vaccine-baits, as estimated from tetracycline deposits in the dentin of canine teeth from young-of-the-year.

Experiment	Fox	(n)	Skunk	(n)	Raccoon	(n)
1984	bc ^a 3.4 ± 2.0	(81)	1.9 ± 1.4	(25)	2.5 ± 1.7	(15)
1985	ab 3.9 ± 2.1	(93)	1.3 ± 0.7	(15)	1.7 ± 1.1	(30)
1986	ab 4.2 ± 2.8	(112)	2.3 ± 1.0	(9)	1.7 ± 0.7	(9)
1987A	bc 3.5 ± 1.9	(49)	1.9 ± 1.1	(10)	1.7 ± 0.8	(53)
1987B	bc 3.3 ± 1.9	(15)	1.5 ± 0.6	(6)	2.0 ± 1.3	(11)
1987C	bc 2.7 ± 1.5	(19)	1.0	(2)	2.0 ± 1.2	(12)
1987D	ab 4.1 ± 2.0	(23)	1.6 ± 0.9	(8)	1.8 ± 1.1	(17)
1987E	c 2.0 ± 1.2	(17)	1.0	(1)	—	

^a Means preceded by the same letter were not different ($\alpha = 0.05$). No differences were detected among means for skunks or raccoons.

The number of tetracycline lines/tooth (Table 7) was lowest for those fox and skunk samples having the lowest overall acceptance (Table 6). For foxes, the Spearman correlation coefficient was $R_s = 0.62$ ($0.05 < P < 0.20$), for skunks, $R_s = 0.70$ ($0.05 < P < 0.10$), and for raccoons $R_s = 0.018$ ($P > 0.80$).

The analysis of bait attractiveness, and indicators of the rate of depletion, are listed in Table 8. The analysis involving only the first days on which an individual fox took a bait did not indicate that any of the experimental baits was clearly superior. In 1987, baits in clear bags stimulated higher

($P < 0.05$) initial acceptance than baits in green bags or no bags. However, during 1984 to 1986, early acceptance was not consistently high when baits were in clear plastic bags.

The regression analysis using all days on which individuals took baits produced very erratic results. The data simply did not fit the model used. In several cases where there were large samples of foxes collected (1976, 1977, 1985), the data did not fit equation (3), because there were many more baits taken in time periods 2 and 3 (days 5 to 12) than in period 1.

Fox acceptance did not vary whether

TABLE 8. Analysis of the temporal pattern of acceptance of rabies vaccine-baits, based on tetracycline marks in the dentin of canines taken from juvenile foxes (*Vulpes vulpes*). The statistic F_0 is an index of the attractiveness of baits, while r is proportional to the rate of depletion of baits in the field (see text).

Experiment	Day of first bait						All bait-days included				
	F_0	95% C.I.	$\ln r$	P	R^2		F_0	95% C.I.	$\ln r$	P	R^2
1976B ^a	bc ^a 8.0	2.3–28.0	a ^a -0.34	0.03	0.56				-0.14	0.15	0.32
1976E ^a	a 14.4	5.2–40.0	a -0.30	0.03	0.63				0.06	0.33	0.16
1976W ^a	bc 6.9	2.9–16.6	a -0.37	0.01	0.76				-0.22	0.06	0.48
1977 ^a	b 10.3	5.3–20.0	a -0.36	0.001	0.84	a 18.6	11.0–31.6	a -0.14	0.04	0.55	
1980B ^b	b 10.6	6.7–16.7	b -0.85	0.001	0.99	a 26.0	15.4–43.9	b -0.55	0.001	0.95	
1980O ^b			-0.84	0.07	0.87	a 15.4	8.1–29.6	a -0.23	0.02	0.69	
1984	bc 7.8	5.8–10.4	a -0.34	0.001	0.96	a 20.8	10.9–40.0	b -1.19	0.02	0.60	
1985	c 6.1	4.4–8.3	a -0.24	0.001	0.91			-0.01	0.87	0	
1986	bc 8.1	4.1–16.0	a -0.46	0.001	0.90	a 33.5	24.9–45.0	a -0.25	0.001	0.93	
1987A	a 14.6	6.7–31.9	b -0.66	0.01	0.93	a 35.1	15.8–78.0	a -0.39	0.001	0.80	
1987B			-0.14	0.32	0.32			-0.23	0.09	0.41	
1987C			0	1.0	0			-0.07	0.57	0.07	
1987D	bc 6.6	2.5–17.8	a -0.31	0.02	0.71	a 19.9	14.2–28.0	a -0.14	0.01	0.75	
1987E	bc 7.1	3.9–12.8	a		0.87			-0.19	0.08	0.58	

^a 30 g ball of ground deadstock meat in clear plastic bag (D. H. Johnston, unpublished data).

^b 30 g ball of grade A ground beef in clear plastic bag (D. H. Johnston, unpublished data).

^c Statistics preceded by the same letter do not differ at $\alpha = 0.05$.

large fields of standing corn and woodland were baited or not (1984 versus 1985, $P > 0.96$). When woods and cornfields alone were baited, fox acceptance was lower than when all habitats received similar baits (1987E compared with 1987A, $P < 0.005$, or 1987B, $P < 0.017$). The 1987A area resembled previous years' experiments in having a clear plastic bag surrounding each bait, whereas the 1987B experiment differed from the 1987E area only in the habitats into which baits were dropped. Density of baits was lower on the 1987E area (Table 1), because baits were dropped at the standard rate, but into fewer habitats.

Neither ground beef nor liver slurry increased acceptance by foxes ($P > 0.40$). Presence or absence of the plastic bag did not affect bait acceptance (1987A versus 1987B, $P > 0.59$). Bag color did not affect acceptance by foxes ($P > 0.25$), nor was there any difference in acceptance between baits in perforated versus intact green bags (1987D versus 1987C, $P = 0.21$), but the sample sizes were small.

Figure 3 shows the time when juvenile foxes picked up their first baits for those experiments where there were at least 25 individuals taken.

In Table 5, acceptance estimates from foxes taken within 28 days of the bait drop were higher ($P < 0.05$) than those taken later in 1984 and 1986, but not in 1985. The 1987 results were erratic, and analysis was difficult due to small samples. Tetracycline-marked foxes were taken regularly outside the air-drop areas (Table 9).

The percent of skunks marked with tetracycline was the same, regardless of differing baits and distribution. Raccoons presented a variable picture in which the results did not relate clearly to the experimental variables (Table 6).

Small mammal experiment

Small mammals contacted 12% ($n = 392$) of the baits on the experimental grid. Only half of those had the sponge exposed by gnawing, enabling the mammal to contact the vaccine. Thirty-seven small mammals

TABLE 9. Rabies vaccine-bait acceptance by red foxes (*Vulpes vulpes*), striped skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*) taken outside air-drop areas, but within 25 km of the boundaries (Huron and Grey Counties, Ontario, Canada).

	Red fox		Skunk		Raccoon	
	%	n	%	n	%	n
1984	16	103	6	47	5	101
1985	12	232	0	80	2	119
1986	12	170	0	52	5	131
1987	13	252	2	136	8	324

were trapped: 22 short-tailed shrews (*Blarina brevicauda*), 13 meadow voles (*Microtus pennsylvanicus*) and two deer mice (*Peromyscus leucopus*). Seven of the shrews and one deer mouse, but none of the meadow voles, had tetracycline in their mandibles.

DISCUSSION

The rabies enzootic zone in Ontario covers more than 98,000 km². Our results indicated that baits distributed at about 20/km² usually achieved 50% or greater acceptance by foxes. The two baits described here are designed for delivery of liquid MLV rabies vaccine, which must be absorbed through the mucosa of the oropharynx to be effective (Black and Lawson, 1973; Baer et al., 1975). The BP bait was more suited to mass-production. The plastic bag was essential to maintain the integrity of ground beef baits, to cushion the fall of sponge baits when they were dropped from the air, and to retain the attractant. The 1987 BP bait was robust enough to be dropped without a bag, and demonstration that lack of the bag and meat attractant did not reduce acceptance by foxes was important. Baits will be much more easily produced and distributed in very large quantities if bags and liver slurry are not required.

Measuring acceptance by foxes

Baits were dropped and foxes trapped during the period when juvenile foxes were dispersing most actively (Voigt, 1987),

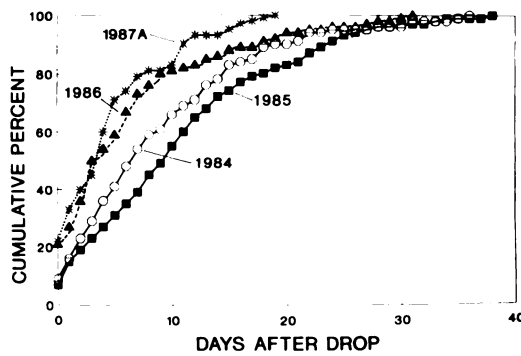


FIGURE 3. Cumulative percent of the days on which juvenile foxes (*Vulpes vulpes*), which ate baits, ate their first bait, plotted as days after baits were dropped. Sample sizes were: 1984, $n = 80$; 1985, $n = 94$; 1986, $n = 114$; 1987A, $n = 42$.

which caused difficulty in interpreting bait acceptance. Dispersal must have reduced the estimates of acceptance. If the baited area were large enough that a majority of dispersing foxes started and ended their movements within the baited area, then we believe acceptance would be higher than indicated in Tables 5 and 6, but we could not estimate how much higher. Average dispersal distances for juvenile foxes in Ontario were 27 km for males, and 7 km for females (Voigt et al., 1985). That indicated a strong possibility of ingress or egress from the study sites. Some foxes trapped within the experimental areas must have arrived there after baits were no longer present. Also, foxes which had eaten baits may have left the area without being trapped. The estimates of acceptance based on animals trapped within 28 days of bait placement are closer to the true value. In 1987, dispersal appeared to alter acceptance from only two of five areas (C, E), although small samples made statistical analysis inconclusive. Because dispersal was a significant factor in 1984 and 1986 (Table 5), we used data only from foxes taken up to 28 days after baits were dropped for statistical comparisons with other experiments.

European measures of bait acceptance were sometimes higher than ours. Schneider et al. (1988) reported on 12 experi-

mental areas in Germany, of which three had higher ($P < 0.05$) acceptance than the best Ontario trials. These were larger areas than we used, so the difference may in part be due to less fox dispersal. However, the baits were different and were placed by hand, at densities of 15 to 20/km², but twice a year, in spring and fall. Fox samples were taken only after the fall baiting. In other European trials, acceptance values fell in the same range as we encountered (Artois, 1987).

Time of acceptance

Figure 3 shows the cumulative profile of dates upon which individual foxes ate their first baits. While that allows easy visual interpretation, such graphs are difficult to compare statistically. If many baits were picked up within 24 hr of distribution, there can be no doubt that foxes found the baits attractive, but rapid depletion of baits by non-target species might produce a curve similar to the profile provided by an attractive bait which was not rapidly depleted.

The contrast between the two regression analyses provided essential information. Two scenarios can be considered. First, if a bait was highly attractive to foxes, then the analysis including only the first days on which individuals ate baits should provide both a significant r value and a high value of F_0 . The significant r value would result from "depletion" of unmarked foxes as more and more of the population had eaten a bait. The high value of F_0 would reflect the initial attractiveness. However, considering that 20 to 200 baits were dropped per fox on the area, foxes alone should not deplete available baits rapidly. (Voigt and Tinline [1982] estimated Ontario fox population densities in the range 0.1 to 1.0 foxes/km², and baits were distributed at about 20/km² [Table 1].) Therefore, the analysis of all bait-days need not produce a significant r estimate. Secondly, if baits were depleted rapidly by non-target species, there should be significant r estimates in both analyses, but the value

of F_0 should not be high. The significant r values would result from the fact that foxes could not find baits as time progressed. Variability about the regression should also be reduced, for the same reason, leading to a higher R^2 .

The regression analyses (Table 8) produced several useful results. In Table 8, F_0 as estimated from the day of first bait only indicated that 1976E and 1987A had high values of F_0 . Two other groups (b and c) were poorly separated. There was no experimental feature common to the high group, but absent in all other experiments. Failure of the data to fit a depletion curve based on all days on which foxes took baits (Table 8) gave two important insights. First, depletion of baits by non-target species could not be rapid enough to reduce overall acceptance by foxes. The values of r , coupled with relatively high R^2 values observed in 1984, 1980B, 1987A, 1986 and 1980O might indicate that depletion was sometimes a problem, but the connection is weak. Secondly, there was a change over time in the fox-bait interaction such that acceptance rose for the first 8 to 12 days. The three most plausible explanations are that foxes learned that baits were edible and searched for more, that foxes cached baits when first found, and ate them later, or that the scent of baits became more attractive up to twelve days. In the case of baits with meat attractants, changes in scent must have occurred.

Caching could explain the observed rise in numbers of baits eaten after 8 to 12 days. One worker (D. H. Johnston, unpubl. data) observed a fox cache four baits, but those were not retrieved after 2 wk. The evidence from tetracycline lines in teeth indicated that caching was not a problem, since most foxes (>70%) ate their first bait in the first 14 days (Fig. 3).

The erratic results of these two analyses, plus yr-to-yr variation in fox dispersal (Table 5) and unexpected variations in overall acceptance (Table 6, 1987C versus 1987D) indicated that variation in factors which we were unable to measure may have in-

fluenced overall acceptance. We did not have local estimates of fox density, or of food resources for foxes, both of which must affect the reaction to baits. If those measures varied from yr to yr, then accurate tests of which characteristics of baits affected acceptance would require replication in ≤ 2 yr. However, the relatively narrow range of acceptances observed indicated that such uncontrolled effects were probably minor.

Replication within 1 yr on different plots may introduce some unexpected variation due to inherent differences between areas. Baits were dropped during the period of most active harvest of the major crops, corn and beans. Harvesting would certainly change the availability of baits, and might also alter fox activity. In some years, at least part of the stubble was plowed under in the 2 wk after baits were dropped. Variation between plots in 1987 might have been affected in that way.

Habitat

Initially we believed that acceptance would be poor if baits dropped into woodlots were covered by falling leaves. The major leaf-fall in the region occurred in early to mid-October, shortly after baits were dropped. Similarly, baits dropped into standing corn seemed likely to be obscured. Placing baits into corn and woods as well as into other habitats did not raise acceptance (1984 versus 1985, $P > 0.50$) during the first 28 days. Dropping baits continuously simplified the distribution process without substantially raising bait density (Table 1). However, we did select habitats by flying along the edges of woodlots, rather than following arbitrary lines regardless of habitat.

Readers are cautioned about comparing bait acceptance reports from different studies. In these experiments baits were neither uniformly nor randomly distributed throughout the area, whereas in Germany, where baits were placed by hand, uniform distribution was reported (Schneider et al., 1988). We used two flight lines

between each pair of roads, and the same distance between baits. However, distance between roads was not uniform, so the dispersion of baits did vary. In addition, places which were not baited varied from experiment to experiment, partly as an experimental variable (1984, 1987E), and partly as a property of the landscape. The probability of a fox encountering a bait probably depends on dispersion as well as density. European workers also attempted to place baits where foxes were thought likely to find them, and hid the baits under leaves or debris to reduce corvid problems. Thus, while overall number of baits/km² seems a simple measure, it must be used with caution.

Skunk and raccoon acceptance

Skunks have smaller home ranges than foxes, and their nightly movements are less (Rosatte, 1987; Voigt, 1987). The acceptance values in Table 6 suggest that all bait combinations used in these experiments were eaten by skunks, but acceptance was poor. It has been shown (D. H. Johnston, unpubl.) that 48 baits/km² resulted in a higher proportion ($P < 0.05$) of skunks eating baits.

Acceptance by raccoons was variable, but the pattern of differences in Table 6 does not relate clearly to the experimental variables (Table 1). Local, day-by-day changes in availability of corn due to harvest activity may have affected the tendency of raccoons to take these baits.

In Table 6, α was arbitrarily set at 0.06 because in the raccoon data, probabilities for four comparisons were between 0.05 and 0.06. For foxes and skunks $\alpha = 0.05$ gave the same result as $\alpha = 0.06$.

Small mammal contacts

Most MLV rabies vaccines induce rabies in a percentage of laboratory mice (Black and Lawson, 1980; Schneider and Cox, 1983; Lawson et al., 1987). The vaccines used in 1985 to 1987 killed 22 to 50% of CD-1 laboratory mice which ate part of a

bait (Lawson et al., 1987, 1989). The 1985 vaccine killed two of 35 wild-caught *Mus musculus*, but left *Microtus pennsylvanicus* and *Peromyscus leucopus* unaffected (Lawson et al., 1987). The 1986 and 1987 vaccine also killed a small percentage of *Microtus* (one of 38) and *Peromyscus* (one of 29) (Lawson et al., 1989). These species, when dying of vaccine-rabies, did not have virus in the salivary glands, and therefore could not pass the disease by biting (Lawson et al., 1989). Scavenging of dead mice by conspecifics did not result in spreading infection among laboratory mice (Black and Lawson, 1980; Baer, 1988). Further, when affected mice were fed to foxes, all the foxes survived and three of six developed antibody to rabies (Black and Lawson, 1980). We sought to evaluate the frequency of small mammal contact with baits in order to assess whether vaccine-rabies posed any threat to small mammal populations. The low number of actual contacts when a very high density of baits was used suggested that there would be no problem. That is reinforced by similar findings from Europe (Wandeler et al., 1982; Schneider and Cox, 1983; World Health Organization, 1986; Artois et al., 1987; Schneider et al., 1988).

Cost

Comparative costs of making and deploying the two types of bait are shown in Table 10. These estimates are probably considerably higher than eventual costs for a large-scale control program. These experimental baits were literally hand-made, by a research team whose total salaries were higher than would be required for a technical team assigned to the same job.

Aerial distribution was a major feature of our approach to mass distribution of baits. The alternative is to place baits individually by hand. In Germany that is done by enlisting local hunters as volunteers (Schneider et al., 1983; Schneider, 1985). In France, a variety of people were hired temporarily (Artois et al., 1987). Given that most of the 98,000 km² of the rabies

TABLE 10. Comparative costs and staff requirements for production and distribution of the sponge and BP baits.

	Sponge bait ^a 1986		BP bait ^b 1987	
	\$CDN ^c	%	\$CDN ^c	%
Expenses per bait				
Bait production	1.24	91	0.62	81
Aerial distribution	0.05	4	0.08	10
Other	0.07	5	0.07	9
Total	1.36		0.77	
Man-hours per bait				
Bait production	0.041	93	0.028	90
Aerial distribution	0.003	7	0.003	10
Total	0.044		0.031	

^a See Figure 1 and methods.

^b See Figure 2 and methods.

^c Canadian dollars (about \$0.85 U.S.).

enzootic area in southern Ontario would have to be treated, we believed that ground placement was either impractical or too expensive. Our total costs/km² were \$29.94 + 0.855 man-hour in 1986, and \$19.25 + 0.621 man-hour in 1987. A small-scale ground distribution experiment in 1988 required 0.65 man-hour/km² for distribution alone, which compares to 0.06 man-hours from the air, assuming 20 baits/km². In France, Artois et al. (1987) reported needing 1.47 to 4.86 man-hours/km² to achieve 15 baits/km², at a total cost of \$52.46 (244 FF)/km², not including costs of serology and rabies diagnosis during evaluation of success (Artois, 1987).

Conclusions

Vaccination will succeed in reducing or eradicating a disease only if a sufficient proportion of the target population can be immunized. In a program aimed at free-ranging wild animals, the proportion of the population which accept baits and the proportion of individuals that eat baits which will be protected from the disease are crucial parameters. Acceptance is affected by several factors, including composition of the bait, and the density and dispersion patterns by which the baits are placed in the field. The two baits tested in these experiments were accepted within the same

range of values as most other baits reported in the literature. Meat attractants did not increase acceptance.

European efforts to eradicate rabies appeared initially successful with vaccination rates as low as 50% (Schneider et al., 1988; Wandeler et al., 1988). Therefore, fox acceptance reported here is sufficient to justify use of these baits in the field, provided the vaccine used immunizes over 80% of all foxes eating a bait. Simulation modelling using these results will also provide support for a decision to begin large-scale baiting to eradicate rabies (Voigt et al., 1985). However, there is no question that higher acceptance ($\geq 80\%$) would be preferable, but difficult to achieve.

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