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Oral Vaccination of Captive Arctic Foxes with Lyophilized SAG2 Rabies Vaccine

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ABSTRACT: Arctic foxes (*Alopex lagopus*) were immunized with lyophilized SAG2 oral rabies vaccine. The effectiveness of this vaccine was determined by serologic response and survival to challenge by rabies virus isolated from a red fox from Alaska (USA). No vaccine virus was found in saliva 1–72 hr after ingestion. At 2 wk after vaccination, all foxes had seroconverted, with rabies virus neutralizing antibody levels of 0.2–3.1 IU ml⁻¹. All vaccinated foxes survived to week 17 after challenge, and hippocampus, pons, and cerebellum were free of rabies virus as determined by direct immunofluorescence testing after death. One of four nonvaccinated foxes survived challenge and was free of rabies virus in neural tissue, and no rabies virus neutralizing antibody was detected in blood. Our results suggest that the lyophilized SAG2 oral rabies vaccine could be effective in arctic and subarctic regions, where freezing air and ground temperatures probably would not reduce its immunogenicity.

Key words: *Alopex lagopus*, arctic fox, oral vaccination, rabies, SAG2.

The concept of using an oral rabies vaccine to vaccinate wild populations of vertebrates was first proposed >30 yr ago (Baer et al., 1971). Many laboratory and field experiments have been conducted to evaluate the effectiveness of oral vaccines in mammals. These include the red fox (*Vulpes vulpes*), gray fox (*Urocyon cinereoargenteus*), coyote (*Canis latrans*), raccoon (*Procyon lotor*), skunk (*Mephitis mephitis*), and dog, the latter in tropical and subtropical regions (Schumacher, 1995), where rabies is endemic. Programs to control rabies in wild red foxes have been effective in several European countries and in southern Ontario, Canada (Wandeler, 1991; Bogel et al., 1992; MacInnes et al., 2001).

The arctic fox (*Alopex lagopus*) is the primary rabies reservoir in most circumpolar regions (Rausch, 1958; Syuzumova,

1968; Secord et al., 1980; Ritter, 1981; Crandall, 1991). Most research had been done on oral vaccination of captive foxes in Alaska (USA) (Follmann et al., 1988). Subsequently, a field trial was conducted in Newfoundland (Canada) (Johnston and Fong, 1992), although the program and method to establish success were not described. Another field trial in northern Greenland was conducted, but results were inconclusive (Hansen, 1996). Further laboratory investigations evaluating oral rabies vaccines in arctic foxes and their safety in nontarget vertebrate species continued in Alaska (Follmann et al., 1992, 1996, 2002). Our laboratory investigations have provided evidence that arctic foxes can be vaccinated by the oral route using both SAD-BHK₂₁ and SAG1 vaccines and that the vaccines available are safe for at least six vertebrate species that are sympatric with arctic fox (Follmann et al., 1996, 2002).

Oral vaccination programs that have proved effective in the wild to control rabies in red foxes used liquid vaccines enclosed in a bait, with bait distribution programs recommended twice per year (World Health Organization, 1992). A spring program is designed to vaccinate adult foxes that have survived the winter and an autumn program to vaccinate young from the previous summer. A vaccination rate of 50–70% has been proposed as the coverage required to prevent an epizootic from spreading through an animal population (Wandeler, 1991). This type of program could fail in arctic regions, where freezing temperatures can occur throughout the year, especially during spring and autumn. A liquid vaccine

could freeze and thereby be ineffective, should it pass directly into the stomach without vaccine virus being absorbed through the buccal and pharyngeal mucosa. It is not unusual for arctic foxes to scavenge frozen carcasses. Therefore, eating frozen vaccine-bait would be expected. Solutions to avoid this problem are a coated vaccine that passes through the stomach and is enterically absorbed (Wandeler, 1991) or a lyophilized vaccine that would not be negatively affected by freezing temperatures and require chewing to expose vaccine virus to buccal and pharyngeal mucosa. The present article evaluates the latter approach in captive foxes. Although the ultimate success of a vaccine and distribution program is to evaluate it in the field, it is necessary to first determine the effectiveness of a new version of a vaccine under controlled conditions.

Fourteen foxes were captured on the North Slope of Alaska in box traps constructed of wire mesh. The University of Alaska Fairbanks (UAF, Fairbanks, Alaska) Institutional Animal Care and Use Committee approved the trapping protocol and subsequent experiment. Foxes were transported to the Experimental Animal Facility of the UAF by truck and housed in a Biosafety Level 3 Facility. Foxes were housed individually in cages and fed commercial dog food daily (IAMS Mini-Chunks, Lewisburg, Ohio, USA) and water ad libitum. After several weeks of acclimation, foxes were anesthetized with a combination of 0.5 ml of xylazine hydrochloride (TranquiVed, VEDCO, Inc., St. Joseph, Missouri, USA) and 0.2 ml of ketamine hydrochloride (Ketaset, Aveco Co. Inc., Fort Dodge, Iowa, USA), and blood samples were obtained from the jugular vein to evaluate for rabies virus neutralizing antibody. This was necessary to ensure that foxes had not acquired natural immunity to rabies, a condition that has been found in several foxes by other researchers (Ballard et al., 2001). Foxes were dosed intramuscularly with Droncit (Bayer Corp., Shawnee Mission, Kansas, USA) for

potential infestations with *Echinococcus* sp. Serum samples, including all those subsequently obtained, were analyzed for rabies virus neutralizing antibody using the rapid fluorescent focus inhibition test (Smith et al., 1973) at the Kansas State University Department of Veterinary Diagnosis (KSUDVD, Manhattan, Kansas, USA).

The SAG2 virus (Lafay et al., 1994) was lyophilized without the bait attractant and imported directly to Fairbanks from Virbac Laboratories (13-emu Rue-L.I.D., 06516 Carros-Cedex, France). The vaccine titer was $10^{-5.5}$ 50% mouse lethal doses (MLD_{50})/0.01 ml, as determined in suckling CD-Ha/ICR mice by the Alaska State Virology Laboratory (ASVL, Fairbanks, Alaska). The lyophilized vaccine was in the form of a wafer $\sim 2.5 \times 2.5 \times 1$ cm in size. The vaccine was coated with a thin layer of ground beef, to insure ingestion by foxes. The vaccine was not enclosed in a sachet or other covering to separate it from the beef, because immediate ingestion was expected. A field application would require a coating around the vaccine to prevent hydration, which could affect its immunogenicity prior to ingestion.

Ten foxes (five males and five females) were anesthetized as described above, and blood samples were obtained for rabies virus antibody determination. After recovery, each fox was fed the lyophilized SAG2 vaccine. All foxes consumed the vaccine within 10 min of delivery. To determine vaccine-virus shedding, oral swabs were obtained from each fox 1, 24, 48, and 72 hr after vaccine ingestion. Harvested swabs were stored in viral transport medium at -70 C.

A dual approach was exercised to determine presence of virus in saliva. Samples were analyzed at KSUDVD for viral RNA (reverse-transcriptase polymerase chain reaction using the 10 g and 304 primers) according to methods described by Trimarchi and Smith (2002). Isolation attempts were conducted in murine neuro-

blastoma cell (NA-C1300) suspensions, as outlined in Webster and Casey (1996).

Consistent with previous challenge experiments (Follmann et al., 1988, 1992), a challenge virus was prepared from a rabid red fox harvested in western Alaska; only the arctic strain of the rabies virus is known to occur in Alaska. Approximately 50 g of fox brain tissue was ground in 0.75% bovine albumin fraction V using Hanks's basic salt solution as the grinding diluent. The suspension was mixed and aliquoted into 50-ml tubes and centrifuged at $330 \times G$ for 15 min at 4 C. Thereafter 3-ml aliquots of the supernatant were dispensed, with mixing, into snap-capped ampoules (Weaton 223684) and immediately frozen at -70 C. One vial was rapidly thawed (37 C) and placed into an ice bath, and a viral titration was initiated in 19–21-day-old Ha/ICR-CDR mice. Three mice were inoculated intracerebrally with 0.01 ml of each dilution (10^0 – 10^{-4}) and observed daily. Moribund animals were harvested (10^0 at day 11, 10^{-1} at day 16, 10^{-2} and 10^{-3} at day 17, and 10^{-4} at day 18). Touch impressions of the brain were prepared for all harvested animals, and all were rabies positive as determined by direct fluorescent antibody (DFA) analysis. The titer was calculated as $MLD_{50} \text{ Ha/ICR-CDR mice} 10^{-3.9/ic/0.01ml}$ (Reed and Muench, 1938).

At 1, 2, 4, and 7 wk after vaccination, foxes were anesthetized and blood samples obtained for rabies virus antibody analysis. After venipuncture at week 7 postvaccination, the 10 vaccinates and four controls (two males and two females) were challenged with 1 ml of 50,000 MLD_{50} rabies virus. The challenge virus was split and injected bilaterally into the masseter muscles. Two weeks later, blood samples were obtained from vaccinated foxes for antibody determination and again at 10 wk after virus challenge. Logistic constraints required the challenge to occur 7 wk after vaccination, although this closely followed protocols from trials with liquid SAD-BHK₂₁ and SAG1 oral rabies vaccines in

which foxes were challenged 9 wk after vaccination (Follmann et al., 1988, 1992). Epizootics in Alaska typically occur during November–March (Ritter, 1981). Challenging foxes 7 wk after vaccination, therefore, closely simulates what could occur in a field application with bait distribution in late August–September.

Foxes were observed daily for signs of rabies. All foxes, except for two controls that died of rabies, were killed with a barbiturate overdose during week 11 after challenge (18 wk after vaccination). Touch impressions of hippocampus, pons, and cerebellum were analyzed for rabies virus by DFA (Goldwasser and Kissling, 1958) at the ASVL.

Before vaccination, all 14 foxes tested negative (<0.05 IU ml^{-1}) for rabies virus neutralizing antibody. No evidence of rabies virus nucleic acid was found in saliva samples collected from foxes 1–72 hr after the ingestion of vaccine. One week after vaccination, seven of 10 foxes had a positive antibody titer, and at 2 wk, all foxes had seroconverted (range, 0.2–3.1 IU ml^{-1} ; Table 1). At 4 wk, titers in some foxes had increased and some had decreased, compared with earlier titers. Fox 343 was exceptional compared with the other foxes, with a titer of 15.7 IU ml^{-1} . The titers of six foxes continued to increase at 7 wk, whereas those of others remained about the same or declined slightly.

Two weeks after challenge (9 wk after vaccination), all foxes except 341 and 343 showed dramatic increases in antibody levels (Table 1). The titer of fox 343 was still high but had declined somewhat from the level on the day of challenge. At week 17, the titers of all foxes except fox 341 (which never achieved a titer >0.5 IU ml^{-1}) decreased dramatically, some to levels approximating or below those recorded at week 7, the day of challenge. All foxes had evidence of antibody at this time, however.

Three of four control foxes (challenged but not previously vaccinated) tested positive for rabies virus in brain tissue. Foxes 349 and 348 died 18 and 37 days, respec-

TABLE 1. Rabies virus neutralizing antibody titers (IU ml⁻¹) in arctic foxes fed lyophilized SAG2 rabies virus vaccine.

Fox number	Week of experiment						
	0	1	2	4	7 ^a	9	17
336	<0.05	2.0	0.4	1.5	2.8	82.4	14.0
337	<0.05	<0.05	2.0	0.6	2.2	69.4	2.8
338	<0.05	0.2	2.4	2.3	2.4	28.2	2.8
339	<0.05	0.1	0.2	2.2	2.8	16.5	2.3
340	<0.05	<0.05	3.1	5.4	4.7	>82.4	5.4
341	<0.05	0.1	0.5	0.2	0.5	0.5	0.5
342	<0.05	<0.05	1.1	1.5	1.1	70.6	2.8
343	<0.05	0.2	0.6	15.7	19.1	16.5	3.4
344	<0.05	0.2	2.3	2.4	2.3	>82.4	3.2
347	<0.05	0.2	0.2	0.5	0.5	80.0	0.7

^a Foxes were challenged with rabies virus after a blood sample was obtained.

tively, after challenge. Fox 346 was moribund on day 26 after challenge and was killed after it was anesthetized and had blood drawn; it had an antibody titer of 1.0 IU ml⁻¹. Fox 345 did not show any signs of rabies after challenge and was killed on day 74; its brain tissue was negative for rabies virus, and no rabies virus neutralizing antibody was present in blood.

The lyophilized SAG2 oral rabies vaccine protected all 10 arctic foxes, as demonstrated by seroconversion and resistance to challenge with a large dose of the arctic strain of rabies virus. These results agree with those of earlier experiments that used liquid SAD-BHK₂₁ and SAG1 oral rabies vaccines (Follmann et al., 1988, 1992). Unlike earlier experiments with liquid SAG1 vaccine (Follmann et al., 1992), saliva samples collected after the ingestion of the lyophilized vaccine contained no rabies virus. Thus, it is unlikely that a fox would expose other foxes or animals to the attenuated rabies virus after ingestion. However, Orciari et al. (2001) reported vaccine virus present in saliva in a dog 1 hr after the ingestion of bait that contained lyophilized SAG2 vaccine.

The 10 vaccinated foxes showed a great deal of variability over time in response to the initial exposure to the vaccine—these results are similar to those obtained in previous experiments with other oral vaccines

(Follmann et al., 1988, 1992). Some foxes responded slowly and never achieved high levels of antibody, whereas others produced a great deal of antibody that declined some weeks after the peak. In all cases, however, the levels of antibody protected foxes from subsequent exposure to rabies virus. The antibody titers recorded for six foxes 2 wk after challenge are the highest ever reported for this species and dramatically illustrate the rapid production of antibody characteristic of the anamnestic response.

The titer of the challenge virus used in our experiment was sufficient to infect three of four control foxes, with virus found in three areas of the brain after death. However, the titer was 1 log lower than the challenge virus used in other experiments with oral vaccines in arctic foxes (Follmann et al., 1988, 1992). It had been determined previously (Follmann et al., 1988) that the titer of the challenge virus needed to achieve 100% mortality in arctic fox controls, 500,000 MLD₅₀, was significantly higher than that needed for other canids. The 50,000 MLD₅₀ dose used in this experiment corroborated that earlier finding, with one fox surviving for 74 days after exposure to the rabies virus and with no virus found in the brain after death. The higher dose was not available for this experiment.

The antibody titer of 1.0 IU ml⁻¹ found in control fox 346 is the first report of rabies-specific antibody present in the blood of a rabid arctic fox. This condition probably resulted from the immune system of the fox responding by the production of antibody after challenge but not quickly enough to overcome viral transport from the masseter muscle to the brain and subsequent viral replication. Alternatively, the fox may have been exposed previously to rabies in the wild and had seroconverted, but the antibody titer was below detection at the time it was first tested. Ballard et al. (2001) reported four of 92 arctic foxes with rabies virus neutralizing antibody in a separate study in the same area where the foxes in our experiment were captured. In a previous experiment, the titer of a fox vaccinated with oral SAD-BHK₂₁ vaccine was not detectable (<1:5) after 55 wk but the fox responded dramatically within 1 wk (1:280) of the administration of a second dose (Follmann et al., 1988). If a similar scenario was the case for fox 346, a control, the massive dose of challenge virus injected into the masseter muscles, which resulted in a short incubation time and accelerated viral replication in the brain, perhaps occurred before the immune system reacted with sufficient antibody to prevent the transport of virus into the brain. This explanation is perhaps feasible under the conditions of our experiment but is unlikely in naturally exposed foxes, because it is doubtful that the amount of virus entering the body would be as massive and direct as that used here.

Fox 345, a control fox that survived challenge, also may have been previously exposed to rabies virus. No rabies virus neutralizing antibody was found at the time of death, 11 wk after challenge, and its brain tissue was negative for rabies virus. This fox may have produced sufficient antibody to withstand infection from the challenge virus, but at the time of death, levels had dropped below the limit of detection. A fox exposed to a similar dose of challenge virus in earlier trials also tested

negative, both for virus in the brain and for antibody in blood, after 17 mo. Had the control foxes been sequentially tested for rabies antibody after challenge, this could have been determined. However, that was not done. The role of cell-mediated immunity in arctic foxes remains unknown but also may be of importance in protecting foxes.

This experiment demonstrated that the lyophilized SAG2 oral rabies vaccine produced by Virbac Laboratories was effective in immunizing captive arctic foxes. This was determined by the presence of rabies virus neutralizing antibodies in the blood of all foxes 2 wk after ingesting the vaccine, the antibodies remaining through 17 wk, and the survival of all vaccinated foxes when challenged by a large dose of rabies virus. This is supported by the results of previous experiments, where all foxes vaccinated with liquid SAD-BHK₂₁ and SAG1 vaccines and challenged with a dose of rabies virus 1 log higher than used in this experiment all survived. On the basis of our results, this vaccine appears to be suited for field application in cold environments where lyophilized vaccine probably would retain its efficacy despite freezing air and ground temperatures. The effect of freeze-thaw cycles on vaccine stability, however, warrants investigation. A field application of this vaccine would require that it be contained in a sachet or other enclosure to protect the vaccine from moisture penetration, which could alter the immunogenicity of the vaccine. Also, it would have to incorporate a suitable bait attractant.

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LITERATURE CITED

- BAER, G. M., M. K. ABELSETH, AND J. G. DEBBIE. 1971. Oral vaccination of foxes against rabies. *American Journal of Epidemiology* 93: 487–490.
- BALLARD, W. B., E. H. FOLLMANN, D. G. RITTER, M. A. CRONIN, AND M. D. ROBARDS. 2001. Rabies and canine distemper in an arctic fox population in Alaska. *Journal of Wildlife Diseases* 37: 133–137.
- BOGEL, K., F.-X. MESLIN, AND M. KAPLAN (EDITORS). 1992. *Wildlife rabies control*. Wells Medical Ltd., Kent, UK, 222 pp.
- CRANDALL, R. A. 1991. Arctic fox rabies. In *The natural history of rabies*, 2nd Edition, G. M. Baer (ed.). CRC Press, Inc., Boca Raton, Florida, pp. 291–320.
- FOLLMANN, E. H., D. G. RITTER, AND G. M. BAER. 1988. Immunization of arctic foxes (*Alopex lagopus*) with oral rabies vaccine. *Journal of Wildlife Diseases* 24: 477–483.
- , ———, AND ———. 1992. Oral rabies vaccination of arctic foxes (*Alopex lagopus*) with an attenuated vaccine. *Vaccine* 10: 305–308.
- , ———, AND ———. 1996. Evaluation of the safety of two attenuated oral rabies vaccines, SAG1 and SAG2, in six arctic mammals. *Vaccine* 14: 270–273.
- , ———, AND D. W. HARTBAUER. 2002. Safety of lyophilized SAG2 rabies vaccine in collared lemmings. *Journal of Wildlife Diseases* 38: 216–218.
- GOLDWASSER, R. A., AND R. E. KISSLING. 1958. Fluorescent antibody staining of street and fixed rabies virus antigens. *Proceedings of the Society for Experimental Biology and Medicine* 98: 219–223.
- HANSEN, E. H. 1996. Oral vaccination of arctic foxes in Greenland: A field trial. VII Annual International Meeting, Advances Towards Rabies Control in the Americas, Atlanta, Georgia. 7 pp.
- JOHNSTON, D. H., AND D. W. FONG. 1992. Epidemiology of arctic fox rabies. In *Wildlife rabies control*, K. Bogel, F.-X. Meslin, and M. Kaplan (eds.). Wells Medical Ltd., Kent, UK, pp. 45–49.
- LAFAY, F., J. BENEJEAN, C. TUFFEREAU, A. FLAMAND, AND P. COULON. 1994. Vaccination against rabies: Construction and characterization of SAG2, a double avirulent derivative of SAD-Bern. *Vaccine* 12: 317–320.
- MACINNES, C. D., S. M. SMITH, R. R. TINLINE, N. R. AYERS, P. BACHMANN, D. G. A. BALL, L. A. CALDER, J. GROSGREY, C. FIELDING, P. HAUSCHILDT, J. M. HONIG, D. H. JOHNSTON, K. F. LAWSON, C. P. NUNAN, M. A. PEDDE, B. POND, R. B. STEWART, AND D. R. VOIGT. 2001. Elimination of rabies from red foxes in eastern Ontario. *Journal of Wildlife Diseases* 37: 119–132.
- ORCIARI, L. A., M. NIEZGODA, C. A. HANLON, J. H. SHADDOCK, D. W. SANDERLIN, P. A. YAGER, AND C. E. RUPPRECHT. 2001. Rapid clearance of SAG-2 rabies virus from dogs after oral vaccination. *Vaccine* 19: 4511–4518.
- RAUSCH, R. 1958. Some observations on rabies in Alaska, with special reference to wild Canidae. *Journal of Wildlife Management* 22: 246–260.
- REED, L. F., AND H. A. MUENCH. 1938. A simple method of estimating fifty percent end points. *American Journal of Hygiene* 27: 493–497.
- RITTER, D. G. 1981. Rabies. In *Alaskan wildlife diseases*, R. A. Dieterich (ed.). University of Alaska Press, Fairbanks, Alaska, pp. 6–12.
- SCHUMACHER, C. L. 1995. The oral delivery of rabies vaccines to dogs. In *Proceedings of the Third International Conference of the Southern and Eastern African Rabies Group*, J. Bingham, G. C. Bishop, and A. A. King (eds.). Harare, Zimbabwe, pp. 150–158.
- SECORD, D. C., J. A. BRADLEY, R. D. EATON, AND D. MITCHELL. 1980. Prevalence of rabies virus in foxes trapped in the Canadian arctic. *Canadian Veterinary Journal* 21: 297–300.
- SMITH, J. S., P. A. YAGER, AND G. M. BAER. 1973. A rapid reproducible test for determining rabies neutralizing antibody. *Bulletin of the World Health Organization* 48: 535–541.
- SYUZUMOVA, L. M. 1968. Epizootiology of rabies among arctic foxes on the Yamal Peninsula. *Problemy Severa* 11: 99–106.
- TRIMARCHI, C. V., AND J. S. SMITH. 2002. Diagnostic evaluation. In *Rabies*, A. C. Jackson and W. H. Wunner (eds.). Academic Press, New York, New York, pp. 307–337.
- WANDELER, A. I. 1991. Oral immunization of wildlife. In *The natural history of rabies*, 2nd Edition, G. M. Baer (ed.). CRC Press Inc., Boca Raton, Florida, pp. 485–503.
- WEBSTER, W. A., AND G. A. CASEY. 1996. Viral isolation in neuroblastoma cell culture. In *Laboratory techniques in rabies*, F.-X. Meslin, M. Kaplan, and H. Koprowski (eds.). World

- Health Organization, Geneva, Switzerland, pp. 96–104.
- WORLD HEALTH ORGANIZATION. 1992. WHO Expert Committee on Rabies. 8th report. WHO technical report series 824. World Health Organization, Geneva, Switzerland, 86 pp.
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