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VACCINATION OF FOXES AGAINST RABIES USING INGESTED BAITS

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Abstract: A method for immunizing foxes against rabies was evaluated. Fifteen of 36 red foxes (*Vulpes fulva*) fed baits impregnated with modified live virus rabies vaccine developed serum rabies neutralizing antibody. The vaccine-bait proved unstable when held at 4 C or 25 C for 96 hours prior to feeding. For safety testing the vaccine virus was administered to opossums (*Didelphis virginiana*), cotton rats (*Sigmodon hispidus*), hamsters (*Mesocricetus auratus*), and chickens (*Gallus domesticus*) in either liquid or bait form. One of ten cotton rats fed liquid vaccine died of vaccine induced rabies. No animals which ate the vaccine-baits died of vaccine induced rabies.

INTRODUCTION

For many years attempts to control sylvatic rabies in wild carnivore populations were based on lowering population densities to remove infected animals and to reduce intraspecies transmission. This approach has not proven adequate.[4] Recently, the concept of population immunization has been explored and various mechanical devices have been used to experimentally vaccinate wild animals, especially foxes.[7] More recently, oral vaccination *via* instillation of liquid vaccine has been evaluated and proven successful in the laboratory.[1,2,3,5,6,9]

This report describes the immunization of captive red foxes with a field-applicable technique using vaccine-impregnated baits, the field evaluation of similar baits without vaccine, and the safety testing of the vaccine in several species.

MATERIALS AND METHODS

Rabies Vaccine

The vaccine used in this study was the ERA strain of rabies virus grown on BHK-21 cells (ERA/BHK-21) described in an earlier report on oral vaccination.[1] Titer of the vaccine at the time of incorporation into baits or when instilled as liquid vaccine into the buccal cavity of animals was $10^{6.5}$ to $10^{7.0}$ 50% mouse intracerebral lethal doses ($MICLD_{50}$'s)/.03 ml in 3-week-old weanling mice.

Baits

For the laboratory experiments, commercial dog biscuits (Milkbone, medium)* were impregnated with either 1.0 ml or 4.0 ml of ERA/BHK-21 vaccine (by dropping liquid vaccine on the surface of the biscuit) and then chilled to —75 C. The cold biscuits were then immersed in a liquid wax mixture (by volume: 49% paraffin, 49% beef tallow,

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* Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.

2% sardine oil; melting point 85 C) to make them more attractive and waterproof. Baits thus prepared were stored at —75 C until used. For evaluation of the field bait acceptance, baits were similarly prepared except that the impregnation with vaccine was omitted.

Animals

The red foxes used in the laboratory evaluation of the oral vaccine were subadult to adult (4 months to 1 year old) animals which had been collected from the wild as pups. Opossums were captured in the wild as adults. Foxes and opossums were held in captivity at least 60 days before inclusion in the study. All were found to be negative for rabies serum neutralizing antibody when tested against 20 $MICLD_{50}$ of fixed virus (Challenge Virus Standard) in a standard mouse neutralization tests.[8] Cotton rats, Syrian hamsters, and chickens used for safety testing of vaccine in non-target species were born and reared to adulthood in captivity.

Challenge Virus

The rabies virus used to challenge the foxes vaccinated orally and intramuscularly (IM) was obtained from the salivary glands of a naturally infected red fox. The challenge dose was 250 $MICLD_{50}$ in 0.50 ml volume administered IM in the right rear leg by a single puncture.

Field Bait Stations

The acceptance of baits by animals in the field was evaluated using bait stations and determining the utilization of these stations through track and bait observation. The study site for the field evaluation was located on Elgin Air Force Base in Okaloosa County, Florida. Habitat types included cut-over pine forest in sandy soil with grass or second growth pine in various stages of development as well as mature pine and pine-oak forests. Bait stations were placed at 0.3 km intervals along secondary dirt roads in suitable animal habitats. The individual stations were formed by clearing and smoothing a 1 m^2 area and placing two baits in the center of the square. Several drops of fox urine were deposited adjacent to the baits at some stations for added attraction.

PROCEDURES

Fox Oral Vaccination

For the laboratory evaluation of the vaccine baits in the target species, 36 foxes were divided into five groups.

In group A, 12 foxes were each fed one vaccine bait containing 1.0 ml of ERA/BHK-21 vaccine in week 0 and again in week 5.

In group B, six foxes were each fed one vaccine bait containing 4.0 ml of ERA/BHK-21 in week 0 and again in week 10.

In group C, six foxes were each fed two vaccine baits each containing 4.0 ml of ERA/BHK-21 vaccine in week 0.

In group D, six foxes were each fed one vaccine bait containing 4.0 ml of ERA/BHK-21 vaccine in week 0. The vaccine baits had been held at 4 C for 96 h prior to feeding.

In group E, six foxes were each fed one vaccine bait containing 4.0 ml of ERA/BHK-21 vaccine in week 0. These baits had been held at 25 C for 96 h before feeding.

All foxes in each group were bled in weeks 1 and 3 and at 3 week intervals for 21 weeks after oral vaccination, and individual sera were tested for rabies antibody by the serum-virus mouse neutralization test.

Approximately 18 months after the first (or only) oral vaccination, foxes in groups A, B, and C and five non-vaccinated control foxes were challenged IM with rabies virus. Foxes were then observed for 150 days. Any foxes that died during the study were tested for rabies by fluorescent antibody (FA) examination of brain tissue and inoculation of mice with brain and salivary gland

tissues. Survivors were euthanized at 150 days post-challenge and similarly tested for rabies.

Field Bait Evaluation

A total of 273 bait nights (one bait station for 1 night) were set for 4 consecutive nights in June 1972. Each bait station was examined at least once a day for evidence of animal contact. Identification of animals which visited the stations was based on track identification.

Safety Testing

Experiments were conducted to determine whether or not the modified live virus vaccine used in this study might induce clinical rabies in foxes or other non-target species. Five adult red foxes were each inoculated IM in the right *quadriceps femoris* muscles with 4.0 ml of ERA/BHK-21 vaccine which had a titer of $10^{6.5}$/.03 ml in 3-week-old mice inoculated intracerebrally (IC). They were bled for serum neutralizing antibody determinations at 1- and 3-week intervals (See Table 2) and observed for 90 days for signs of clinical disease.

Liquid vaccine was instilled by pipette into the buccal cavity of 10 each of the following species; chickens, 1.0 ml each; opossums, 1.0 ml each; hamsters, 0.3 ml each; and cotton rats, 0.3 ml each. Ten additional cotton rats were each fed one bait biscuit containing 4.0 ml of the vaccine. All animals were observed for 120 days, and any animal that died during this period was necropsied and tested for rabies by FA examination of brain tissue and where appropriate, salivary glands, brown fat, and lung tissues; animals which survived were sacrificed at 120 days and brain tissue tested for rabies by FA examination.

RESULTS

Fox Oral Vaccination

In group A, five of 12 foxes developed rabies antibody, four by 3 weeks after the first vaccination, and one by week 6, after the second vaccination (See Table 1). Two of the seropositive foxes died of causes other than rabies during the 21-week observation period. Two of the 3 surviving seropositives foxes retained antibody titer throughout the 21 week observation period.

In group B, four of six foxes developed antibody, all by 3 weeks post-vaccination. One had a minimal titer and on only one occasion. The other three retained titers throughout the observation period, although one of these died in week 20 of causes other than rabies.

In group C, all six foxes developed antibody by 3 weeks post-vaccination, and all retained antibody titers throughout the observation period, though one fox had only a minimal titer by 21 weeks post-vaccination.

In groups D and E, no foxes converted to seropositive throughout the observation period.

Following the IM challenge with "street" virus one vaccinated fox (number 17, group B) and one control fox died of rabies, 94 and 105 days, respectively, post-challenge. Virus was isolated from the brain of both animals and from salivary glands of the vaccinated fox. No virus was isolated from foxes which survived and were sacrificed 150 days post-challenge.

Safety Testing

None of the foxes inoculated IM with 400 million $MICLD_{50}$ of the ERA/BHK-21 vaccine virus became ill during the observation period. Antibody appeared rapidly and reached high levels in all animals (Table 2). None of the 10 chickens, hamsters, or opossums fed liquid vaccine developed illness throughout the observation period. One of the 10 cotton rats orally vaccinated with 0.3 ml of liquid vaccine died on day 12 after a 2 day illness. Brain tissue from this rat was positive for rabies by FA and mouse inoculation tests; lung, salivary gland, and brown fat tissues were negative by mouse inoculation tests. None of the cotton rats fed the vaccine-impregnated baits became ill during the observation

TABLE 1. Rabies Serum Neutralizing Antibody Titers in Foxes After Oral Vaccination.

Group	Fox	Weeks Post-Vaccination							
		1	3	6	9	12	15	18	21
		▼		▼					
A	1	0	625*	3125	1400	280	800	280	350
	2	0	18	11	3	D+	—	—	—
	3	0	0	9	33	11	3	0	0
	4	0	625	480	800	D	—	—	—
	5	60	6230	56	15	18	4	11	7
	6-12	NO CONVERSIONS							
		▼				▼			
B	13	0	3	3	0	3	45	70	160
	14	0	480	625	480	1400	1150	480	D
	15	0	480	280	350	2400	350	350	280
	16	0	3	0	0	0	0	0	0
	17-18	NO CONVERSIONS							
		▼							
C	19	0	230	625	750	625	480	350	280
	20	0	70	1150	625	1400	1150	350	230
	21	0	3	280	160	160	NT‡	230	45
	22	0	3	33	280	350	230	230	45
	23	0	3	480	NT	230	160	160	56
	24	0	3	70	33	9	11	11	3
		▼							
D	25-30	NO CONVERSIONS							
		▼							
E	31-36	NO CONVERSIONS							

* reciprocal of end point dilutions.
▼ indicates when vaccine baits were fed.
\+ Died of causes other than rabies.
‡ NT — Not Tested.

TABLE 2. Antibody Titers in Foxes Inoculated IM With ERA/BHK-21.

Fox No.	Weeks Post-Vaccination					
	1	2	3	6	9	12
83	0	>625*	5,900	5,900	5,900	5,900
85	0	>625	7,000	2,400	1,400	1,150
86	0	>625	800	1,750	1,400	800
87	0	>625	15,625	4,200	4,200	1,400
88	0	>625	16,500	12,000	15,625	7,000

* reciprocal of end point dilutions.

period. All of the animals which survived were negative for rabies when examined 120 days post-challenge.

Field Bait Acceptance Evaluation

Of the 273 bait nights, 72.5% (198 of 273) were visited by one or more animals (Table 3). Foxes visited 65.7% (130 of 198) of those stations visited, other animals, 34.3% (68 of 198). Foxes ate, or otherwise removed, the baits from 46.5% (127 of 273) of all bait stations, from 80.3% (120 of 158) of stations where bait was taken, and from 97.3% (127 of 130) of stations visited by foxes.

Other animals, especially raccoons, were more inclined to visit stations and leave bait uneaten. Raccoons, which visited only 14.1% (28 of 198) of the stations visited, left bait uneaten at 71.4% (20 of of 28) of these stations. Only 4.8% (13 of 273) of all bait stations were visited by unidentified animals, and only 5.1% (14 of 273) of the stations were visited by more than one species of animals within a 24-hour observation period. No significant difference was found in the visitation rates between bait stations with fox urine or those without.

TABLE 3. Results of Field Bait Acceptance Evaluation.

Species	Baits Taken	Baits Disturbed	Stations Visited Only	Total
Gray fox (*Urocyon cineroargenteus*)	104	0	0	104
Red fox (*Vulpes fulva*)	23	2	1	26
Bobcat (*Lynx rufus*)	4	1	1	6
Raccoon (*Procyon lotor*)	8	18	2	28
Striped skunk (*Mephitis mephitis*)	4	3	0	7
Unidentified	3	8	2	13
Multiple species	12	2	0	14
TOTAL VISITED	158	34	6	198
STATIONS NOT VISITED				75

DISCUSSION AND CONCLUSIONS

Earlier reports have described the immunization of foxes against rabies by oral instillation of liquid vaccine. The study described in this report was an attempt to adapt a successful laboratory procedure to field conditions.

In an earlier laboratory study, the oral administration of liquid ERA/BHK-21 vaccine had produced sero conversions in 100% of foxes given a dose containing only 10,000 $MICLD_{50}$ of virus.[1] The relatively low conversion rate seen in foxes in this study given larger doses of the same type of vaccine show that considerable loss of efficacy results when the liquid vaccine is incorporated into the biscuit bait. The complete failure to produce seroconversions in fox groups D and

E when the bait was held for 96 h at 4 C and 25 C indicates that the vaccine in the baits is relatively unstable. This bait-vaccine form would be unacceptable under most field conditions without the addition of a stabilizer.

The benefit to be derived from a second vaccination remains unclear. In fox groups A and B, which were each fed second doses of vaccine, no significant booster effect was produced by the second vaccination. In group A, one of eight previously negative foxes converted after the second dose, but the four foxes that had converted after the first vaccination showed no significant response to the second dose. In group B, no additional foxes converted after the second vaccination, but an elevation in titer was apparent in the antibody profiles of the three foxes that had converted after the initial vaccination.

A comparison of groups B and C, both receiving the same amount of vaccine, suggests that when the total dose is given at one time it is more effective than when it is divided between two doses. Age may also be an important factor since groups A and B were sub-adults (4-5 months old) and groups C, D, and E adults at time of vaccination. It is obvious that with this technique a relatively large amount of vaccine must be administered at one time to produce satisfactory conversion rates and antibody profiles. This is perhaps analogous to the "minimal effective dose" found necessary to produce satisfactory results with conventional parenteral vaccination of live virus vaccines.

The failure of the challenge with street virus to produce significant mortality in control foxes was surprising. In previous studies, the fox LD_{50} has been calculated at ranges from <5 to 100 $MICLD_{50}$[8,10,11] but the challenge method there was by multiple *masseter* muscle inoculation to increase virus-nerve contact. The single puncture technique used in this study and the *quadriceps* muscle site both probably contributed to elevating the fox LD_{50} above the 250 $MICLD_{50}$ used in this challenge. While evaluation of protection was thus not accomplished, extrapolation from other studies would suggest that most if not all of those foxes that developed demonstrable antibody were probably protected against any challenge that might be incurred under field conditions.

The field bait acceptance evaluation was conducted in an area known to contain relatively dense populations of foxes and other carnivores. The high degree of acceptance of the bait by foxes and its rejection by non-target species suggests that this type of bait is highly satisfactory when only foxes are the intended targets. It should be noted, however, that the removal of baits from sites by foxes does not necessarily mean that the baits were eaten immediately if at all. Foxes are known to "cache" food for later consumption, and it is possible that some baits were "cached" rather than eaten (Sargent, A. L., 1974, personal communication). We need to learn more about the "caching" behavior of foxes if baits of limited stability will be used in future studies.

Results of the safety testing indicate that the ERA/BHK-21 virus would probably be non-pathogenic for the target species. Massive doses of virus administered IM produced very high antibody levels and no disease. None of the 36 foxes fed oral vaccine developed rabies from the vaccine. The rabies death of one cotton rat fed liquid vaccine raises the question of safety in this and perhaps other species. Although none of the cotton rats that ate the vaccine-impregnated bait developed rabies, the numbers were small, and more extensive safety testing seems indicated.

In summary, the technique employed in this study for vaccinating foxes in the field shows more promise, but additional research is needed to produce a more stable and more efficacious bait vaccine. Additional safety testing will also be required to prove conclusively whether this or other live vaccines can safely be distributed in the field.

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