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## STUDIES ON THE ORAL INFECTIVITY OF RABIES VIRUS IN CARNIVORA<sup>[1]</sup>

R. O. RAMSDEN<sup>[2]</sup> and D. H. JOHNSTON<sup>[2]</sup>

**Abstract:** Mature and immature red foxes (*Vulpes vulpes*) and striped skunks (*Mephitis mephitis*) were fed varying numbers of white mice infected with street isolates and a fixed strain of rabies virus. Rabies deaths and the development of serum neutralizing antibody to rabies virus occurred in both species. The epizootiological implications of these findings are discussed.

### INTRODUCTION

Rabies virus infection through aerosol, nasal and oral exposure of laboratory rodents has been confirmed by a number of investigators.<sup>7,8,12,21,22</sup> Evidence of oral infection of wild carnivores with rabies virus is available;<sup>2,5,6,10,11,16,19</sup> however, the potential of this route of infection in the epizootiology of the disease is relatively unexplored.<sup>4,13</sup>

Baer et al.<sup>3</sup> were among the earliest workers to consider the possibility of oral infection in foxes. One of five red foxes administered a fixed rabies virus by stomach tube developed serum neutralizing antibody (SN) but none died of rabies. Kantorovich et al.<sup>16</sup> fed 9 g of rabies infected mouse brain to each of 19 arctic foxes (*Alopex lagopus*). None of the foxes developed SN antibody nor clinical signs of rabies. Black and Lawson<sup>5</sup> immunized red foxes by stomach tube administration of an attenuated rabies virus (ERA strain) and demonstrated rabies deaths in the same species when fed street rabies virus.<sup>6</sup> Baer et al.,<sup>2</sup> using the ERA attenuated rabies virus, successfully immunized both red and grey foxes (*Urocyon cinereoargenteus*) by dropping liquid vaccine directly on the oral mucosa.

Debbie et al.<sup>10</sup> found that 40 of 53 red foxes were successfully vaccinated when 1 ml of commercial ERA was placed directly in the mouth and Mayr et al.<sup>19</sup> immunized six red foxes with the WIRAB attenuated strain of rabies virus through drinking water or application to the mouth by pipette. There is, therefore, sufficient indication that animals can be vaccinated by ingestion of a suitable high titered vaccine and efforts are now underway to find a suitable vehicle for the presentation of the vaccine<sup>11</sup> to wildlife as a means of rabies control.

Studies involving the transmission of street rabies virus by ingestion have been more limited. Kovalev et al.<sup>18</sup> recorded rabies deaths in two of three arctic foxes fed rabies infected mice. Another fox died of rabies after being fed the carcass of a rabies infected domestic rabbit. Bell and Moore<sup>4</sup> fatally infected 6 of 18 mature striped skunks by feeding each a mouse infected with a bat isolate of rabies virus. Five ferrets (*Mustela putorius*) also fed one rabies infected mouse each and 48 domestic cats fed up to 25 infected mice each did not develop SN antibody nor clinical rabies.

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Differences in host susceptibility and oral infectivity of various strains of rabies virus have been noted and probably account for the unpredictability of the oral route.<sup>8,10,21</sup> This paper describes the results of feeding mice infected with a street isolate of rabies virus to immature and mature red foxes and striped skunks; also the feeding of mice infected with a fixed strain CVS<sub>50</sub><sup>3</sup> rabies virus to mature red foxes and striped skunks.

#### MATERIALS AND METHODS

Red foxes were obtained from trappers in rabies-enzootic southern Ontario. Foxes used in trials I and II were estimated by comparison with standard weight and body measurements to be approximately 16 weeks old and had been captive for 6 weeks when they were fed rabies-infected whole mouse carcasses. Trial III foxes were approximately 48 weeks of age at the time of oral exposure and had been captive for approximately 36 weeks before oral exposure. Striped skunks used in the study were obtained from a local laboratory colony. All of the skunks in trial IV had been captive for at least 7 months and were considered mature, though of unknown ages. Immature skunks used in trials IV and V were bred and raised within the colony. Throughout the study animals of a single trial were maintained in the same room but in individual wire mesh cages, each fitted with a solid metal partition between adjacent cages which prevented direct physical contact.

The street isolates of rabies virus were obtained from field cases of rabies which occurred in southern Ontario. Isolates W742-71 and W 498-72 were from skunks and W 372-71 from a red fox. The fixed strain used was the challenge virus standard (CVS<sub>50</sub>) maintained by

intracranial (IC) passage in 3 week old Swiss white mice. The single fixed strain and field isolates were prepared as a 10% brain tissue suspension in buffered saline, pH 7.4, with 0.75% bovine serum albumin fraction V. This suspension was inoculated IC in 0.03 ml volumes into 3 week old Swiss white mice.<sup>4</sup> Infected mouse carcasses from mice freshly dead or killed when moribund were stored at -20 C until used in oral infectivity trials. For each of the trials the number of mouse intracerebral 50% lethal doses (MICLD<sub>50</sub>) fed was determined by assaying rabies virus titers in the pooled brain tissues from six infected mice. Titers were calculated by the method of Reed and Muench.<sup>20</sup>

Oral exposure of foxes and skunks was accomplished by withholding food for 24 hours prior to the feeding of frozen rabies virus-infected mouse carcasses. Test animals were given up to five mouse carcasses each per feeding, with 30 min between each feeding. Animals which consumed mice readily were given up to 25 infected mice in a single day.

Serum was collected from foxes and skunks before and after oral exposure and was examined for rabies SN antibody<sup>1</sup> at a single serum dilution of 1:5 throughout the study. Fresh serum was inactivated at 56 C for 20 min and a 2.5 fold dilution of serum was mixed with an equal volume of CVS virus suspension calculated to contain approximately 200 MICLD<sub>50</sub> per 0.03 ml. The mixture and appropriate controls were incubated 90 min at 37C before inoculation IC into 3 week old Swiss white mice, 0.03 ml/mouse.

Foxes and skunks which survived oral exposure to rabies virus were challenged 16 weeks after oral exposure by intramuscular (IM) inoculation with a fox street isolate.<sup>5</sup> The calculated dose of challenge was 15.8 Fox IMLD<sub>50</sub>.

<sup>3</sup> Obtained from the Canada Dept. of Agriculture, Health of Animals Branch, Hull, Quebec, Canada.

<sup>4</sup> (Connaught strain) Connaught Medical Research Laboratories, Toronto, Ontario.

<sup>5</sup> This fox isolate had been previously titrated in foxes and was provided by Connaught Medical Research Laboratories, Toronto, Ontario, Canada.

<sup>6</sup> Obtained from the New York State Dept. of Health, Albany, New York, U.S.A.

All test animals were examined for rabies using the direct fluorescent antibody (FA) technique described by Goldwasser et al.<sup>18</sup> and by Dean.<sup>9</sup> Fluorescein conjugated antibody produced in hamsters was used in the examinations.<sup>[6]</sup> Results of FA examinations were periodically confirmed by the mouse inoculation test.<sup>17</sup>

### RESULTS

None of the four immature foxes in trial I, fed from  $10^{7.7}$  to  $10^{8.8}$  MICLD<sub>50</sub> of CVS rabies virus, developed clinical rabies. However, two of these foxes developed rabies SN antibody and resisted later IM challenge with a street rabies virus. The three remaining foxes of this group died of rabies following IM challenge.

Two immature foxes in trial II, fed the greatest number of mouse carcasses infected with a street isolate of rabies virus containing  $10^{5.1}$  and  $10^{5.4}$  MICLD<sub>50</sub>, died of rabies. Rabies SN antibody was not detectable in the surviving foxes all of which died following IM challenge with a street isolate fox rabies virus.<sup>[5]</sup>

Foxes of trial III were 48 weeks old when they were fed approximately the same amount of street rabies virus as caused deaths of the two 16 week old foxes in trial II. Clinical rabies was not observed in trial III but rabies SN antibody developed in one fox which ate 35 rabies virus infected mice.

Skunks of trial V were fed from 5 to 65 CVS rabies virus infected mice and 10 of 13 exposed died of rabies. Two of the three survivors developed rabies SN antibody. One of the control animals which was not fed rabies infected mice also developed rabies SN antibody. Since all orally exposed animals were in the same room and the 10 skunks died within a short period of time, it is speculated that aerosol transmission might have occurred. Intramuscular challenge of the survivors of this trial was not informative because only one of the three inoculated controls died of rabies.

The seven immature skunks of trial IV were fed from  $10^{6.8}$  to  $10^{5.9}$  MICLD<sub>50</sub> of a street isolate rabies virus. The four mature skunks of trial VI were fed from  $10^{4.6}$  to  $10^{6.2}$  MICLD<sub>50</sub> of a street isolate rabies virus. No rabies deaths occurred in either group and only one, a mature skunk, developed SN antibody. Survivors were not challenged.

### DISCUSSION AND CONCLUSION

Rabies deaths occurred only among immature foxes and skunks after oral exposure. Development of rabies SN antibody after oral exposure was found in both immature and mature foxes and skunks. These results suggest that mature animals may be more resistant to oral infection and confirms similar observations made by Debbie et al.<sup>10</sup> Other workers have demonstrated that neonatal mice were more susceptible than adult mice to both IC and IM inoculation of the rabies virus.<sup>7,12</sup> Differences in oral susceptibility of laboratory rodents<sup>8,21</sup> and foxes<sup>10</sup> to different rabies virus strains have also been observed.

In our studies it is impossible to separate the influences of virus strain and virus titer as they may have affected oral infectivity. The CVS<sub>56</sub> strain was most successful in causing rabies deaths of skunks when fed at dosages of from 100 to 1000 times the dosages obtainable with the street virus. In the fox feeding trials, CVS<sub>56</sub> was fed at 100 to 1000 times the dosage of street virus, yet only a street virus at its highest levels resulted in rabies deaths.

Several authors have demonstrated street rabies virus transmission by the oral route in the red fox, arctic fox and striped skunk.<sup>4,6,18</sup> Our research confirms their observations. Previous workers have not, however, reported the presence of SN antibody in carnivores consuming street isolates of rabies virus, only in those exposed to attenuated strains.<sup>2,8,9,16,18</sup> Occurrence of SN antibody following oral exposure may account in part for presence of SN antibody at low levels in carnivores in rabies enzootic areas.<sup>23,24</sup>

TABLE 1. Red Foxes fed Rabies Virus-Infected White Mice.

Trial No.	Fox Age	Strain of Rabies Virus Used	Total Mice Fed	Total Calculated MICLD* <sub>50</sub>	Antibody After Oral Exposure***	Rabies Deaths Following:	
						Oral Exposure	IM Challenge
I	16 wks	Fixed strain (CVS <sub>60</sub> )	control	0	—	—	+
			5	7.7	—	—	+
			10	8.0	—	—	+
			15	8.2	+	—	—
			20	8.3	+	—	—
II	16 wks	Street Isolate (W 372-71)	control	0	—	—	+
			control	0	—	—	+
			control	0	—	—	+
			5	4.5	—	—	+
			10	4.8	—	—	+
			15	5.0	—	—	+
			20	5.1	—	+	+
			35	5.4	—	+	+
III	48 wks	Street Isolate (W 372-71)	control	0	—	—	ND**
			control	0	—	—	ND
			30	5.3	—	—	ND
			35	5.4	+	—	ND

— did not occur

+ occurred

\* Virus dose presented as log<sub>10</sub> x 50% mouse intracerebral lethal doses

\*\* ND Not Done

\*\*\* SN antibody titers to rabies at a serum dilution of 1:5 were considered positive.

TABLE 2. Striped Skunks fed Rabies Virus-Infected White Mice.

Trial No.	Skunk Age	Strain of Rabies Virus Used	Total Mice Fed	Total Calculated MICLD* <sub>50</sub> 's	Rabies Deaths Following:	
					Oral Exposure	IM Challenge
IV	16 wks	Street Isolate (W 498-72)	control	0	—	ND**
			control	0	—	ND
			14	5.8	—	ND
			14	5.8	—	ND
			14	5.8	—	ND
			15	5.8	—	ND
			16	5.8	—	ND
			17	5.9	—	ND
V	28 wks	Fixed Strain (CVS <sub>50</sub> )	control	0	—	+
			control	0	—	—
			control	0	+	—
			5	7.1	—	—
			5	7.1	—	—
			10	7.4	—	—
			15	7.6	—	—
			15	7.6	—	—
			20	7.7	—	—
			20	7.7	—	—
			25	7.8	+	—
			25	7.8	+	—
VI	Mature	Street Isolate (W 742-71)	control	0	—	ND
			control	0	—	ND
			1	4.6	—	ND
			5	5.3	—	ND
			5	5.3	—	ND
			15	5.8	+	ND
			15	5.8	—	ND
			40	6.2	—	ND
			40	6.2	—	ND
			40	6.2	—	ND

For footnotes, see Table 1.

The pathogenesis of transmission by ingestion of rabies virus was not studied and the possibility of viral inoculation resulting from trauma to the oral mucosa exists. However in at least two previous studies, trauma to oral mucosa was not required for infection.<sup>2,22</sup> Other studies have indicated that nasal or nasopharyngeal infection of rodents occurs more readily than oral infection.<sup>14,21</sup> Immunization of the red fox against rabies has been accomplished by dropping liquid ERA vaccine virus on the oral and lingual mucosa, but approximately 200 doses of the same vaccine were needed

to immunize when given by stomach tube.<sup>2,5</sup> These results may be attributable to the superficial nature of olfactory nerve fibers in oral and nasal mucosa described by Johnson and Mims.<sup>15</sup>

Ingestion of rabies infected carcasses by carnivores has been proven, in this study and others, to be a possible means of rabies transmission. In cold climates frozen carcasses of rabid animals may provide a source of infection to scavengers for long periods.<sup>28</sup> These reservoirs of virus may be in part responsible for new foci of wildlife rabies and the perpetuation of the disease in the north.

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#### LITERATURE CITED

1. ATANASIU, P. 1966. Quantitative assay and potency test of antirabies serum. In: *Laboratory Techniques in Rabies*. World Health Organization, Geneva, 2nd ed., pp. 167-172.
2. BAER, G. M., M. K. ABELSETH and J. G. DEBBIE. 1971. Oral vaccination of foxes against rabies. *Am. J. Epidemiol.* 93: 487-490.
3. BAER, G. M., S. B. LINHART and D. J. DEAN. 1963. Rabies vaccination of foxes. In *Annual Report of the Division of Laboratories and Research*, New York State Department of Health, Albany.
4. BELL, J. F. and G. J. MOORE. 1971. Susceptibility of carnivora to rabies virus administered orally. *Am. J. Epidemiol.* 93: 176-182.
5. BLACK, J. G. and K. F. LAWSON. 1970. Sylvatic rabies studies in the silver fox (*Vulpes vulpes*). Susceptibility and immune response. *Can. J. Comp. Med.* 34: 309-311.
6. BLACK, J. G. and K. F. LAWSON. 1973. Further studies of sylvatic rabies in the fox (*Vulpes vulpes*). Vaccination by the oral route. *Can. vet. Jour.* 14: 206-211.
7. CASALS, J. 1940. Influence of age factors on susceptibility of mice to rabies virus. *J. exp. Med.* 72: 445-451.
8. CORREA-GIRON, E. P., R. ALLEN and E. S. SULKIN. 1970. The infectivity and pathogenesis of rabies virus administered orally. *Am. J. Epidemiol.* 91: 203-215.
9. DEAN, D. J. 1966. The fluorescent antibody test. In: *Laboratory Techniques in Rabies*. World Health Organization, Geneva, 2nd ed. pp. 59-68.

10. DEBBIE, J. G., M. K. ABELSETH and G. M. BAER. 1972. The use of commercially available vaccines for the oral vaccination of foxes against rabies. *Am. J. Epidem.* 96: 231-235.
11. DEBBIE, J. G. 1974. The use of inoculated eggs as a vehicle for the oral rabies vaccination of red foxes (*Vulpes fulva*). *Infection and Immunity* 9: 681-683.
12. FISCHMAN, H. R. and F. E. WARD, III. 1968. Oral transmission of rabies virus in experimental animals. *Am. J. Epidem.* 88: 132-138.
13. GOLDWASSER, R. A. and R. E. KISSLING. 1958. Fluorescent antibody staining of street and fixed rabies virus antigens. *Proc. Soc. exptl. Biol. Med.* 98: 219-223.
14. HRONOVSKY, V. and R. BENDA. 1969. 1. Experimental inhalation infection of laboratory rodents with rabies virus. 2. Development of inhalation rabies infection in suckling guinea pigs. *Acta Virol.* 13: 193-197, 197-202.
15. JOHNSON, R. T. and C. A. MIMS. 1968. Pathogenesis of viral infections of the nervous system. *New Eng. J. Med.* 278: 23-30, 84-92.
16. KANTOROVICH, R. A., G. V. KONOVALOV, I. A. BUZINOV and V. P. RIUTOVA. 1963. Experimental investigations into rage and rabies in polar foxes, natural hosts of the infection. I. An experimental morphological study of rage in polar foxes. *Acta Virol.* 7: 554-560.
17. KOPROWSKI, H. 1966. Mouse inoculation test. In: *Laboratory Techniques in Rabies*. World Health Organization, Geneva, 2nd ed. pp. 69-80.
18. KOVALEV, H. A., V. A. SEDOV and A. S. SHASHEN'KO. 1971. Experimental study of certain ways in which rabies is transferred. *Proceedings 19th World Veterinary Congress, Mexico City* 2: 711-712.
19. MAYR, A., H. JAEGER and H. HAACKE. 1972. Orale Immunisierung von Fuschsen gegen Tollwut. *Zentbl. Vet. Med. B.* 19: 615-625.
20. REED, L. F. and H. MUENCH. 1938. A simple method of estimating fifty per cent end points. *Am. J. Hyg.* 27: 493-497.
21. SEROKOWA, D. 1969. Food-borne infection with rabies virus under experimental conditions. *Epidem. Rev.* 23: 122-134.
22. SOAVE, O. A. 1966. Transmission of rabies to mice by ingestion of infected tissue. *Am. J. vet. Res.* 27: 44-46.
23. SIKES, R. K. 1962. Pathogenesis of rabies in wildlife. I. Comparative effect of varying doses of rabies virus inoculated into foxes and skunks. *Am. J. vet. Res.* 23: 1041-1047.
24. TIERKEL, E. S. 1969. Rabies. *Adv. Vet. Sci.* 5: 183-226.
25. VALADAO, F. G. 1961. Ensaio sobre a conservacao do virus rabico as temperaturas ambiente e da geleira. *Anais do servicos de veterinaria de Mocambique.* 7: 77-82.

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