# Immunization of Foxes *Vulpes vulpes* by the Oral and Intramuscular Routes with Inactivated Rabies Vaccines

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## ABSTRACT

**Inactivated rabies vaccines prepared from common vaccine strains of virus were inoculated into foxes by the intramuscular and intestinal route. There were differences among the vaccines in the duration of antibody produced after intramuscular administration.**

**Inactivated vaccines deposited directly into the lumen of the duodenum by means of a fiberscope caused seroconversion in some foxes, especially following a booster dose, but the antibodies produced were for the most part of short duration.**

**The ERA® modified live virus vaccine, in contrast, produced a satisfactory and long lasting antibody after intestinal instillation.**

## RÉSUMÉ

**Cette expérience consistait à administrer à des renards, par les voies intramusculaire et intestinale, des vaccins antirabiques inactivés, préparés à partir de souches vaccinales usuelles. Ces divers vaccins stimulèrent la production d'anticorps d'une durée variable, à la suite de leur administration intramusculaire.**

**Le fait de déposer ces vaccins directement dans la lumière du duodénum, à l'aide d'un fibroscope, entraîna la production d'anticorps transitoires, seulement chez certains renards et surtout après une dose de rappel, tandis que l'utilisation du vaccin antirabique atténué ERA® se solda par l'élaboration d'une concentration satisfaisante d'anticorps durables.**

## INTRODUCTION

The current rabies problem in Ontario is a result of an epizootic that was first diagnosed in the Northwest Territories in 1947 and finally spread throughout Canada (11). The number of rabies positive cases in animals diagnosed each year in Ontario has remained relatively constant over the last 19 years. Rabies Surveillance Reports issued by Agriculture Canada record more than 1 000 cases annually. These numbers are probably conservative, as in the main, they represent specimens in which a known human or domestic animal exposure has taken place. The red fox *Vulpes vulpes* remains the most important wildlife vector, accounting for some 40-50% of the cases diagnosed each year.

Because of the importance of the red fox in disseminating rabies in Ontario and in other parts of the world, there has been an interest in immunizing this species against rabies. A number of workers in Canada, the United States and Europe ( 1,2,3,4,6,13) have investigated this possibility using attenuated live virus vaccines administered by the oral route. Attenuated live viruses may cause rabies in certains species when administered orally (4,7,12,14). This complication has resulted in the realization that the use of a live virus in a bait, that may be distributed on a large scale in the wild, would require extensive safety testing in all species found in the baiting area.

Recently, Nicholson and Bauer (7) have reported on the administration of inactivated rabies vaccine to rats by the enteric route. Their results showed that the inactivated vaccine was not particularly effective by this route.

This paper reports on results obtained when foxes were vaccinated by the oral and intramuscular routes with inactivated rabies vaccines.

## MATERIALS AND METHODS

### ANIMALS

The foxes used were raised in captivity and were of the wild red genotypes commonly found in southern Ontario. The foxes ranged in age from one to five years and were randomly stratified into the various experimental

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groups so that each sex age class (male, female, juvenile, yearling and adult) was represented.

The groups were divided into the following categories: a) Fiberscope instillations of vaccines into the intestine (six groups), b) fiberscope instillation of tissue culture fluids, to simulate vaccine, i.e. placebo control (one group), c) intramuscular injection of vaccines (four groups) and d) untreated environmental control held under same conditions as above groups (one group). Mice for virus titration, inactivation and National Institutes of Health (NIH) tests were White Swiss (Connaught Strain) and were approximately five weeks of age and weighed 12-16 g when used. Guinea pigs for vaccination and challenge studies were Connaught Strain and weighed approximately 350 g.

VIRUSES

Vaccines used in the trials were prepared from the Street Alabama Dufferin (SAD), high egg passage (HEP), challenge virus standard (CVS), and Pitman Moore (PM) strains of vaccine virus (9). The vaccines were obtained commercially, by donation, or prepared at Connaught Laboratories Limited. The SAD strain was grown both in MRC-5 cell line and in $BHK_{21}$ cell line. The vaccines were inactivated using betapropiolactone or other suitable methods used by the manufacturer or donor.

Challenge viruses used in the tests were either street virus obtained from rabies positive fox salivary glands supplied by Dr. K. Charlton, Animal Diseases Research Institute of Agriculture Canada, or challenge virus standard (CVS) originally obtained from the National Institutes of Health, United States Public Health Service. The fox salivary glands were ground and suspended in sterile distilled water supplemented with 10% inactivated horse serum containing 1 000 units of penicillin and 2 mg streptomycin/mL. The suspension was stored in 1 mL amounts in sealed vials in the vapour phase of liquid nitrogen. The CVS virus was propagated in mouse brain, ground, suspended in 20% suspension in phosphate buffered saline (PBS) containing 10% egg yolk and 1 000 units of penicillin and 2 mg of streptomycin/mL. The virus was dispensed in 1 mL amounts and frozen in vapour phase of liquid nitrogen.

DILUENTS

Diluent for making virus dilutions was sterile saline containing 2.5% normal horse serum, 500 units of penicillin and 1 mg of streptomycin/mL. Phosphate buffered saline was used for making dilutions for the NIH test (5). Reconstitution of lyophilized vaccine was made with sterile distilled water.

TEST SAMPLES

Blood samples were collected from the jugular vein of foxes after administration of 0.5 to 1 mL Ketaset[1] containing 100 mg of ketamine HCl/mL. Samples were collected prior to and after vaccination. Sera were removed and stored at -20°C until tested. The samples were tested for rabies neutralizing antibodies by the modified rapid fluorescent focus inhibition test (RFFIT) (15).

Potency tests to determine antigenic value of the vaccines were carried out using the NIH test.

ANALYSIS

Fifty percent end-points for virus titrations were calculated according to the method of Reed and Muench (8).

Statistical analysis of the experimental results was carried out according to the Kruskal-Wallis one-way analysis of variance (10).

FIBERSCOPE ADMINISTRATION

The introduction of vaccine into the intestine of foxes was carried out using a veterinary fiberscope[2] with a diameter of 12.5 mm. Foxes were starved for 24 h and water was removed for 16 h prior to anesthesia. The anesthetic used was a mixture of Atravet[3] and Ketaset (1 mL Atravet to 10 mL of Ketaset) administered intramuscularly in a 1.5 to 2 mL dose, depending on size of fox. At the same time, Atropine Sulphate[4] 6 mg/mL and Tigan[5] were injected intramuscularly in 0.5 and 1.1 mL amounts respectively. After a suitable state of anesthesia was obtained the mouth was held open with a speculum, and the fiberscope (lubricated with a sterile lubricant)[6] was introduced into the esophagus and gently manipulated into the stomach (a distance of 68-72 cm). The end of the fiberscope was positioned at the pyloric sphincter.

A catheter[7] was introduced into the fiberscope and threaded into the duodenum, a distance of 15 cm. Flushing of the optics throughout the procedure with 2 to 5 mL of distilled water was necessary on some occasions to effect clearance. A 10 mL plastic syringe containing vaccine was then attached to the catheter and the vaccine introduced slowly into the duodenum. The syringe was removed and a second syringe containing 2 mL of saline was attached and used to flush the catheter. The catheter was removed followed by the fiberscope.

The animals were placed in a recovery room before being removed to the holding area. The fiberscope was rinsed in tap water after each operation while a new catheter was used for each vaccine. The catheters were finally steril-

[1]Rogar/STB, London, Ontario.
[2]Model VFF-80, American Optical, Southbridge, Maryland.
[3]Acepromazine maleate, Ayerst Laboratories, Montreal, Québec.
[4]Atropine sulphate, Glaxo Laboratories, Montreal, Québec and Toronto, Ontario.
[5]Trimetho benzamide hydrochloride, Hoffman-LaRoche Ltd., Vaudreuil, Québec.
[6]KY sterile lubricant, Johnson and Johnson, Montreal, Québec and Toronto, Ontario.
[7]American Cystoscope Makers Inc., Stamford, Connecticut.

**TABLE I. Results of Intramuscular Vaccination of Foxes with Inactivated Rabies Vaccines**

| Vaccine | Number with Seroconversion/Number Tested (GMT I.U./mL)[a] | | |
|---|---|---|---|
| | Days Postvaccination | | |
| | 0 | 28 | 90-104 |
| 1 | 0/8 (0) | 7/8 (0.17) | 5/8 (0.13) |
| 2 | 0/8 (0) | 8/8 (0.35) | 7/8 (0.41) |
| 3 | 0/8 (0) | 6/8 (0.41) | 1/8 (0.04) |
| 4 | 0/8 (0) | 8/8 (1.77) | 8/8 (0.56) |
| 5 | 0/9 (0) | 8/9 (0.35) | 1/8 (0.02) |
| Control | 0/8 (0) | 0/8 (0) | 0/8 (0) |

[a]Geometric Mean Titre in International Units/mL

ized using Benzalide[8] 1:20 overnight. The fiberscope was disinfected using a solution of Surgical Bridine[9] 500 mL, isopropyl alcohol 250 mL, and water 250 mL.

INTRAMUSCULAR ADMINISTRATION

Those foxes receiving the vaccine by intramuscular inoculation were injected in the left hind leg in the region of the biceps femoris. The vaccine was deposited deeply into the musculature in a 1 mL amount.

## RESULTS

TRIAL 1

The vaccines were inoculated intramuscularly into groups of eight rabies seronegative foxes. The animals were bled prior to and at 28 and 90-104 days after vaccination. Statistical analysis of the results (Table I) of the 90-104 day bleeding showed that vaccine 4 produced a significantly (at 5% level) higher antibody level than vaccines 3 and 5. However, no significant difference was observed between other vaccines.

TRIAL 2

The vaccines used in trial 1 were instilled in 10 mL amounts into the duodenum of eight to ten foxes per group using the fiberscope as described. The animals were bled prior to vaccination and at various periods postvaccination and postbooster. A booster dose of vaccine was given at day 49 or 103. A commercial serial of ERA® live virus and a placebo consisting of tissue culture fluids were also inoculated by this route to act as positive and negative controls on the technique. Very few animals responded after the initial administration of inactivated vaccine (Table II). However, after a second dose, 50% of the animals inoculated with vaccine 4 had rabies antibodies as determined by the RFFIT test. It should be noted that by day 55-90 postbooster, antibodies could not be detected in most of the animals.

**TABLE III. National Institutes of Health Potency Results of Inactivated Rabies Vaccine**

| Vaccine | Potency (I.U./mL)[a] |
|---|---|
| 1 | 2.14 |
| 2 | 1.95 |
| 3 | 2.91 |
| 4 | 2.14 |
| 5 | 7.97 |

[a]International Units/mL

TRIAL 3

Table III shows the NIH potency values expressed as international units per 1 mL of the various vaccines used in the trials. Although there appears to be a wide range in results, there is no significant difference in potency among the vaccines. These results would indicate that all the vaccines had an adequate potency for intramuscular use.

TRIAL 4

Each of the vaccines used in the trials was inoculated intramuscularly into a group of five guinea pigs using a 0.25 mL dose. These animals were challenged three weeks later with a 0.2 mL intramuscular inoculation of street rabies virus. There was no significant difference in protective value among the vaccines on test, all showing a protection against the street virus (Table IV).

**TABLE II. Results of Intestinal Instillation of Inactivated Rabies Vaccines and Controls in Foxes**

| Vaccine | Number with Seroconversion/Number Tested (Titre Range: I.U./mL)[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Days Postprimary | | | Days Postbooster[b] | | |
| | 0 | 28 | 90 | 379 | 7 | 28 | 55-90 |
| 1 | 0/9(0) | 0/9(0) | N.A. | N.A. | 1/9(0.24) | 1/9(0.22) | 0/9(0) |
| 2 | 0/8(0) | 0/8(0) | N.A. | N.A. | 1/8(3.65) | 1/8(0.46) | 1/8(0.2) |
| 3 | 0/8(0) | 1/8(0.26) | N.A. | N.A. | 2/8(1.3-2.2) | 2/8(0.66-0.77) | 1/8(0.26) |
| 4 | 0/10(0) | 1/10(0.26) | N.A. | N.A. | 5/10(0.18-5.5) | 4/10(0.09-2.68) | 1/10(0.36) |
| 5 | 1/8(0.02) | 2/8(0.15-1.41) | N.A. | N.A. | 2/8(0.66-3.4) | 2/8(0.39-0.75) | 0/8(0) |
| ERA®[c] | 0/8(0) | 7/8(0.15-15.7) | 6/8(0.55-16.5) | 5/7(0.22-2.41) | N.A. | N.A. | N.A. |
| Placebo | 0/8(0) | 0/8(0) | 0/8(0) | 0/8(0) | N.A. | N.A. | N.A. |

[a]Range I.U./mL — International units per 1 mL
[b]Booster: Vaccine 1 and 2 — Day 103
Vaccine 3, 4 and 5 — Day 49
[c]Live virus vaccine titre $10^{5.4}$ mouse $LD_{50}$mL
N.A. Not applicable

[8]Benzalide, Burns Veterinary Supply Ltd., Downsview, Ontario.
[9]Povidone-iodine, Allen and Hanburys, Montreal, Québec and Toronto, Ontario.

**TABLE IV. Challenge Results of Guinea Pigs Inoculated with Inactivated Rabies Vaccines by the Intramuscular Route**

| Vaccine | Results ($PD_{50}$)[a] |
|---|---|
| 1 | 1:200 |
| 2 | 1:631 |
| 3 | 1:316 |
| 4 | 1:200 |
| 5 | 1:200 |
| Reference 183[b] | 1:32 |

[a]Dilution of vaccine which protected 50% of animals against virulent challenge
Challenge — rabies positive fox salivary glands > 19 guinea pig $LD_{50}$
[b]Standard NIH reference vaccine

## DISCUSSION

Five inactivated rabies vaccines were potency tested in mice and guinea pigs and administered to foxes by the intramuscular and intestinal routes. There were no significant differences in the potency of the vaccines, as measured by the NIH test. All strains of inactivated rabies virus protected guinea pigs against the endemic strain of street virus. In spite of this, vaccine 4 gave significantly better results, as determined by duration of antibodies, than vaccines 3 and 5 when foxes were vaccinated by the intramuscular route. Duration of antibody is more important than initial antibody response in those animals which may only receive one dose. Vaccine 4 also caused seroconversion by more foxes than did the other inactivated vaccines when they were administered directly into the duodenum. The reason for the variable and unpredictable rate of seroconversions in the enteric vaccinated animals along with the relatively short duration of antibody is not known at this time, but is the subject of continuing studies. The ERA® live virus vaccine produced a satisfactory and long lasting antibody response after only one administration.

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