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Rabies Detection in Road-killed Skunks *(Mephitis mephitis)*

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Abstract

Three diagnostic techniques (1) microscopic examination for Negri bodies, (2) mouse inoculation and (3) fluorescent antibody tagging were compared as to their ability to detect rabies virus in 14 experimentally infected striped skunks *(Mephitis mephitis)* exposed to normal summer weather conditions after death. The fluorescent antibody technique correctly identified rabies virus longer than either of the other methods. Rabies incidence in 61 road-killed skunks collected on the day following death in southeastern North Dakota was 26 per cent.

Introduction

The striped skunk is a frequent mammalian highway casualty in North Dakota. Howell[1] attributed the high frequency of striped skunk road-kills to the animal's apparent confusion when caught in the headlights of an automobile. Verts,[6] however, attributed the disproportionate number of road-kills to the speed of highway traffic and the fact that skunks habitually stand their ground when being pursued and overtaken.

Knowledge of skunk rabies incidence usually is limited to reported cases involving contact with humans or domestic animals. It is unlikely that such data reflect true incidence levels. A method of monitoring the true incidence of rabies on a regional level would be of public health significance. Such a method must permit specimen collection from a large area with minimal time and effort. If road-killed skunks represent an unbiased sample of rabies incidence in a population, they may provide a comparatively inexpensive source of specimens.

A two-year study was initiated in 1968 on an 11,000 square mile study area in southeastern North Dakota to determine the potential value of road-killed striped skunks in monitoring rabies incidence. Road-killed skunks were collected from the study area and matched temporally and spatially with a trapped sample. Damage due to mechanical injury, and decomposition enhanced by high summer temperatures, not only prevented the collection of many road-killed skunks but also cast doubt on the validity of diagnoses obtained from the collected carcasses. To evaluate the ability of standard diagnostic tests to detect rabies under these normal, yet sub-optimal, conditions we initiated a series of exposure tests in the summer of 1969.

This paper reports the results of the exposure tests and estimates rabies incidence in road-killed skunks in light of these results.

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Materials and Methods

Eighteen descented captive skunks were injected intramuscularly with a 10 per cent mouse brain suspension of live rabies virus. The skunks were injected in groups of six at two-week intervals. The animals were maintained in the laboratory until advanced stages of rabies were apparent, then sacrificed with an overdose of pentobarbital sodium. An incision was made in the skin at the base of the neck; pulling this skin flap forward exposed the skull. The top of the skull was removed and a brain sample collected. The skin flap was returned to its original position and the incision swabbed with an insect repellent salve (Myzin Smear). The carcass was then exposed to the elements on a simulated highway surface. All animals were sacrificed in early evening to approximate field conditions. Brain samples were collected three times each day at 0800, 1400 and 2000 hours; sampling ceased when the brain was completely decomposed. A record of temperatures during the trials was kept by means of a recording thermograph.

All samples were tested by three diagnostic techniques: (a) microscopic examination for Negri bodies using Sellers' stain,[5] (b) intracerebral mouse inoculation,[2] and (c) fluorescent antibody tagging[3] (FA).

Results

Two of the 18 skunks injected with rabies virus failed to develop symptoms and remain under observation. Two of the 16 skunks used in the trials were negative for all tests and have been excluded from further consideration. For the 14 remaining skunks, five separate trials were required due to variation in incubation periods and infection dates. The mean daily temperature during the trials varied from 52 to 85 F. The results of these trials (Fig. 1) may be summarized as follows:

All 14 animals were FA positive after 24 hours exposure. After 36 hours 11 animals were FA positive and six of these were still positive after 42 hours. No FA positives were recorded beyond 42 hours of exposure.

Seven of 14 skunks were Sellers' positive after 24 hours; four were Sellers' positive after 36 hours; two after 42 hours; and one after 69 hours. Positive tests in excess of 36 hours exposure resulted from trials run when the mean daily temperature was 52 F. No Negri bodies were recorded beyond 20 hours exposure when mean daily temperatures exceeded 80 F.

The mouse inoculation results were similar to those for Negri bodies. Only at low temperatures (mean temperature 52 F.) were positive tests obtained in excess of 36 hours. Little difference was noted between the tests run at 55, 66, 80 and 85 F.

Discussion

Data from Negri body and mouse inoculation tests indicated a relationship between mean daily temperatures and positive diagnoses; no such relationship was apparent for FA tests (Fig. 1).

Since the time between samples was not constant and since the various methods reflect different portions of the daily temperature regime, numerical comparison of the raw data was not justified. To permit comparison, elapsed time from death to the last positive test for each sample was grouped into 12 hour periods from which mean values (Fig. 1) were calculated. The effectiveness of each of the three techniques was evaluated by comparing the 24 hour period in which final positive diagnosis occurred. The ability of the three techniques to detect rabies virus on the second day of exposure was significantly different ($P<0.005$,

$X^2 = 13.24$, d.F. $= 2$); FA demonstrating rabies after the other two tests were no longer effective. Furthermore, FA is the preferred technique when exposure to temperature greater than 60 F. is encountered (Fig. 1). It follows that FA is recommended in all cases when specimen condition is questionable.

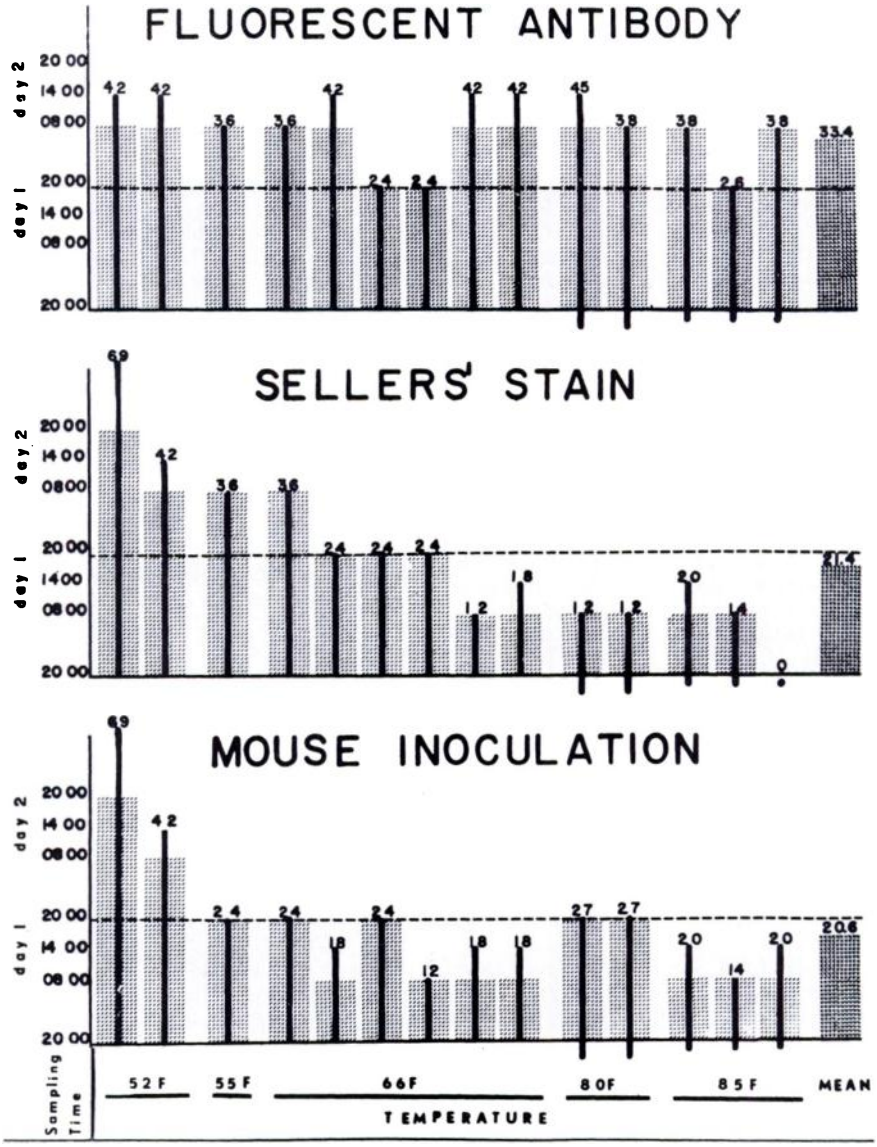

FIGURE 1. *Time after death, in hours to the last positive rabies diagnosis for 14 rabid skunks. Temperatures are mean values for the first 24 hours after death. Bars beginning below 2000 hours indicate starting times of 1700 and 1800 hours.*

Application of Results

Road-killed skunks were subjectively classified into one of four exposure categories: 0-12 hours, 12-24 hours, 24-48 hours, and over 48 hours. On the basis of the controlled exposure tests, only road-killed skunks collected within 24 hours of death can be relied upon to give accurate results. We have, however, examined a number of skunks known to have been dead in excess of 48 hours, which tested positive for rabies by all of the diagnostic methods. In all such cases, the temperature at the time of exposure was either below 50 F. or the animals were protected from direct exposure to the sun.

For the purpose of evaluating rabies incidence in road-killed skunks, we rejected all carcasses which, at the time of collection, were judged to have been more than 24 hours old unless mean daily temperatures were below 50 F.

Rabies diagnostic tests for road-killed skunks collected in 1968 and 1969 (Table 1) demonstrated that the rejection of road-kills after the first day of exposure was reasonable. This restriction resulted in the exclusion of 14 carcasses over the two years. None of the rejections, however, had tested positive for rabies.

We believe that estimates (Table 1) of rabies incidence which exclude samples exposed greater than 24 hours while increasing the incidence rate (26 versus 21 per cent) also increase the accuracy of the incidence determination.

Although such estimates are relatively high, we have obtained estimates of a 15-20 per cent incidence rate from other population samples drawn from this region. Our interpretation of these incidence levels will be presented in a future paper.

TABLE 1. *Rabies incidence levels in samples of road-killed skunks, from southeastern North Dakota.*

	Total Collected Carcasses		Carcasses less than one day old	
	Number	% Infected	Number	% Infected
1968	24	21	22	23
1969	51	22	39	28
Total	75	21	61	26

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