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Ineffectiveness and Comparative Pathogenicity of Attenuated Rabies Virus Vaccines for the Striped Skunk (*Mephitis mephitis*)

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ABSTRACT: Three attenuated rabies virus vaccines (SAD-B_{19}, ERA/BHK-21, AZA 2) were compared for efficacy and safety in the striped skunk (*Mephitis mephitis*) by the oral and intranasal routes. The SAD-B_{19} and ERA/BHK-21 vaccines were given orally; all three vaccines were given intranasally. Oral administration of SAD-B_{19} and ERA/BHK-21 vaccines induced neither seroconversion nor significant protection against rabies challenge. One skunk which consumed a SAD-B_{19} vaccine-laden bait succumbed to vaccine-induced rabies. Intranasal instillation of the three vaccines resulted in the deaths of two of six (AZA 2), three of six (ERA/BHK-21) and six of six (SAD-B_{19}) skunks.

Key words: Rabies, oral vaccination, pathogenesis, skunk, *Mephitis mephitis*, experimental study.

Skunks (predominantly the striped skunk, *Mephitis mephitis*) are the major rabies reservoir throughout North America (Charlton et al., 1988). Despite significant progress both in the laboratory and in the field over the past decade in the development of effective baits and oral rabies vaccines for the control of vulpine rabies (reviewed by Baer, 1988; Schneider et al., 1988; Wandeler, 1988), no oral attenuated rabies vaccines have proved efficacious and safe for the skunk. The objectives of these experiments were to simultaneously compare, in the striped skunk, the immunogenicity and safety of three different attenuated rabies virus vaccines that are either under consideration or currently in use for fox rabies control in Europe and North America.

Equal numbers of male and female skunks, 1.5- to 2-yr-old, were obtained from Ruby's Fur Farm (New Sharon, Iowa 50207, USA). They were individually housed in stainless-steel cages and were provided food and water ad-libitum, with husbandry as described (Charlton and Casey, 1979). For blood collection and intranasal vaccine instillation, skunks were sedated with a combined intramuscular inoculation of ketamine hydrochloride (Rogar/STB Inc., London, Ontario, Canada L4V 1T2) at 20 mg/kg and acepromazine maleate (Ayerst Laboratories, Montreal, Quebec, Canada H4R 1J6) at 0.6 mg/kg. The SAD-B_{19} vaccine, used extensively for fox oral rabies vaccination in the Federal Republic of Germany and elsewhere in Europe, consisted of a liquid preparation of plaque-purified SAD-B_{19} virus passaged upon BHK cells, as previously described (Schneider and Cox, 1983) and was obtained from the WHO Collaborating Center for Rabies Surveillance and Research (Tübingen, Federal Republic of Germany). The ERA/BHK-21 vaccine, used for fox rabies control in southern Ontario, was an ERA virus strain propagated upon a BHK-21 C13 cell line, as described (Lawson et al., 1989) and was obtained from Connaught Laboratories (Willowdale, Ontario, Canada M2R 3T4). The AZA 2 vaccine, which has a reduced pathogenicity for adult mice by intracerebral inoculation, was a small plaque 8-azaguanine mutagenized ERA strain mutant prepared as described (Tolson et al., 1990), and was propagated as for the ERA/BHK-21 vaccine. Challenge virus consisted of a

10% suspension of submandibular salivary glands collected from naturally-infected Ontario skunks (ADRI, Nepean, Ontario, Canada K2H 8P9), prepared as previously described (Charlton and Casey, 1979). The titer was $1 \times 10^{7.5}$ $MICLD_{50/g}$. Blood samples, collected for the determination of rabies virus-neutralizing antibodies (VNA), were obtained from skunks prior to vaccine administration and at various intervals thereafter as indicated. Sera were stored frozen at −20 C until tested for rabies VNA by the fluorescence inhibition microtest (Zalan et al., 1979) using ERA virus as challenge, with results calculated in international units (IU/ml). Rabies diagnosis was confirmed post-mortem using the fluorescent antibody test (FAT) upon brain tissue as described (Goldwasser and Kissling, 1958).

To determine the potential efficacy of $SAD\text{-}B_{19}$ and ERA/BHK-21 vaccines for skunks, animals were divided into three groups: two groups of eight skunks each received a sponge bait (Lawson et al., 1987) containing 4.0 ml of either $SAD\text{-}B_{19}$ ($1 \times 10^{9.5}$ $TCID_{50}$/ml) or ERA/BHK-21 ($1 \times 10^{9.0}$ $TCID_{50}$/ml) vaccine; a third group of four skunks received 1.0 ml of $SAD\text{-}B_{19}$ vaccine directly per os, by slowly administering liquid vaccine by needleless syringe to sedated animals. Skunks were observed daily and bled for VNA on days 14, 28, 60 and 90. On day 90, skunks were sedated and inoculated with 0.3 ml of challenge virus ($1 \times 10^{7.5}$ $MICLD_{50}$/ml) into the right abductor digiti quinti muscle, equivalent to approximately 200 skunk LD_{50} doses. Animals were observed daily and euthanized when definitive clinical signs of rabies developed.

None of the skunks given $SAD\text{-}B_{19}$ and ERA/BHK-21 vaccine-laden baits or $SAD\text{-}B_{19}$ vaccine per os developed rabies VNA following vaccination. Skunks given vaccine orally remained healthy until the time of challenge, except one skunk died 15 days after administration of the $SAD\text{-}B_{19}$ vaccine-laden bait. Vaccine-induced rabies in this skunk was determined by virus isolation from brain material and confirmed by analysis with rabies-specific anti-nucleocapsid and anti-glycoprotein monoclonal antibodies as consistent with the characterization of SAD/ERA fixed rabies virus strains (Dietzschold et al., 1988). In addition, a submandibular salivary gland from this skunk yielded rabies virus at a concentration of $1 \times 10^{5.0}$ TCID/g of tissue. In the remaining $SAD\text{-}B_{19}$ and ERA/BHK-21 vaccinates, two of seven skunks in the $SAD\text{-}B_{19}$ bait group survived challenge, whereas all other skunks, including controls, succumbed to street rabies virus infection 16 to 30 days postchallenge. The two surviving skunks in the $SAD\text{-}B_{19}$ bait group were both negative for rabies VNA and rabies virus by the FAT at the time of euthanasia, 90 days postchallenge.

In a related experiment designed to study the comparative safety of attenuated rabies vaccines, skunks were divided into three groups of six animals each. Skunks were sedated, placed in dorsal recumbency in a bio-safety hood and were allowed to slowly aspirate 0.5 ml of either undiluted $SAD\text{-}B_{19}$, ERA/BHK-21 or AZA 2 vaccine into the right nostril (vaccine concentrations as above; AZA 2 vaccine given at $1 \times 10^{8.0}$ $TCID_{50}$/ml). Thereafter, skunks were observed and treated as in the above study, but were not challenged. The intranasal route of exposure was used as an extreme measure of safety, second, perhaps only to intracerebral inoculation in sensitivity. Intranasal exposure may occur (albeit rarely) during natural consumption of vaccine-laden baits.

In comparison, intranasal instillation of the three attenuated rabies virus vaccines produced mortality within 2 to 3 wk of inoculation in each group: two of six skunks with AZA 2, three of six skunks with ERA/BHK-21, and six of six skunks with $SAD\text{-}B_{19}$ vaccine. All rabies-suspect skunks were positive for rabies by the FAT of brain tissue but salivary glands were negative. Only one of the three surviving skunks in the ERA/BHK-21 group seroconverted to rabies by day 14 (2.8 IU/ml). Three of the

four surviving skunks in the AZA 2 group demonstrated rabies VNA by day 30 (5.6–44.4 IU/ml). Only one skunk in the SAD-B_{19} group seroconverted by day 14 (0.3 IU/ml) but succumbed nonetheless, as did the remaining five skunks in this group. Surviving skunks in the ERA/BHK-21 and AZA 2 groups were euthanized on day 90. All were negative for rabies virus by the FAT.

These experimental results definitively confirm previous suggestions (Tolson et al., 1988, 1990) of the general inadequacy of several conventional attenuated rabies vaccines given orally to skunks, even at dosages 1,000-fold in excess of those found minimally protective for foxes (Schneider and Cox, 1983). Moreover, these experiments detail the potential pathogenicity of such vaccines by the intranasal and oral routes in skunks. Whereas modified-live rabies vaccines administered orally are known to induce rabies in a variable proportion of different rodent species (Lawson et al., 1987; Schneider et al., 1988; Wandeler, 1988), vaccine-induced rabies by the SAD-B_{19} vaccine delivered via bait and recovery of vaccine virus from carnivore salivary glands has not been previously reported even after extensive laboratory safety evaluations and field trials of SAD-B_{19} virus in several European countries. Both of the ERA and ERA/BHK-21 vaccines have also been intensively studied for safety and immunogenicity in a number of Canadian target and non-target species (Lawson et al., 1987, 1989).

Clearly, caution is advised in the consideration of the field deployment of conventional rabies biologicals that retain residual pathogenicity in a major target, vector or non-target species. Conclusions regarding the relative attenuation or virulence of a given rabies vaccine for one host species are not readily extendable to others without direct testing. For example, the AZA 2 vaccine has a reduced virulence for adult mice even by direct intracerebral infection. Nevertheless, similar "apathogenic" rabies virus variants are lethal to skunks by intranasal or intracerebral inoculation (Charlton et al., 1988; Tolson et al., 1990). Although the use of individually administered inactivated rabies virus vaccines may provide a measure of skunk rabies control under special circumstances, such as in urban habitats (Rosatte et al., 1987), to date only bio-engineered vaccines, such as a vaccinia-rabies glycoprotein recombinant virus vaccine, have been shown safe and efficacious for skunks when consumed in bait and when administered by other routes (Tolson et al., 1987). Further studies are needed to develop equally suitable and inexpensive recombinant (e.g., canine adenovirus-based) or sub-unit vaccines safe and effective for skunks, the construction of relevant baits specific towards skunks to minimize vaccine wastage and the applied ecological research necessary towards comprehensive, effective skunk rabies control.

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