Safety and Immunogenicity of a Vaccine Bait Containing ERA® Strain of Attenuated Rabies Virus

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ABSTRACT

Ninety percent of foxes fed commercial ERA® vaccine in a specially designed bait developed rabies serum neutralizing antibodies. The vaccine bait did not cause clinical signs of rabies when consumed by foxes, raccoons, skunks, dogs, cats, cattle and monkeys. When presented, in the laboratory, to wild rodents of the species *Microtus*, *Mus musculus* and *Peromyscus*, the vaccine baits caused vaccine-induced rabies only in *Mus musculus*. Laboratory mice of the CD-1 and CLL strain were susceptible to vaccine-induced rabies; however, studies showed that transmission of virus to other animals did not occur. These studies suggest that the vaccine bait described could be useful in a rabies control program in areas where foxes and wild dogs are the principal vectors.

Key Words: Rabies, safety, immunogenicity, ERA® vaccine, bait, foxes.

RÉSUMÉ

Cette expérience a démontré que 90% des renards qui avaient ingéré le vaccin antirabique ERA®, dissimulé dans un appât approprié, développèrent des anticorps sériques neutralisants contre le virus de la rage. L'ingestion de l'appât précité par des renards, des ratons laveurs, des mouffettes, des chiens, des chats, des bovins et des singes n'entraîna pas le développement de signes de rage. L'ingestion de cet appât, au laboratoire, par des rongeurs sauvages des espèces *Microtus*, *Mus musculus* et *Peromyscus* causa la rage seulement chez *Mus musculus*. Les souris des souches CD-1 et CLL s'avérèrent susceptibles à la rage de laboratoire, contrairement aux autres rongeurs expérimentaux. Cette expérience laisse par conséquent entrevoir l'utilité de l'appât précité dans un programme d'éradication de la rage, dans les régions où les renards et les chiens errants en sont les principaux vecteurs.

Mots clés: rage, innocuité, immunogénicité, vaccin ERA®, appât, renards.

INTRODUCTION

Rabies remains a significant public health problem in many countries, including Canada. In Ontario alone, 1500-2000 rabies positive animals are diagnosed each year according to data released by Agriculture Canada. In many countries, wildlife are the main vectors of rabies, exposing pets, other domestic animals and humans to the disease.

Control measures such as reduction of the wildlife population have not been particularly effective, and vaccination of wildlife against rabies has received more attention in recent years (1,2). Studies performed in Europe, the United States and Canada have shown that it is possible to vaccinate foxes successfully by the oral route using modified live virus vaccines (3-10). Debbie *et al* (7) showed the ERA® strain (SAD) of rabies virus to be particularly effective for this purpose. Steck *et al* (11) and Schneider *et al* (12) have conducted controlled field trials in Switzerland and West Germany, respectively, using the SAD strain of rabies virus grown to a high titer in a BHK-21 cell line.

In any field program requiring the distribution of modified live virus, the safety of that virus becomes very important. The ERA® strain of rabies virus, which was licensed in 1964 for vaccination of domestic animals by injection, has enjoyed a worldwide reputation for safety and efficacy, although vaccine-induced rabies in cats has been reported (13). In laboratory studies, Lawson *et al* (14) reported on the stability and lack of reversion to virulence of the strain by 20 serial intracerebral back passages of the virus in dogs.

Black *et al* (6) extended these observations by serially passaging the virus intracerebrally in foxes and reported on the safety of the vaccine in a number of species fed vaccine bait.

The purpose of this paper is to report the safety and efficacy of the ERA® strain as a vaccine against sylvatic rabies, with special reference to the use of a novel sponge bait.

MATERIALS AND METHODS

ANIMALS

Foxes *(Vulpes vulpes)*, red or silver forms, were ranch bred and supplied as required. Foxes in the trial varied from four months to five years of age. Skunks (adult) were obtained from a commercial supplier (Ruby's Fur Farm, New Sharon, Iowa). Raccoons (adult), which had been captured in the wild, were supplied by the Ontario Ministry of Natural Resources. Monkeys *(Macaca mulatta)* were obtained from a commercial supplier (Charles River Research Primates, Port Washington, New York). Laboratoy mice, White Swiss strain, were obtained from Connaught Laboratories Limited and the CD-1 strain from a

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Submitted November 3, 1986.

commercial supplier (Charles River Canada Inc., St. Constant, Quebec). The mice in the various trials were four to eight weeks old and weighed 10-16 g. A laboratory-reared strain of *Microtus* was supplied by the Ontario Veterinary College, Guelph, Ontario. *Mus musculus* and *Peromyscus leucopus* were field trapped and supplied by the Ontario Ministry of Natural Resources. Dogs and cats were litter mates obtained from the rural farming community. The dogs were approximately 12 weeks and the cats 12-16 weeks of age. Cattle (Holstein steers) were obtained from the rural farming community and weighed approximately 200 kg. The animals were usually kept singly in individual cages. Dogs and cats were held in individual cages during bait consumption, after which they were held as groups in isolated rooms. The cattle were placed in individual rooms during bait consumption, otherwise as a group in an isolated room.

VIRUSES

The vaccine used in the trials was derived from commercially-prepared SAD strain of rabies virus that has been designated as ERA® (15) and was propagated in primary pig kidney cells in Hanks' solution with 0.5% lactalbumin hydrolysate, supplemented with 5-7% bovine serum, pH 7.0-7.3. A commercial stabilizer was added before storage at -30°C. Potency, as determined by intracerebral inoculation of mice and recorded as median mouse intracerebral lethal dose ($MICLD_{50}$), varied from $10^{5.5}$ to $10^{6.5}$ per mL.

DILUENTS

The diluent used to make tissue suspensions and viral titrations for potency tests was sterile saline containing 2.5% normal horse serum, 500 units of penicillin and 1 mg streptomycin/mL. Vaccine dilutions for dose response and animal tests were made in tissue culture media (described above) with 10% egg yolk added as a stabilizing agent.

TEST SAMPLES

Blood samples were collected from the jugular vein of foxes after intramuscular administration of 0.5 to 1 mL Ketaset (Rogar/STB, London, Ontario) containing 100 mg of Ketamine HCl/mL. Samples were collected before and at various time periods after vaccination. Sera were removed and stored at -20°C until tested for rabies neutralizing antibodies by the modified rapid fluorescent focus forming inhibition test (RFFIT) (16).

BAIT

The bait used in these studies was composed of a 31 × 31 × 38 mm cube-shaped polyurethane sponge (Engineered Foam Products Canada Ltd., Weston, Ontario) which was coated three to four times with a beef fat (Minor Meats Ltd., Lowbanks, Ontario) and wax (Fisher Scientific, Don Mills, Ontario) mixture. The coated sponges were sterilized by irradiation (Steri-Rad, Markham, Ontario). The vaccine was injected in 14 mL volumes into the sponge, the hole sealed with the fat-wax mixture and the baits were stored at -20°C.

PROCEDURES

The vaccine virus was used in liquid form or after lyophilization. The lyophilized vaccine was used as a powder or after reconstitution with an appropriate volume of distilled water to give the required amount of virus. The vaccine was administered by intramuscular inoculation of the pelvic limbs, stomach tube, dose syringe into the oral cavity, orally by drinking and eating in liquid or lyophilized form, as a lyophilized bait in a plastic pouch covered with a sardine oil attractant (Table I) and as a liquid in a sponge bait (Tables II, III and VI). In some trials dimethyl sulfoxide (DMSO), diethylaminoethyl dextran (DEAE-dextran) and hyaluronidase were added to enhance absorption of the vaccine virus (Table I). The virus was also presented to foxes and cats in the form of vaccine-induced rabid mice (Table V).

Brains and salivary glands were removed aseptically and frozen at -20°C until tested. Brain tissue was checked for rabies antigen by either the fluorescent antibody test (FA) or by intracerebral mouse inoculation of a 10% suspension of brain. Salivary glands were prepared as 10% suspensions and checked for rabies antigen by mouse inoculation. The inoculum was 0.03 mL for intracerebral and 0.05 mL for intramuscular administration.

Mouse potency tests to determine live virus content of samples were performed by the method of Koprowski (17). A rapid fluorescent antigen test (RFAT) for potency was performed according to the method described by Abreo (18), the results of which closely approximate those of the mouse potency test.

TABLE I. Safety Trials of ERA® VAccine in Various Species

Species	Number	Route of Administration	Vaccine Virus	Dose[a] Log $MICLD_{50}$	Observation	
					Days	Clinical Signs
Fox	18	intramuscular	liquid	6.3-7.5	180	none
	12	stomach tube	liquid	6.6-7.7	28	none
	31	dose syringe	liquid	6.0-7.6	28	none
	33	oral	lyophilized	6.2-8.6	28	none
	21	oral	liquid	6.0-8.5	28	none
	105	plastic bags	lyophilized	5.9-8.7	28	none
Skunks	3	dose syringe	liquid	7.3	27	none
	3	dose syringe	liquid + 10% DMSO[b]	6.7	27	none
	3	dose syringe	liquid + 0.5% hyaluronidase	7.1	28	none
Dogs	7	dose syringe	liquid	6.2-7.2	28	none
Cattle	3	dose syringe	liquid	6.5	28	none
	3	dose syringe	liquid + 5% DMSO[b]	6.5	28	none
	3	dose syringe	liquid +DEAE dextran[c] 50 mg/mL	6.5	28	none
Monkeys	21	dose syringe	liquid	7.4-82.	180	none

[a]Mouse intracerebral $dose_{50}$ per animal
[b]DMSO = Dimethyl sulfoxide
[c]Diethylaminoethyl-dextran

TABLE II. Results Obtained When Sponge Baits Containing ERA® Vaccine Were Fed to Various Species of Animals other than Rodents

Species	No. on Test	Baits[a] Fed	Number of Animals Consuming This Number of Baits 0 1 2 3 4 5	Clinical Signs	Rabies Antigen in Brain at Day 90	Number With Antibody[b]/ Number Tested
Fox	10	5	- - - - 2 8	none	negative	8/10
Skunks	10	5	- - - 1 4 5	none	negative	0/10
Raccoons	10	5	- - - 1 3 6	none	negative	0/10
Dogs	9	5	- - - - - 9	none	negative	2/9
Cats	12	5	2 2 4 2 - 2	none	negative	1/12
Cattle	5	5	4 - - - - 1	none	negative	0/5
Monkey	10	2	5 2 3 - - -	none	negative	1/10

[a]Titer of baits $10^{5.6}$ tissue culture infective $dose_{50}$/mL — bait contained 14 mL
[b]Antibody level, at 0.5 International Units per mL as determined by RFFIT

TABLE III. Summary of Results Obtained When Sponge Baits Containing ERA® Vaccine Were Fed to Rodent Species

Strain/Species	No. on Test	Observation Period Day	Dead/Fed	Rabies Pos[a]	Other[b]	Antigen[c] Brain Survivors
CD 1 (3-4 weeks)[d]	50	30	11/50	10/50 (2 0)[e]	1/50	0/39
CD 1 (6-8 weeks)	50	30	4/50	4/50 (8)	0/50	0/46
Microtus[d]	50	30	23/50	0/27 (0)	23/50	0/27
Microtus	33	30	1/33	0/32 (0)	1/33	0/32
Peromyscus	82	49	1/82	0/81 (0)	1/82	0/81
Mus musculus	34	42	2/34	2/34 (5.9)	0/34	0/32
CD 1 (3-4 weeks)	50	30	22/50	13/43 (3 0)	9/50	0/28
CLL (3-4 weeks)	50	30	16/50	8/44 (1 8)	8/50	0/34

[a]Rabies antigen demonstrated in brain by fluorescent antibody test or by virus isolation in mice
[b]Deaths due to stress or other causes
[c]Fluorescent antibody test
[d]Virus isolation from brains of mice sacrificed at day 7, 15 and 30 were negative by intracerebral inoculation of 15-16 gram mice
[e]% rabies positive shown in parenthesis

TABLE IV. Virus Isolation From Brain and Salivary Glands of Vaccine-induced Rabid Mice

Strain/Species	Number Rabies Positive/Number Tested		
		Salivary Glands	
	Brain[a]	Intracerebral[b]	Intramuscular[c]
CD 1 (3-4 weeks)	10/11	0/11	N.D.
CD 1 (6-8 weeks)	4/4	1/4	0/4
Mus musculus	2/2	0/2	0/2
CLL (3-4 weeks)	8/10	0/10	0/10
CD 1 (3-4 weeks)	13/14	0/14	0/14

[a]Tested by intracerebral inoculation of 15-16 gram CLL mice or fluorescent antibody
[b]Intracerebral inoculation of 15-16 gram CLL mice with 0.03 mL
[c]Intramuscular inoculation of 15-16 gram CLL mice with 0.05 mL

TABLE V. Results of Feeding Vaccine-induced Rabid Mice to Foxes and Cats

Species	Number on Test	Treatment	Observation Period	Results
Fox *(Vulpes vulpes)*	6	6 mice/day for 6 days[a]	240 days	No rabies symptoms seen
Cat *(Felis catus)*	10	3 mice/day for 7 days[a]	97 days	No rabies symptoms seen

[a]Mice in advanced stages of vaccine-induced rabies sacrificed by cervical dislocation. Viral titers of the brains approximately $10^{5.7}$ $MICLD_{50}$/mL (10% suspension)

ANALYSES

Fifty percent end-points for virus titrations were calculated according to the method of Reed and Muench (19).

RESULTS

From 1968 to 1973 infectivity of the ERA® strain of rabies virus was extensively investigated in a variety of species, the results of which are shown in Table I. Foxes receiving $10^{6.3}$ to $10^{7.5}$ $MICLD_{50}$ of vaccine virus by intramuscular inoculation of the pelvic limb did not show symptoms of rabies over a six month observation period. A total of 202 foxes received vaccine virus orally; 64 received liquid vaccine, 12 by stomach tube, 31 by dose syringe and 21 by oral consumption while 138 received lyophilized vaccine. None of the animals developed clinical signs over a 28 day observation period. Similarly, nine skunks, seven dogs, nine cattle and 21 monkeys did not show clinical symptoms when given the rabies vaccine virus orally by dose syringe.

In trials to test the safety of vaccine in sponge baits, foxes, skunks, raccoons, dogs and, to a lesser degree, cats, ate the baits quite readily, whereas, one of five cattle ate five baits and five of ten monkeys ate one to two baits (Table II). None of the animals developed clinical signs of vaccine-induced rabies during the 90 day observation period. Examination of the brain of surviving foxes, skunks, raccoons, dogs, cats and monkeys did not show rabies antigen when tested by FA. Rabies virus was not isolated when suspensions of brain and salivary glands taken at day 7, 15, 30, 60 and 90 from foxes, skunks and raccoons were inoculated into mice.

Vaccine baits with titers of $10^{5.2}$ to $10^{6.5}$ $MICLD_{50}$/mL were presented to the three wild rodent species tested: only *Mus musculus* showed vaccine-induced rabies (2/34) after consuming vaccine baits (Table III). Vaccine-induced rabies occurred in the more highly susceptible laboratory CD-1 mice in which the incidence varied from 8 to 30%. None of the survivors showed rabies antigen in the brain by the FA test. Table IV shows the results obtained when a suspension of the salivary glands from vaccine-induced

rabid mice was inoculated intracerebrally or intramuscularly into mice; after intracerebral inoculation, rabies occurred in only one of 41 samples. In this instance, 2/10 mice receiving the 10% suspension showed rabies clinical signs and the brains of these mice contained rabies antigen. In another sample, in the same trial, 1/10 mice showed rabies-like clinical signs, however, rabies could not be confirmed by FA examination of the brain nor by intracerebral mouse passage. Virus could not be demonstrated when these salivary gland suspensions were inoculated intramuscularly into mice.

In transmission studies, 12 normal mice, placed in contact with an equal number of mice inoculated intracerebrally with ERA® vaccine, did not develop rabies and rabies antigen was not found on FA examination of the brains at day 60. All 12 of the inoculated mice died of rabies and rabies antigen was found on FA examination of the brain.

When vaccine-induced rabid mice were sacrificed by cervical dislocation and fed to rabies seronegative foxes and cats, none of those animals developed clinical signs of rabies during the observation period (Table V). Three of the foxes developed antibody levels as a result of consuming the mice and were subsequently shown to be protected against challenge. None of the cats showed antibody and the brains were negative by fluorescent antibody when examined at the end of the observation period.

A dose response study in foxes in which groups of eight to ten foxes received vaccine baits containing 14 mL volumes of vaccine with titers of $10^{6.1}$, $10^{5.3}$, $10^{4.4}$ or $10^{4.2}$ $MICLD_{50}$/mL, showed an antibody response in 90, 77, 40 and 0 percent of foxes, respectively (Table VI). The protective dose $_{50}$ was $10^{4.7}$ $MICLD_{50}$/mL.

TABLE VI. Results of Dose Response Curve in Foxes Fed Sponge Baits Containing ERA® Vaccine

Group	Number of Foxes	Vaccine Titer[a] FAT[b]	Vaccine Titer[a] Mouse[c]	Number Seroconverting[d]/Number on Test 28 Days Postvaccination
1	10	5.8	6.1	9/10 (3.65-40.4)[e]
2	10	5.4	5.3	7/9[f] (0.83-49.9)
3	10	4.7	4.4	4/10 (0.35-6.72)
4	8	4.4	4.2	0/10

[a]Recorded as Log per mL
[b]Median tissue culture infectious $dose_{50}$
[c]Median mouse intracerebral lethal dose
[d]Antibody level at least 0.3 International Units per mL as determined by RFFIT
[e]Range of antibody titers in the RFFIT
[f]One animal died of gastric ulceration prior to 28 day bleeding

DISCUSSION

Commercial ERA® rabies vaccine, because of its worldwide acceptance for efficacy and safety in pets and domestic animals, would seem to be an appropriate vaccine for use in the immunization of wildlife. The vaccine has been extensively tested for safety in the highly susceptible fox as well as in a number of other wildlife species under laboratory conditions. None of these animals developed clinical signs of rabies, nor was rabies antigen detected in either brain or salivary glands of the species tested.

Vaccine-induced rabies can, however, occur in some rodent species, especially the highly susceptible laboratory strains of mice, after consumption of vaccine baits. This occurrence is not considered a serious deterrent to the use of the vaccine baits, since there is ample evidence that the virus is not transmitted within the species or to other animals, a finding supported by the work of Winkler *et al* (20). Virus isolations from salivary glands of vaccine-induced rabid mice occurred only occasionally at a very low titer as shown in Table IV. Inoculation of these suspensions intramuscularly into mice failed to transmit the virus in this highly-susceptible species. Similarly, the virus was not transmitted when vaccine-induced rabid mice were fed to cats and foxes (Table V).

Previous work by Black *et al* (4-6) has shown that commercial ERA® vaccine given orally or in bait form produced antibodies in foxes which were subsequently protected against challenge. The current work describes a vaccine bait which was acceptable to a variety of species and capable of stimulating the development of rabies serum neutralizing antibodies in a high percentage of foxes fed a single bait.

In another study (results not shown) the ERA® vaccine virus was propagated in the BHK-21 cell line to a high titer, incorporated in the bait and fed to dogs. Four of six dogs fed a single bait developed serum rabies virus neutralizing antibodies ranging from 0.48 to 9.61 international units per mL.

The results reported confirm the safety and immunogenicity of the ERA® vaccine. A program in the field, consisting of the delivery of bait for the immunization of wildlife, could play a role in rabies control in locations in which foxes or wild dogs are the main vectors of the disease, for example, southern Ontario and India.

ACKNOWLEDGMENTS

The authors would like to acknowledge the assistance of Mr. A. Casey of the Animal Diseases Research Institute, Agriculture Canada, Nepean, Ontario, for carrying out the rabies fluorescent antibody test on brain specimens from the various species. The assistance of Dr. J.B. Campbell of the Department of Microbiology, University of Toronto, in providing antibody results is also gratefully acknowledged. The technical assistance of H. Chiu, N. Rumine, H. Pedel, G. Wratten and R. Hertler in the preparation, testing and providing of baits was much appreciated, as was the assistance of D. Flemming, S. Kolenko and M. Matson in the handling of animals on the various experimental trials. The technical advice and assistance of Mr. T. Reynolds of Novopharm Ltd. was also very much appreciated and is acknowledged.

The supply of wild rodent species by Mr. R. Bramwell of the Ontario Ministry of Natural Resources and the laboratory reared *Microtus* by Dr. I. Barker and Mr. G. Jones of the Ontario Veterinary college was very much appreciated.

The authors acknowledge the support and advice of Dr. C.D. MacInnes, Supervisor, Wildlife Research Section, Ontario Ministry of Natural Resources,

and of the Rabies Advisory Committee of the Ministry (Dr. A.J. Rhodes, Chairman; Dr. K.M. Charlton, Dr. K. Nielsen, Vice-Principal D.G. Sinclair, Dr. S.M. Smith, Dr. L.P. Spence and Dr. D.S. Willoughby, Members; Dr. C.D. MacInnes, Secretary).

The support of Drs. K.J. Dorrington and M.J. Walcroft on the project was much appreciated and is acknowledged.

This work was financed by grants from the Ontario Ministry of Health (1968-1973) and contracts from the Ontario Ministry of Natural Resources (1979-1986); some of the latter from funds provided by the Provincial Lottery (1979-1983).

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