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Source: Journal of Wildlife Diseases, 11(2) : 170-176

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-11.2.170>

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## A RABIES EPIDEMIC IN RECENTLY CAPTURED SKUNKS<sup>1</sup>

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**Abstract:** An epidemic of rabies occurred within a colony of captive skunks (*Mephitis mephitis*). One of the animals had been infected with the virus in the wild and developed clinical illness nearly 7 weeks after capture. She transmitted the virus to three of her five offspring and one other adult. The disease spread to additional skunks when orphaned infants were adopted by lactating females. Although the animals were in close contact with each other, the epidemic spread slowly. Furious rabies generally did not occur and frequently rabid skunks were found dead without any clinical signs of the disease having been observed.

### INTRODUCTION

Although a vaccination program has resulted in control of rabies among domestic animals, the disease remains endemic in certain wildlife populations. In the upper Mississippi and Ohio River Valleys, the skunk has emerged as the most frequent vector of rabies. Therefore, an investigation into the role of skunks as reservoirs for the maintenance of rabies virus in nature was undertaken.

One observation, to be described in detail elsewhere, was that antibodies against rabies virus could be detected by several serological procedures in the sera from many skunks but generally not in sera from other small carnivores living in the same geographic area.<sup>4</sup> As a part of the study pregnant skunks were captured from the wild and held in captivity in order that transfer of immunoglobulins to offspring could be determined. An epidemic of rabies which occurred within a colony of these animals is described in this report.

### MATERIALS

Pregnant striped skunks were live-trapped in Story County, Iowa, during the last 2 weeks of March, 1972. Serum titers for rabies antibodies were determined by the passive hemagglutination (PHA) procedure<sup>5</sup> and nine of the animals were retained for additional observation: three skunks without a titer, two with titers of 1:2, three with titers of 1:4 and one with a titer of 1:8; four additional skunks had no titers and were released. The skunks were ear-tagged for identification, their scent glands were removed and the animals were vaccinated against canine distemper, hepatitis and leptospirosis (Enduracell D-H-L, Norden Laboratories) and feline distemper (Felocine, Norden Laboratories). They were housed on a wood shavings-covered concrete floor in individual pens approximately 1.5 m x 2.0 m, constructed from plywood panels 1 m high. Nesting boxes were provided for each animal. A diet of dry dog food and eggs, with occasional carrots, cabbage and mice, was fed.

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Between May 2 and May 12, seven of the skunks gave birth to litters of five to eight offspring each. At 4 weeks of age the young animals were ear-tagged and vaccinated with  $\frac{1}{2}$  of a dog dose of Enduracell D-H-L and  $\frac{1}{2}$  of a cat dose of Felocene. All animals showed seroconversion in response to both vaccines. Blood was collected for rabies antibody titration by the PHA and plaque reduction serum neutralization (PR)<sup>8</sup> procedures from the young skunks at birth and at 4 weeks, 2 months and 8 months of age, and from adults at the same times.

The brains and salivary glands of skunks that died were examined for rabies antigens by the fluorescent antibody staining (FA) procedure.<sup>1</sup> Brains that appeared negative for rabies virus by that method were further tested for the virus by mouse inoculation.<sup>3</sup>

#### RABIES EPIDEMIC

For convenience the adult skunks are identified in this report by pen number and juveniles by the pen number followed by a letter. Young animals fostered by another adult retain the number designation of their biological mother. The location of the pens is shown in Figure 1.

Skunk #1 was captured from a farm yard on March 31, 1972, in apparent good health except for a lesion 14 mm in diameter on the left lateral aspect of her muzzle. She delivered eight young 32 days later on May 2. These were extremely small infants and matured more slowly than those in the other six litters. Whereas skunks normally are born with short hair coats, these remained completely hairless for more than a month.

Although the wound that skunk #1 had when she was caught had apparently healed, facial edema was observed on May 17, 47 days after capture, and gradually increased. Beginning on May 18 (day 48 postcapture), the animal showed a severe thirst and increasing anorexia.

On May 19 (day 49), skunk #1 escaped from her pen with her offspring. She and seven infants were recaptured

and returned to the pen; the eighth infant was not found. During the next 2 days the dam abandoned her young and fled her pen three more times, entering the pen of skunk #3 on two occasions. Skunk #3, which had no young, left her pen and no evidence of fighting between the two animals was observed. Both skunks were returned to their own pens.

Skunk #1 entered the pen of skunk #2 and her litter of five on May 21 (day 51). The two adults fought but the young of skunk #2, in their nesting box, were not contacted by the intruder. Skunk #1 voluntarily returned to her own pen where she was found dead on the following day. A weeping puncture wound was observed on the right side of the dead animal's muzzle. A laboratory diagnosis of rabies was made by fluorescent antibody staining of impression smears of brain stem, hippocampus and cerebellum, but viral antigen was observed only in the cerebellum. Rabies was confirmed by mouse inoculation.

Prior to a laboratory diagnosis of rabies in skunk #1, surviving abandoned offspring were toe-clipped for identification and placed with skunks #5 and 6 as foster mothers. Three were adopted by skunk #5 and two by skunk #6. The young were left in these pens after their dam was identified as rabid in order to obtain additional information on the epidemiology of rabies. Two infants had died of maternal neglect and inability to adapt to hand-raising before the decision was made to attempt adoption by other lactating skunks. No wounds could be detected on the carcasses of these young and no rabies antigen was detected by FA staining of brain impression smears.

On June 12, 22 days after fighting with skunk #1, skunk #2 showed a slight limp. Examination of the animal revealed a slightly hyperemic left hind foot pad and a sub-cutaneous abscess near the left eye. The latter yielded, upon culture, *Streptococcus equisimilis*. With the exception of a severe thirst, there were no other signs of illness. Several hours later the animal appeared somewhat uncoordinated and restless but made no effort to attack humans or her young. She was found dead in her pen on the following

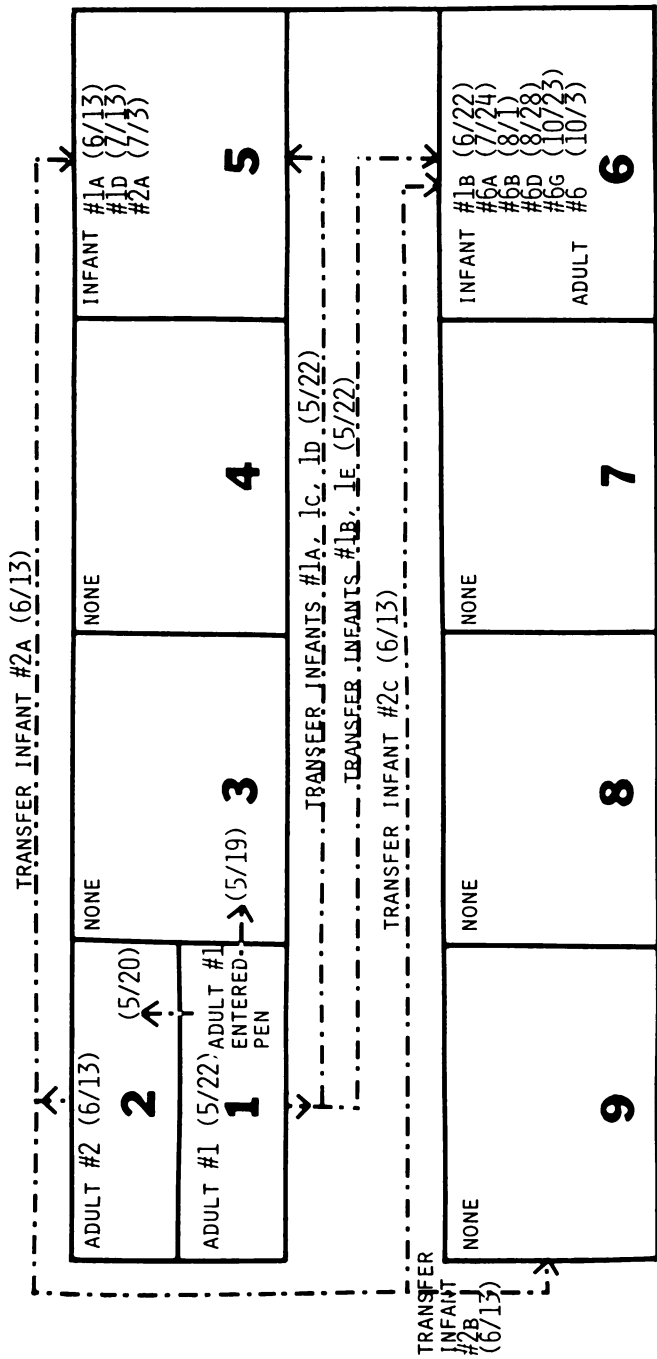


FIGURE 1. Pen arrangement of skunks in the study. Broken lines indicate movement of animals. Animal identifications in upper left corner of each pen are rabid skunks, with date of death from the disease in parenthesis.

morning. Rabies was diagnosed by FA, staining of brain impression smears.

Young of skunk #2 were marked with blue dye for identification and transferred, one each, to pens #5, 6 and 9. Two offspring in this litter had died approximately 2 weeks earlier as a consequence of accidental injury during care of the animals.

The progress of the epidemic of rabies is summarized in table 1. Skunks originally in pens 3, 4, 5, 7, 8 and 9 did not develop the disease although two offspring of skunk #1, after being transferred to pen 5, became rabid; offspring of skunk #2 were fostered by skunks #5 and 9; and skunk #1 herself, during her illness, entered the pen of skunk #3.

Serologic titrations by PHA and PR procedures showed no development of rabies antibodies by any of the skunks that were not rabid. Sera from animals showing clinical signs of rabies, drawn shortly before death, had titers from completely negative to greater than 1:5000. The animal with the highest titer was partially paralyzed in the rear quarters for 16 days prior to death—she was euthanized in terminal stages of rabies after having shown promise of recovery. Non-rabid adults maintained the titers they had at the time of capture. Skunks #1 and 9 had PHA and PR titers of 1:4 at capture and skunk #2 had no detectable rabies specific antibody at that time.

With the exception of the first skunk, with which fluorescence was observed only in the cerebellum and was very limited, very extensive and intensive reactions were obtained by FA staining of brain impression smears. Most salivary glands that were tested also showed much specific fluorescence. Numerous virions were also observed by electron microscopic examination of fixed brain tissue.

#### DISCUSSION

The epidemic of rabies in the skunk colony spread slowly although animals were in intimate contact with each other. Generally a rabid skunk infected only a single animal, if any at all. One juvenile,

1b, probably infected two pen mates; skunk #1, with a clinical illness of 5 days characterized by wandering tendencies, transmitted virus to three of her five offspring as well as to another adult.

Transmission of rabies virus may have been by bite in all cases although wounds were generally not observed and scars were not detected on pelts. Transmission via the milk, from skunk #1 to three of her five offspring and from skunk #2 to one of her three offspring, cannot be ruled out. Similarly aerosol transmission, especially within nesting boxes, or transmission of virus via saliva shed onto shared food cannot be discounted. There was no evidence for *in utero* transfer of virus from skunk #1 to fetuses. Her litter size was normal, offspring did not shed virus in absence of clinical signs of disease and the young were susceptible to rabies at a later challenge. However, when compared with the other young skunks, the infants were very small at birth and matured more slowly.

In biting incidents it is believed, on the basis of observations of the behavior of the animals, that the rabid animal was rarely the aggressor. Only skunk #6 showed signs of furious rabies, attacking pen mates without provocation, but even this animal made no effort to pursue victims if they attempted to escape.

Most of the rabid animals died without showing any clinical signs of illness. One skunk (6d) was partially paralyzed for 16 days and appeared to be recovering when she suddenly became very weak. When she died, during bleeding by cardiac puncture, her serum titer for rabies antibodies was greater than 1:5000 by both procedures used for testing. The primary case in the infection (#1) showed wandering tendencies for 3 days prior to her death, but these had initially been attributed to an effort on her part to remove her young from frequent observations by curious humans. At no time did she become vicious towards humans nor was she more docile than originally. It is believed that she fought with skunk #2 only when the latter tried to drive the intruder away from her young. Similar defensive acts were

TABLE 1. Progress of epidemic of rabies within the skunk colony.

Animal #	Probable Source of Infection	Incubation Period	Duration of Illness	Clinical Signs of Illness Suggestive of Rabies	Rabies Diagnosis (FA)
1	Primary Case	>6 weeks	May 17-22	Tendency to wander, severe thirst, anorexia, facial edema.	+
2a,b	—	—	—	None (accidental traumatic injury).	—
2	1	22 days	June 12-13	Severe thirst, slight limp, slight incoordination; sub-cutaneous abscess near left eye, slightly hyperemic left hind foot pad.	+
1a (pen 5) <sup>□</sup>	1	32 days	June 21-22	Slight weakness in rear legs one evening; found dead next morning.	+
1b (pen 6)	1	32 days	June 22	None.	+
1c (pen 6)	—	—	July 1	None (multifocal granulomatous hepatitis).	—
2c (pen 5)	2 (1a) <sup>□</sup>	20 days (11 days)	July 3	None.	+
1d (pen 5)	1 (1a)	41 days (11 days)	July 3	None.	+
2d (pen 9)	—	—	July 19	None (heart damage during bleeding via cardiac puncture).	—
6a	1b	32 days	July 24	None.	+
2e (pen 6)	—	—	August 1	None (suppurative peritonitis due to <i>Proteus</i> ).	—
6b	1b	40 days	August 1	None.	+
6c	—	—	August 7	Limping gait due to swollen, tender feet; small hard nodule on skull muscle.	—

TABLE 1 — Continued

Animal #	Probable Source of Infection	Incubation Period	Duration of Illness	Clinical Signs of Illness Suggestive of Rabies	Rabies Diagnosis (FA)
5a	—	—	August 15-18	Facial edema, protuberant eyes, blind for approximately 6 weeks. Elevated temperature.	—
6d	6a	19 days	August 12-28	Severe weakness in hind legs, tail in stiffly erect position; would eat only soft foods, became emaciated. No personality change.	+
1e (pen 5)	—	—	September 11	None (chronic prolapse; peritonitis).	—
6e	—	—	September 15	None (sub-acute purulent bronchio-pneumonia).	—
6	6d, (6a,b)	36 days (71, 63 days)	October 1-3	Periodic severe agitation during which it would attack other skunks but not pursue fleeing victims. Lost eye in fighting, profuse nasal hemorrhage.	+
6f	—	—	October 10	Massive abscesses on left front shoulder and right rear abdomen; blood clot in left neck. (Staphylococcal septicemia).	—
6g	6	20 days	October 21-23	Very nervous, paced with very "jerky" movement (also for 2 days, 1 week earlier), very docile in interim; ate little since October 1-2 fights with skunk #6.	+
9a	—	—	November 26	None (respiratory infection).	—
3; 5; 5b 9; 9b, c, d, e, f, g	—	—	December 30	None (euthanized).	—

1 Pen numbers to which infant skunk was transferred.

2 Other possible sources of infection.

observed when any adult skunk was put into a pen with a mother and offspring. Young animals were readily adopted into new families.

The juvenile skunks were not descended but only on three occasions during the 8 months did they scent. None of these was associated with attacks by rabid animals.

The probable incubation period for rabies virus in the animal colony ranged from 19 to 41 days with a mode of 32 days. The skunk that introduced the virus harbored it for at least 7 weeks before showing signs of infection. It is possible that the animals in captivity suffered more severe exposures due to their confinement in a restricted area. Very extensive fluorescent antibody staining of brain impression smears indicated the presence of much viral antigen.

There was a low rate of transmission of rabies between animals in very close contact, most of the rabid skunks were not aggressive and the duration of illness of the animals in this epidemic was short. In view of this it is remarkable, if this epidemic was indeed characteristic of rabies in free-living skunks, that the disease remains such a serious problem among skunks in this country. On the other hand, the nature of the illness of the animals in this epidemic would indicate that the incidence of rabies in skunks likely is much greater than statistics suggest, as the majority of the rabid animals likely are not identified but rather die, undetected, in their dens. The infrequent occurrence of recaptures of skunks from one year to the next<sup>4</sup> would suggest that the mortality rate for skunks in the area is high; the contribution of rabies to this rate can only be speculative but possibly is significant.

#### Acknowledgements

We gratefully acknowledge the assistance of Dr. David L. Graham in the post-mortem examination of many of the animals in this study and that of Ruth Hopper and Carmen Ellis for serologic titrations of rabies antibodies.

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Received for publication 8 May 1974