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EXPERIMENTAL RABIES INFECTION IN WILD RODENTS

WILLIAM G. WINKLER[1], NATHAN J. SCHNEIDER[2], WILLIAM L. JENNINGS[3]

Abstract: The potential for wild rodents to serve as inapparent rabies reservoirs in nature has not been well evaluated. In this study five species of rodents were inoculated intramuscularly with rabies virus derived from naturally infected wild animals. Inoculated rodents were observed for behavioral changes, and those which died were tested for rabies. Differences in species susceptibility and salivary gland virus tropism were noted and discussed as these factors might affect the epidemiological potential of rabies in wild rodents.

INTRODUCTION

Rodents have never been shown to be involved in the epidemiology of wildlife rabies. However, the hypothesis that rodents might play a role in wildlife rabies epidemiology is an attractive one, since the existence of such a large unrecognized reservoir could explain such enigmas as the occurrence of sporadic isolated cases of carnivore rabies or the dogged persistence of rabies in an area despite exhaustive attempts to eradicate it from the commonly recognized carnivore reservoirs.

Only occasionally do rabies positive rodents and lagomorphs[4] appear in the national rabies surveillance reports. In the seven year period 1964-1970 during which 28, 319 rabid animals were reported in the United States, rabies was diagnosed in only 39 rodents.[1] There is some question whether many of these 39 may not have been diagnostic errors.[9]

The paucity of *bona fide* rodent rabies cases in the surveillance literature has not been well explained. Approximately 35% of all animals examined for rabies in diagnostic laboratories are rodents. Experimental data do not indicate that rodents are especially refractory to infection. The experimental infection of laboratory rodents by parenteral inoculation is a commonplace procedure.[5] Infection by other routes including ingestion, inhalation, and rectal instillation has also been reported.[2,4,6,7,8]

Experimental infection of laboratory rodents with rabies is well described, but experimental or natural infection in wild rodents is not. In this study the authors measured the susceptibility of several wild rodent species (one a laboratory strain of a common wild species) to infection with different rabies isolates to which they might conceivably be exposed in nature. Salivary glands of the experimentally infected rodents were tested to evaluate the possibility of these rodents' transmitting rabies to other animals by bite.

MATERIALS AND METHODS

Rabies viruses

Bat origin rabies virus was isolated from the mandibular salivary glands of a naturally infected Florida yellow bat *(Dasypterus floridarus)* collected near

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[4] "Rodent" as used in the reports, includes the closely related lagomorphs—rabbits, hares and pikas.

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Tampa, Florida. The titer of the pooled salivary glands was $10^{4.7}$ mouse intracerebral 50% lethal dose ($MICLD_{50}$) per 0.03 ml at the time of original isolation. Raccoon rabies virus, obtained from the paired mandibular salivary glands of a naturally infected raccoon *(Procyon lotor)* collected near Inverness, Florida, originally had a titer of $10^{5.0}$ $MICLD_{50}$ per 0.03 ml. Fox rabies virus, obtained from the paired mandibular salivary glands of a naturally infected red fox *(Vulpes fulva)* collected in northwestern Virginia, originally had a titer of $10^{6.3}$ $MICLD_{50}$ per 0.03 ml.

In all cases, the inoculum used to infect rodents was a suspension of the original tissues rather than mouse passaged material. Serial ten-fold dilutions of virus inoculum were prepared using 10% horse serum in phosphate buffered saline, pH 7.2, and containing 1000 Units of penicillin and 2 mg of streptomycin per ml. At the time of inoculation, all viruses were titrated by intracerebral inoculation in 3-week-old Swiss white mice to determine the exact amount of virus given.

Experimental Animals

Gray squirrels *(Sciurus carolinensis)* were trapped near Orange Park, Florida. Kangaroo rats *(Dipodomys merriami)* and cactus mice *(Peromyscus eremicus)* were collected near Las Cruces, New Mexico. Cotton rats *(Sigmodon hispidus)* and laboratory white rats *(Rattus norvegicus)*, Hartley strain, were obtained from laboratory animal colonies and had been born and reared in captivity.

Gray squirrels included both juveniles and adults, which were randomly distributed among the experimental cohort groups. All ithers were adult animals.

All animals were held in individual cage quarantine for at least 30 days prior to experimental inoculation.

Virus Inoculation and Animal Observation

Gray squirrels were divided into six groups of five animals each. Each animal was inoculated intramuscularly (IM) in the left cervical muscles with 0.10 ml of rabies virus inoculum. Three groups received bat origin virus containing 64, 640, or 6400 $MICLD_{50}$'s, and three groups received raccoon origin virus containing 33, 330, or 3300 $MICLD_{50}$'s.

Each of the other four species of rodents was divided into four groups of seven to nine animals. All were inoculated IM in the quadriceps femoris region of the right leg with 0.03 ml of inoculum containing 6.3, 63, 630, or 6300 $MICLD_{50}$'s of fox origin rabies virus.

All inoculated animals were observed daily for behavioral changes, and any animals that died after inoculation were tested for rabies. Gray squirrels were observed for 142 days, all other rodents for 75 days.

Rabies testing

Brain and mandibular salivary glands of all animals that died after inoculation with rabies virus were tested for rabies by intracerebral (IC) mouse inoculation and fluorescent antibody (FA) tests. The paired mandibular salivary glands of each animal were pooled and tested as a single unit. Initial screening of tissues was by IC mouse inoculation of a 20% tissue suspension in 10% horse serum - antibiotic diluent. Specimens found positive on screen testing were then titrated in 10-fold dilutions to determine the amount of virus present in tissue.

RESULTS

Seven of the 15 squirrels inoculated with bat origin virus and 13 of the 15 squirrels inoculated with raccoon origin virus died of rabies (Table 1). The 50% lethal dose (LD_{50}) for squirrels inoculat-lethal dose (LD_{50}) for squirrels inoculated with bat origin virus was approximately 980 $MICLD_{50}$; with the raccoon origin virus it was <33 $MICLD_{50}$.

Twelve of the 20 rabid squirrels had demonstrable virus in the salivary glands. Two squirrels inoculated with bat origin virus had small amounts of rabies virus in the salivary glands (<1 log10 $MICLD_{50}$/0.03 ml), whereas of the 10 salivary gland positive squirrels inocu-

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lated with raccoon origin virus, six had large amounts of rabies virus in the salivary glands (3-4 $\log_{10}$ $MICLD_{50}$/0.03 ml).

Incubation periods in the infected squirrels ranged from 18 to 86 days, longest in squirrels that received the smallest dose of virus.

Clinical signs of rabies in the squirrels appeared to be unrelated to the source of infecting virus. About half of the squirrels infected with both the raccoon and bat origin viruses died without demonstrable clinical illness. The other half died after exhibiting signs of furious rabies for approximately 24 hours. In the furious state these squirrels displayed the most bizarre behavior the authors have observed in any of many species of animals infected with rabies. The squirrels were extremely aggressive, attempted to attack persons who approached, gnawed metal in their cages, and frequently engaged in self-mutilation, chewing tails and feet in some cases to the point of amputation.

Of the four species of rodents inoculated with fox origin virus, all 30 kangaroo rats, 29 of 32 cotton rats, 26 of 36 white rats, and 18 of 33 cactus mice died of rabies (Table 2). The LD_{50} with the fox origin virus was 6.3 $MICLD_{50}$ for kangaroo rats and cotton rats, 24 $MICLD_{50}$ for white rats and 125 $MICLD_{50}$ for cactus mice. Only kangaroo rats and white rats developed demonstrable rabies virus in salivary glands. Ten of the 17 salivary-gland-positive kangaroo rats had large amounts of virus (3-4 $\log_{10}$ $MICLD_{50}$/0.03 ml), whereas none of the three salivary gland positive white rats had as much as 1 $\log_{10}$ $MICLD_{50}$/0.03 lm in the salivary glands.

Incubation periods varied somewhat with the species infected; in kangaroo rats, 10-26 days; cactus mice, 15-34 days; white rats, 12-71 days; and cotton rats 11-73 days. Longest incubation periods characteristically were related to lowest doses of virus.

Clinical signs in the four species infected with fox origin virus did not vary by species. Most individuals exhibited progressive ascending paralysis of 3-6 days' duration terminating in death. No agrressive behavior was observed.

DISCUSSION AND CONCLUSIONS

From the results of the experimental infections it is apparent that the five species of rodents tested are susceptible to rabies infection, and at least two, gray squirrels and kangaroo rats, may have appreciable amounts of virus in mandibular salivary glands when infected.

TABLE 1. Results of Gray Squirrel Rabies Exposure Experiment

Virus type and dose $MICLD_{50}$	Rabid/ exposed	Positive sal. gland/rabid	Range of Virus Titer in Salivary Glands*			
			<1	1-2	2-3	3-4
bat 64	1/5	0/1				
bat 640	2/5	1/2	1			
bat 6400	4/5	1/4	1			
Total	7/15	2/7	2	0	0	0
raccoon 33	4/5	3/4			1	2
raccoon 330	4/5	3/4			2	1
raccoon 3300	5/5	4/5		1		3
Total	13/15	10/13	0	1	3	6

*Expressed as $\log_{10}$ $MICLD_{50}$/0.03 ml.

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TABLE 2. Results of Other Rodents Rabies Exposure Experiment

Species	Virus dose* $MICLD_{50}$	Rabies/ Exposed	Pos. Sal. Gl./ Rabid	Range of Virus Titer in Salivary Glands** 1	1-2	2-3	3-4
Kangaroo rat	6.3	7/7	6/7		1		5
	63.0	8/8	7/8		1	2	4
	630.0	7/7	2/7	1			1
	6300.0	8/8	2/8	1	1		
	Total	30/30	17/30	2	3	2	10
White rat	6.3	2/9	1/2	1			
	63.0	6/9	0/6				
	630.0	9/9	1/9	1			
	6300.0	9/9	1/9	1			
	Total	26/36	3/26	3	0	0	0
Cotton rat	6.3	5/8	0/5				
	63.0	8/8	0/8	None positive			
	630.0	8/8	0/8				
	6300.0	8/8	0/8				
	Total	29/32	0/29				
Cactus mouse	6.3	1/7	0/1				
	63.0	4/9	0/4	None positive			
	630.0	7/9	0/7				
	6300.0	6/8	0/6				
	Total	18/33	0/18				

* All four species received fox salivary gland origin rabies virus.
** Expressed as Log_{10} $MICLD_{50}$/0.03 ml.

The gray squirrel experiment demonstrated that the source of infecting virus may be an important factor in determining both susceptibility and distribution of virus in tissues of infected animals. The raccoon isolate was more virulent (>1 log_{10}) than the bat isolate and produced high titers of virus in the salivary glands of infected squirrels, whereas the bat isolate did not. The relatively small virus dose required to infect squirrels with the raccoon isolate suggests that they might readily be infected in nature by a rabid raccoon. It appears that squirrels are much less susceptible to natural infection with bat rabies if the yellow bat isolate used in this experiment is representative of all bat isolates. The aggressive behavior seen in half of the rabid squirrels suggests that if natural infection does occur some individuals could be expected to attack other animals, even larger carnivores, and so spread infection between species.

The experiments with the other four

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species of rodents exposed to fox origin virus demonstrated that differences in species susceptibilities do exist in wild rodents. Kangaroo rats were obviously the most susceptible of the rodents exposed to the fox isolate. This is also the only species in which infected animals had appreciable amounts of virus in the salivary glands. Like the squirrels infected with the raccoon isolate, kangaroo rats infected with this fox isolate had good potential for transmitting rabies by bite. Unlike the squirrels, rabid kangaroo rats exhibited no aggressive behavior. However, aggressive behavior may not be a prerequisite to rabies transmission by small rodents. Bell and Moore have demonstrated that rabies transmission to carnivores by ingestion of rabid rodents is not difficult.[3]

It is easy to recognize the potential for moribund rabid rodents which constitute normal prey species of carnivores to serve as a source of infection for these predatory species.

The differences in responses between the various experiments suggest that one should be careful in making general statements about rodent rabies. It is common practice to categorize all rodents as responsive or unresponsive to some parameter of rabies infection based on experimental infection studies performed with limited numbers and types of animals and virus inoculums. Obviously this can lead to errors, since rodent species do not all respond similarly to a particular rabies isolate or to different isolates.

The ease with which wild rodents can be experimentally infected with rabies and the potential which some rodents have for transmitting rabies, as shown in this study, are not supported by surveillance or epidemiological data from the field. It would appear that other factors may operate in nature to prevent rodents from serving as effective rabies reservoirs. One possible explanation is that rodents bitten by rabid carnivores may seldom survive long enough to succumb to rabies infection. Additional studies are indicated to evaluate the possible role of rodents in wildlife rabies epidemiology.

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