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# Safety Studies of the Oral Rabies Vaccine SAD B19 in Striped Skunk (*Mephitis mephitis*)

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ABSTRACT: Safety of the modified live rabies virus vaccine, SAD B19, was studied in striped skunks (*Mephitis mephitis*). Seven skunks received $10^{7.9}$ foci formatting units by direct oral administration. In four cages, a vaccinated animal was placed with a control animal, the other three vaccinated skunks were housed individually. Saliva and nasal swabs were collected 1, 2, 4, 24, 48, and 72 hr post-vaccination. From all vaccinated and control animals ($n$ = 11) blood samples were collected 0, 28, 56, 84, and 296 days post-vaccination. Three of seven vaccinated skunks seroconverted. None of the control animals had detectable levels of rabies virus neutralizing antibodies. Also no vaccine virus was isolated from the nasal and saliva swabs collected from any animal. Thus, SAD B19 was innocuous for skunks in our study after direct oral administration at field concentration.

*Key words:* Experimental study, *Mephitis mephitis,* oral vaccination, rabies, skunk.

Oral vaccination of red foxes (*Vulpes vulpes*) against rabies has been very effective in controlling vulpine rabies (Stöhr and Meslin, 1996; Müller and Schlüter, 1998; MacInnes et al., 2001). Also rabies control by means of oral vaccination of raccoons (*Procyon lotor*), coyotes (*Canis latrans*), and raccoon dogs (*Nyctereutes procyonoides*) has been successful (Reinius, 1992; Fearneyhough, 1999; Smith et al., 1999). Unfortunately, the striped skunk (*Mephitis mephitis*) is not included in this list, although it is a major rabies reservoir species in North America. In 1999, 29.4% ($n$ = 2,076) of all reported rabies cases in the United States were skunks (Krebs et al., 2000). Many experimental and commercial-produced live-modified or recombinant-based oral rabies virus vaccines have been tested in skunks with contradictory results (Tolson et al., 1987, 1988, 1990; Rupprecht et al., 1990; Charlton et al., 1992). One of eight skunks died from vaccine-virus induced rabies after consuming a SAD B19 vaccine-bait in one of these studies (Rupprecht et al., 1990). However, the SAD B19 vaccine virus was innocuous for other members of the mustelid family including domestic ferret (*Mustela putorius furo*), mink (*Mustela vison*), and stone marten (*Martes foina*) after oral administration (Vos et al., 1999). Under experimental conditions no pathogenicity was observed in more than 20 other mammalian species after oral administration of SAD B19 with the exception of certain rodent species (Schneider and Cox, 1983; Müller et al., 1998; Vos et al., 1999). However, the possibility of accidental intranasal inoculation in rodents cannot be ruled out completely under these circumstances due to the relatively high vaccine volume administered. Rodents, originating from areas where SAD B19 vaccine baits had been distributed, all tested negative for rabies (Brochier et al., 1988; Schneider, 1989). Furthermore, since 1983 more than 85 million SAD B19 vaccine baits were distributed in 15 European countries and no case of vaccine-virus induced rabies has been reported from those countries (Vos et al., 2000). These contradictory findings led to the decision to repeat the safety studies of SAD B19 in striped skunks.

Four female and seven male, 2–4 mo old, striped skunks from three litters were purchased from a commercial source (Metazoa, Meteren, The Netherlands). The animals were observed for several weeks prior to vaccination to reveal any illness. During this period the litters were housed in separate outdoor wire cages with wooden nest boxes at the outdoor animal enclosure of the Experimental Animal Facility of IDT GmbH (Rosslau, Germany). The animal experiment was performed according to the German Welfare



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Act of 25 May 1998 and the experimental design was approved by the appropriate authorities.

The animals were fed commercial cat food, fruits, and occasionally fresh meat (chicken, rats, and mice); water was given ad libitum. All animals were bled prior to vaccination (B0) and tested negative for rabies virus neutralizing antibodies by rapid fluorescence focus test (RFFIT). Seven animals received 1.0 ml of working seed virus of the current commercially available SAD B19 vaccine virus ($10^{7.9}$ focus formatting units (FFU) per ml) by direct oral administration; the vaccine virus was squirted directly in the oral cavity of the unanesthetized animal. This virus titer was close to the highest titre ($10^{8.2}$ FFU/ml) that has been achieved according to the established manufacturing protocols for the commercial production of SAD B19. The SAD-strain (Street-Alabama-Dufferin) was isolated from a rabid dog in Alabama in 1935 (Sacramento et al., 1992). The SAD B19 virus strain was derived by adaptation of the SAD strain passaged in mouse brain on cloned BSR cells (Schneider and Cox, 1983).

All skunks were kept individually in cages for 1 day post-vaccination to prevent possible horizontal transmission during the first day. Subsequently, one vaccinated and one unvaccinated control animal were placed together in four cages and three other vaccinated animals were kept individually. Blood samples were collected from vaccinated and control animals 28 (B1), 56 (B2), 84 days (B3), and 296 days (B4) post-vaccination; 1.0 ml of blood was collected by clipping of a claw. The serum samples were evaluated by RFFIT (Smith et al., 1973), with modifications of Cox and Schneider (1976). If one or more fluorescing cells were detected in the lowest dilution (1:4), the specimen was regarded as negative for rabies virus neutralizing antibodies. Antibody titers were converted to international units (IU) by comparison with a standard immunoglobulin (Staatliches Kontrollinstitut fuer Arzteneimittel, Berlin, Germany). Saliva and nasal secretions were collected by swabbing the oral and nasal cavity for 1–1.5 min with a cotton wool cylinder 1, 2, 4, 24, 48, and 72 hr post-vaccination. After swabbing, the cotton wool cylinder was placed in the holding tube (Cultiplast®, LP Italiana Spa, Milano, Italy). The holding tubes contained 2.0 ml modified minimal essential medium Eagle (MEM) and a mixture of gentamicin (50 mg/l) and amphotericin B (2.5 mg/l). The tubes were stored at −20 C. After thawing, 0.5 ml of the liquid was removed. One microflask (25 $cm^2$) was used for each sample. Every microflask contained 10 ml cell suspension BSR clone 13 ($10^{5.5}$ cells per ml) in MEM mixed with 10% newborn calf serum (NCS). The sample (0.5 ml) was added to the microflask and incubated at 35 C for 6 days. On the fourth day the medium was changed with MEM plus 1% NCS. Subsequently, two cavities of an 8-well microscope slide (Lab-Tek® chamber slide, Nunc GmbH & Co., Wiesbaden, Germany) were filled with 0.5 ml of liquid from the microflask and 0.1 ml cell suspension BSR clone 13 ($10^{6.0}$ cells per ml) plus 10% NCS. The slides were put into an incubator (35 C, 5% $CO_2$) for 48 hr. After draining, the cells were fixed with 80% acetone 30 min at room temperature. The slides were drained again and dried. Fluorescein isothiozyanate-labeled anti rabies IgG (Centocor Inc., Malvem, Pennsylvania, USA) was added. The slides were stained for 30 min at 37 C and analyzed for rabies virus by the fluorescent antibody test (FAT) as described by Dean et al. (1996). If one or more fluorescing cells were detected, the specimen was regarded as positive for rabies virus. The sensitivity of this method was 1 FFU per 0.5 g saliva.

In contrast to the previous safety studies of SAD B19 vaccine virus in skunks (Rupprecht et al., 1990), no illness was observed in the vaccinated animals. Three of seven vaccinated animals developed virus neutralizing antibodies; animal 1—6.0 (B1), 80.0 (B2), 47.6 (B3), and 14.2 (B4)

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IU/ml; animal 2—3.0 (B1), 23.8 (B2), 28.3 (B3), and 16.8 (B4) IU/ml; animal 3—0.5 (B1), 14.2 (B2), 14.2 (B3), and 6.0 (B4) IU/ml. None of the control animals developed detectable levels of rabies virus neutralizing antibodies. The difference in pathogenicity between the two studies could simply be dose dependent. In the Rupprecht et al. (1990) study, the titer of the vaccine virus, produced for experimental purposes only, was extremely high ($10^{9.3}$ $TCID_{50}$) in comparison to our study ($10^{7.9}$ FFU). However, the vaccine virus used in our study was propagated according to reproducible manufacturing protocols, which deviated from the protocols used for the production of the vaccine virus administered to skunks by Rupprecht et al. (1990). Certain genetic determined properties are selected for during virus propagation using different protocols and materials (cell lines, cell culture conditions, etc.), and could explain the observed difference in pathogenicity (Wandeler, 2000). However, the commercially produced SAD B19 vaccine virus used in this study did not induce illness in striped skunks after oral administration.

Recently, another modified-live virus vaccine used for oral vaccination of wildlife, SAG2, was tested in skunks and all animals were protected against a subsequent challenge (Rupprecht et al., 1999). These findings contradict previous studies that showed skunks were generally refractory to oral vaccination by modified-live viruses (Tolson et al., 1988, 1990; Rupprecht et al., 1990). Recombinant vaccinia virus vaccine expressing the rabies virus glycoprotein (VRG) has been reported to be effective in skunks after oral administration (Tolson et al., 1987) however, during subsequent trials skunks apparently developed only low rates of seroconversion (Charlton et al., 1992). Another recombinant-based rabies vaccine, a human adenovirus type 5 expressing the rabies glycoprotein, was shown to be highly effective in skunks (Charlton et al., 1992). Unfortunately, this virus was isolated from saliva and feces of orally vaccinated skunks up to 10 days post-vaccination (Charlton et al., 1992; Lutze-Wallace et al., 1995). In our study, no vaccine virus could be detected in the saliva and nasal secretions of the vaccinated animals, indicating that no horizontal transmission from vaccinated to control animals took place. Hence, the modified-live virus vaccine SAD B19 may be safer and more effective in skunks after oral vaccination than previously believed.

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