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Further Studies on the Susceptibility of Raccoons (*Procyon lotor*) to a Rabies Virus of Skunk Origin and Comparative Susceptibility of Striped Skunks (*Mephitis mephitis*)

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ABSTRACT: Two raccoons (*Procyon lotor*) were inoculated in the masseter muscles with $10^{5.9}$ mouse intracerebral lethal dose$_{50}$ (MICLD$_{50}$) of a rabies virus isolated from a naturally infected Iowa (USA) striped skunk (*Mephitis mephitis*). Five striped skunks were inoculated with either $10^{0.7}$ or $10^{2.1}$ MICLD$_{50}$ of the same isolate. All five skunks died within 35 days following inoculation. Both raccoons survived 273 days without adverse effects, and virus was not isolated from saliva samples taken at between 25 and 273 days following inoculation.

Key words: Rabies virus, raccoons, *Procyon lotor*, skunks, *Mephitis mephitis*, inoculation, experimental study.

Rabies in North America generally is associated with particular terrestrial wildlife reservoir species within defined geographic areas (Pacer et al., 1985; Smith, 1989). One reason for this compartmentalization of rabies within single animal species may be differential species susceptibility to given virus strains (Sikes, 1962; Parker and Wilsnack, 1966). Hill and Beran (1992) found that raccoons (*Procyon lotor*) remained clinically normal for 90 days after intramuscular inoculation with up to 63,200 ($10^{4.8}$) mouse intracerebral lethal dose$_{50}$ (MICLD$_{50}$) of a rabies virus isolated from the salivary glands of a naturally infected Iowa striped skunk (*Mephitis mephitis*). Our objective was to determine the effects of a larger dose of the same virus for a longer period of time in raccoons. The comparative pathogenicity of the challenge virus was evaluated by inoculation into striped skunks.

The rabies virus in this study was the same skunk virus used by Hill and Beran (1992). Materials and methods used were identical to those reported in that study, unless otherwise noted. Using antigenic reaction patterns of monoclonal antibodies directed against the nucleocapsid protein (Smith, 1989), this skunk virus was identical to the north central USA skunk antigenic variant described by Smith et al. (1986). Five male 5-mo-old licensed fur farm-reared striped skunks (Ruby's Fur Farm, New Sharon, Iowa, USA) and one male and one female adult wild-trapped raccoon (Boone County, Iowa) were used in the experiment. All animals were tested for rabies serum neutralizing antibodies (SNA) prior to inoculation.

The rabies inoculums for the skunks consisted of 1 ml of the skunk salivary gland suspension diluted to virus titer of $10^{0.7}$ (two skunks) or $10^{2.1}$ MICLD$_{50}$ (three skunks). Both raccoons were inoculated with 1 ml of the virus suspension at a titer of $10^{5.9}$ MICLD$_{50}$. Animals were observed daily for changes in behavior. In animals which died, a blood sample for monitoring antibody response was collected at the time of death. Surviving animals were anesthetized and sampled on days 25, 57, 87, 197, and 273. Saliva samples were collected from raccoons by swabbing the oral mucosa and tonsillar areas with sterile cotton swabs.

Neither raccoon developed fatal rabies. No adverse effects were observed in either animal, and both animals gained weight throughout the 273 day observation peri-

TABLE 1. Rabies virus-neutralizing antibody titer and response to experimental inoculation with skunk rabies virus in raccoons and skunks.

Group	Animal number	Inoculum ($MICLD_{50}$)[a]	Days to onset of clinical signs	Response to inoculation[b]	RFFIT titer[c]
Raccoons	1	$10^{5.9}$		S	5.7
	2			S	<5.0
Skunks	1	$10^{6.7}$	24	D (35)	162
	2		22	D (31)	6
Skunks	3		17	D (31)	724
	4	$10^{2.1}$	18	D (27)	322
	5		17	D (37)	1,719

[a] Groups were inoculated with 1 ml of a skunk rabies virus suspension (0.5 ml in each masseter muscle). $MICLD_{50}$ = Mouse intracerebral lethal $dose_{50}$.

[b] S = Survived, D = Died. Day of death is shown in parentheses.

[c] Inverse virus-neutralizing antibody titer on day of death, or peak antibody titer at 25 days post-inoculation as determined by the rapid fluorescent focus inhibition test (RFFIT). All animals had virus-neutralizing antibody titer <5.0 at the time of inoculation. A titer of <5.0 was considered negative.

od. Rabies virus was not isolated from any of the saliva samples. One raccoon developed a virus-neutralizing antibody titer of 5.7 by day 25 post inoculation (Table 1) which declined to a titer of <5.0 by day 57. All five skunks developed fatal rabies. The mean incubation period was 23 days with the lower dose and 17.3 days with the higher dose of virus (Table 1). Clinical disease lasted an average of 13.6 days (range = 10 to 21 days). All skunks developed SNA by the time of death.

Species differences in susceptibility and virus secretion may be important in the compartmentalization of rabies in single reservoirs in host-associated geographic areas (Sikes, 1962; Parker and Wilsnack, 1966). We provide additional evidence that raccoons and skunks have very different susceptibilities to the skunk virus, supporting the hypothesis that differential species susceptibility to different rabies virus strains is an important factor in the compartmentalization of rabies. Raccoons appeared to be relatively resistant to inoculation with even very large doses of skunk rabies virus. Survival of at least 273 days following inoculation was substantiated.

The raccoon is plentiful in areas of North America where the north central U.S. skunk rabies antigenic variant is endemic, yet only a small number of rabies cases in raccoons are reported (Hall, 1981; Pacer et al., 1985; Centers for Disease Control, 1989). Based on our work and that of Hill and Beran (1992), this can be explained by the relative resistance of raccoons to even very large doses of skunk virus.

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