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IMMUNIZATION OF ARCTIC FOXES (*ALOPEX LAGOPUS*) WITH ORAL RABIES VACCINE

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ABSTRACT: Arctic foxes (*Alopex lagopus*) were successfully immunized against rabies using an orally-administered, liquid SAD-BHK_{21} live virus vaccine in a sausage bait. Immunization was determined by serologic response and by resistance to challenge with an arctic rabies virus strain. Virus was not shed in saliva following oral vaccination, indicating that arctic foxes would not infect other foxes after ingesting this vaccine. High antibody levels were present in all experimental foxes 2 wk following initial vaccination. A booster vaccination at 56 wk induced a significant serologic response within 1 wk, suggesting an anamnestic response but titers began to decline within 8 wk in most foxes. Foxes were observed for 16 mo following the challenge and exhibited no symptoms of rabies. The SAD-BHK_{21} rabies vaccine in a sausage bait system has a strong potential for vaccinating wild populations of arctic fox.

Key words: Oral vaccination, rabies, arctic fox, *Alopex lagopus*, field study.

INTRODUCTION

Rabies has been present in the Arctic for over a century (Rausch, 1958; Crandall, 1975; Ritter, 1981), with the arctic fox (*Alopex lagopus*) the main reservoir (Cowan, 1949; Rausch, 1958, 1972; Syuzyumova, 1968; Crandall, 1975; Secord et al., 1980; Odegaard and Krogsrud, 1981). As the number of people in the Arctic and Subarctic increases the probability for increased rabies exposure probably also increases.

Rabies is endemic in arctic foxes but apparently becomes epizootic regionally every 3 to 4 yr at higher latitudes, coincident with the declining phase of the population cycle (Ritter, 1981). However, epizootics can occur annually (Rausch, 1972). Although Freuchen (1935) suggested that rabies regulates arctic fox populations and Cowan (1949) that it regulates populations of arctic canids generally, the conclusions of Elton (1942) and Rausch (1958, 1972) do not support this view. Instead, rabies seems to be one of the mortality factors involved with population declines of arctic fox when prey numbers decline.

The population of the arctic fox in mainland areas is closely tied to the availability of brown lemmings (*Lemmus sibiricus*) and other rodents (Braestrup, 1941; Siivonen, 1948; Rausch, 1958; Macpherson, 1969) which are cyclic in the Arctic (Rausch, 1958; Pitelka, 1967). Population densities of arctic foxes cycle in response to food availability, with declines in numbers often precipitous. During these "crashes" many foxes die of rabies; up to 70% of the foxes sent to the Alaska Northern Regional Laboratory (Division of Public Health, Fairbanks, Alaska 99706, USA) in one such year were diagnosed as rabid (D. Ritter, unpubl. data), although these collections are biased because they represent animals suspected of being diseased when they are killed or found dead. Kantorovich (1964) reported a 75% incidence of rabies in arctic foxes in the Union of Soviet Socialist Republics during an epizootic, with none of the surveyed animals exhibiting symptoms of rabies. The stress on the foxes of reduced food supply during the harsh arctic winter appears also to increase their susceptibility to rabies. Most rabid foxes are reported in November through March (Ritter, 1981), coincident with the lowest rodent availability. Concentration of foxes at large food sources

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during this period would facilitate disease transmission (Rausch, 1958; Crandall, 1975).

Arctic fox rabies control has not been attempted due to the relatively low human population in far northern regions. Extensive control programs have been directed at the red fox (*Vulpes vulpes*) in Canada and Europe. However, these programs, which have included extensive hunting, trapping and poisoning (Lewis, 1975; Bacon and Macdonald, 1980; Bogel et al., 1981), have been quite expensive (Baer, 1975; Lewis, 1975). They also have been ineffective in controlling the spread of rabies, because of the high mobility of fox populations (Lewis, 1975). These types of animal control would certainly be even more expensive and less effective in remote northern regions. The only current rabies program in Alaska is for immunization of domestic animals (Middaugh and Ritter, 1982) to reduce rabies transmission from foxes to domestic dog to persons.

The efficacy of an oral rabies vaccine was first determined in 1971 (Baer et al., 1971) and has been extensively tested on the red fox (Debbie et al., 1972; Black and Lawson, 1973; Winkler et al., 1975; Winkler and Baer, 1976), especially in Europe (Mayr et al., 1972; Blancou et al., 1982; Hafliger et al., 1982; Kiefert et al., 1982; Schneider and Cox, 1983; Wachendorfer et al., 1984; Pepin et al., 1985). Early testing was done on captive animals, but Steck et al. (1981, 1982) and Schneider et al. (1983) conducted successful immunization programs on wild red foxes. Other field tests are under way in Canada and the Soviet Union. An oral immunization program with suitable baits should be quite effective since foxes maintain their immunity for long periods, and at least insular populations of arctic and red foxes, and red foxes in the contiguous United States, southern Canada and Europe, would remain as immune residents thus minimizing immigration of susceptible foxes. In addition, the program probably would be cost effective compared to other programs at high latitudes, where logistic costs and wages are very high. Costs of any rabies control program would depend on various factors including the size of the area to be treated and the means of bait deployment. The costs of a field vaccine-bait program in Switzerland during 1978 to 1980 were about U.S. $1.50/bait and $23.15/km^2 (using 1986 exchange rates) (Steck et al., 1982). These costs were considered low compared to other expenses involving rabies control (Steck et al., 1982).

This paper reports the immunization of captive arctic foxes using oral rabies vaccine in a sausage bait. If this method were effective in wild foxes, it could be applied in restricted areas around remote villages and industrial sites to protect humans during recurring epizootics.

MATERIALS AND METHODS

Fourteen arctic foxes (four males) were live-captured (six in winter, eight in summer) on the central arctic coast of Alaska (70°20'N, 148°20'W) by use of baited wire cage traps. Foxes were held in outdoor cages, fed a combination of dry dog chow (Eukanuba, The IAMS Company, Lewisburg, Ohio 45338, USA) and canned cat food (Blue Mountain, The Hervin Company, Tualatin, Oregon 97062, USA), and provided water ad libitum. About 1 mo after capture, the foxes were anesthetized with 20 mg xylazine hydrochloride (Rompun, Bayvet Division, Miles Laboratories, Inc., Shawnee, Kansas 66201, USA), and blood samples taken from the jugular vein were analyzed for rabies antibodies using the Rapid Fluorescent Focus Inhibition Test (RFFIT) (Smith et al., 1973). This test entailed the addition of equal volumes of a challenge rabies virus (CVS strain) to 1:5 and greater dilutions of arctic fox serum to which were added BHK_{21} cells. The RFFIT measures the capacity of serum antibodies to block infection of the BHK cells by the CVS virus, as determined by the reduction in fluorescence of cells in the microscopic field. A reduction of 50% or more of the fields exhibiting fluorescence is considered an indicator of the presence of neutralizing antibody in the serum of arctic foxes.

Of the 14 foxes six (two males) were selected as the experimental group receiving the SAD-BHK_{21} vaccine, five (one male) were selected as the control group for the challenge experiment, and three (one male) were selected to determine the lethal dose for the challenge experiment.

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Foxes were housed indoors at the Institute of Arctic Biology (University of Alaska, Fairbanks, Alaska 99775, USA) for all experiments in an isolation facility operated according to biosafety III practices. The facility had individual cages for each animal with steel mesh floors over concrete to facilitate urine and feces removal. All sewage drained through kill tanks, the negative air flow system passed cage-room air through absolute filters, and ultraviolet lights protected doorways.

Following a 24 hr fast, six foxes (two males) selected as the experimental group were fed a 10 cm long sausage bait enclosing a sealed plastic straw containing 1.4 ml of liquid SAD-BHK_{21} live rabies virus vaccine, as previously described (Hafliger et al., 1982). Because of its size, foxes had to bite the bait in order to swallow it. The plastic straw was punctured when the sausage was bitten and chewed, and the vaccine then went onto the buccal mucosa. The buccal mucosa was swabbed (in five of the six foxes) at 1, 3, 6 and 24 hr and 1 wk after bait administration. Saliva samples were diluted in viral transport media and injected intracerebrally (Johnson, 1969) in 1- to 3-day-old mice to test for the presence of rabies virus in saliva. Dead mice were tested for rabies using the fluorescent antibody (FA) technique (Goldwasser and Kissling, 1958) conducted at the Alaska Northern Regional Laboratory. In addition, nine serial blood samples were taken from foxes to test for rabies antibodies between 1 and 55 wk after vaccination. These were analyzed at the Centers for Disease Control (Lawrenceville, Georgia 30246, USA). Thirteen mo after the initial ingestion of the baits, the five previously vaccinated foxes (one had died of unrelated cause) were fed a second sausage bait containing the same vaccine dose. Blood samples were obtained 1, 3, 6 and 9 wk following the booster.

The rabies virus used to challenge arctic foxes was isolated from the salivary glands of a red fox from Alaska that was positive for rabies. Salivary gland tissue from this fox was injected intracerebrally into 21-day-old HaICR mice yielding an MLD_{50} titer of $1 \times 10^{5.3}$. To determine the dose necessary to produce 100% mortality in control foxes for the challenge experiment, two arctic foxes were injected with 1 ml of 50,000 MLD_{50} (LD_{50} of 1×10^{-2}) rabies virus bilaterally into the masseter muscles. One fox died within 4 days of multifocal pneumonia and bronchitis; the other survived, and brain tissue was negative for rabies by FA and MI analyses following euthanasia at 20 mo after injection of rabies virus. A third arctic fox given a 50,000 MLD_{50} dose of rabies virus died of rabies after 1 mo. To determine the adequacy of the virus preparation, red foxes were selected because they are susceptible to significantly lower doses (12 to 1,000 MLD_{50}) of virus in challenge experiments (Baer et al., 1971; Black and Lawson, 1973; Winkler, 1975). Doses of 50,000 and 500,000 MLD_{50} (LD_{50} of 1×10^{-1}) were injected into each of two red foxes and mortality in both occurred at 21 days, indicating the suitability of the virus preparation. As a result, a 500,000 MLD_{50} preparation was selected for the challenge experiment to produce 100% mortality in the control group of challenged arctic foxes.

At 9 wk after the booster dose the five experimental foxes and five control (non-immunized) foxes were injected with a 500,000 MLD_{50} dose of rabies virus, injected bilaterally into the masseter muscles. Surviving foxes were maintained for 16 mo.

The cerebellum, pons, hippocampus and parotid salivary gland from dead foxes were tested for rabies virus by the FA method. In addition, brain tissues from two control foxes that were negative for rabies by FA were injected intracerebrally into young mice. Surviving foxes were euthanized at 16 mo following challenge, and their brain and salivary gland tissues were analyzed for rabies using the FA method.

RESULTS AND DISCUSSION

None of the foxes had rabies antibodies prior to vaccination. When foxes were given the sausage bait containing the vaccine, they were closely observed to determine whether bait ingestion occurred. All foxes consumed the bait within 1 hr, some devouring the bait immediately after chewing it, plastic straw included, while others chewed it more cautiously yet also consumed the entire bait. There was no question that foxes had to chew the bait to ingest it and that all foxes were exposed to the liquid vaccine. Foxes responded similarly 13 mo later when given a booster vaccination, although some left pieces of plastic straw on the cage floor and one fox did not touch the bait within 2 hr but had consumed it completely within 19 hr.

All saliva samples taken from foxes after bait ingestion were negative for rabies virus, indicating that virus was not detectable in saliva even 1 hr after feeding on SAD-BHK_{21} vaccine. These results suggest that arctic foxes would not be infective to other animals after they ingest this vaccine should they bite another animal. In a field

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TABLE 1. Rabies antibody titers in arctic foxes (*Alopex lagopus*) fed SAD-BHK$_{21}$ vaccine in a bait.

Fox no.	Week 0	1	2	6	11	16	21	26	42	55	56[a]	57	59	62	65[b]
301	<1:5	<1:5	1:50	1:50	1:50	>1:56	1:56	1:50	1:50	1:45	—	1:280	1:280	1:210	1:56
302	<1:5	1:10	1:170	1:42	1:56	>1:56	1:56	1:50	1:56	1:16	—	1:280	1:290	1:280	1:280
304	<1:5	<1:5	1:50	<1:5	<1:11	<1:11	1:11	1:25	<1:11	1:16	—	1:280	1:280	1:125	1:56
306	<1:5	1:60	1:260	1:200	1:75	1:95	1:95	1:56	1:56	1:56	—	1:250	1:900	1:280	1:280
307	<1:5	1:9	1:85	1:95	—[c]	—	—	—	—	—	—	—	—	—	—
308	<1:5	1:9	1:56	<1:5	<1:11	<1:11	1:11	<1:5	<1:11	<1:5	—	1:280	1:480	1:280	1:56

[a] Booster administered.
[b] Rabies virus challenge.
[c] No sample collected; fox died of unrelated causes.

study using the oral SAD-BHK$_{21}$ rabies vaccine to immunize red foxes (Steck et al., 1982), no vaccine virus was isolated from foxes or other mammals trapped following bait distribution. They concluded that the SAD strain of rabies virus would have little probability of becoming established in wild mammals as a result of a vaccine-bait delivery system.

One wk after vaccination, four of the six foxes developed antibodies (Table 1). At 2 wk postvaccination, all foxes had developed titers of 1:50 or higher. There was considerable variation in titers among foxes, possibly resulting from differences in the amount of chewing during ingestion, from individual variation, or from other factors. This vaccine must be absorbed through the buccal mucosa (Baer et al., 1971, 1975). Vaccine introduced past the mouth, such as by gastric installation, does not lead to successful immunization (Baer et al., 1975). It is essential therefore, that the bait be large enough for the fox to chew it and not simply to swallow it without chewing.

At 6 wk, two of the foxes (animals 304 and 308) no longer had detectable rabies antibodies, while three foxes maintained a detectable antibody through week 55 (Table 1). In similar experiments, red foxes retained protective antibody titers for up to 26 wk, although individual titers varied (Debbie et al., 1972; Mayr et al., 1972; Winkler et al., 1975; Schneider and Cox, 1983).

The five foxes representing the experimental group responded dramatically to an oral booster vaccination given at 56 wk (Table 1). The rapid serologic response to the booster suggested an anamnestic response, although the decline in titers 9 wk later did not reflect the characteristic maintenance of titer.

Nine wk after the booster vaccination the five vaccinated and five control foxes were challenged with the arctic strain of rabies virus. All control foxes died within 16 days of exposure; the earliest was at 7 days. Brain tissues of three control foxes

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were positive for rabies by FA testing, while those of the other two foxes, both dead by day 8 postchallenge, were negative by both FA and mouse inoculation. These were found to have canine distemper as determined by FA analysis. Salivary gland tissue was negative for rabies by FA test for all control foxes. The five vaccinated foxes survived a 16-mo observation period after rabies challenge, and brain and salivary gland tissues were negative for rabies by FA analysis when harvested following euthanasia at 16 mo postchallenge.

The source of the canine distemper virus that was found by FA analysis in the brain tissues of three control foxes is unclear. All foxes, except the five control foxes and the three used to determine the lethal dose for the challenge experiment, were vaccinated with a canine distemper live virus vaccine (Adenomune-7, Biologics Corporation, Omaha, Nebraska 68134, USA), including the two red foxes. None of the vaccinated animals exhibited symptoms of distemper following vaccination, which is consistent with previous work on red foxes (Halbrooks et al., 1981; Montali et al., 1983). It is highly improbable that the arctic foxes vaccinated for canine distemper in the experimental group would have shed virus while sharing the cage room with the control group, especially since the distemper vaccination was given 25 mo prior to the rabies challenge experiment. Also, it is highly unlikely that animals previously occupying the experimental cage room would have been the source of the virus because the room was completely disinfected between experiments. Blood samples taken from the control animals prior to the challenge experiment had no canine distemper antibody as determined by serum neutralization tests conducted at the Washington Animal Disease Diagnostic Laboratory (Pullman, Washington 99165, USA). These results strongly suggest that these foxes did not contract the disease in the wild prior to capture. The only other potential source of the virus was contamination of the animal quarters in which the animals were held prior to their placement in the infectious disease area. The main animal quarters are more accessible to people, and it is possible that this area became contaminated. Had foxes been exposed to canine distemper virus just prior to the challenge experiment, symptoms would not have occurred until after residency in the infectious disease area. On the other hand, foxes may have been exposed to the virus earlier but not sufficiently to elicit seroconversion in the foxes, thus the absence of antibody. However, given the stress of the move, of exposure to a new room and cages and of handling for the experiment, the distemper virus became virulent in these animals. Whatever the source, this problem did not detract from the demonstration that arctic foxes can be effectively immunized against rabies using an oral vaccine.

These experiments showed that the SAD-BHK_{21} rabies vaccine administered in a sausage bait immunized arctic foxes against rabies, as determined by serologic response and by resistance to challenge with a large dose of rabies virus. Although individual serologic responses among animals varied, even those with "lower" antibody titers were protected when challenged 9 wk after a booster vaccination. The booster vaccination given 56 wk after the initial immunization produced a rapid and marked serologic response. In addition, the antibody level did not decline as rapidly as it did after the initial immunization. The SAD-BHK_{21} rabies vaccine administered in a sausage bait system has a strong potential for immunizing arctic fox populations.

Additional studies should be conducted before any effort is made to control rabies in wild arctic fox populations using the bait vaccine system described here. Because the SAD-BHK_{21} vaccine is a live virus vaccine its affect on potential non-target species and the affect of multiple ingestions over a short period of time, should be determined. A field study on an ice-free island in Alaska would be an ideal location to

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evaluate the efficacy of this system to immunize a wild population of arctic foxes. A blood marker system to monitor bait ingestion, which would be essential to properly conduct an experiment to immunize a wild population of arctic foxes, has already been developed using captive animals (Follmann et al., 1987).

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