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DEMONSTRATION OF RABIES VIRUS-SPECIFIC ANTIBODY IN THE SERA OF FREE-RANGING IOWA RACCOONS (*PROCYON LOTOR*)

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ABSTRACT: Between 1984 and 1988, a study was conducted to evaluate the frequency of rabies virus neutralizing antibodies in raccoons (*Procyon lotor*) in two counties in Iowa. Nine hundred eighty five raccoons were trapped and tagged in Guthrie and Cerro Gordo counties during the spring, summer and fall of each year. Sex, age and weight were recorded for each animal and a blood sample was collected. Serum samples were tested for the presence of serum neutralizing antibodies (SNA) by the rapid fluorescent focus inhibition test (RFFIT), mouse serum neutralization test (MSN), and an indirect fluorescent antibody (IFA) technique for detecting immunoglobulin G. Fifty-one raccoons (5%) were found to have SNA by the RFFIT. Thirty-six serum samples (24 with RFFIT antibody titer >3.0, and 12 <3.0) were also tested by the MSN, with results correlating well with the RFFIT results ($r = 0.86$, $P < 0.01$, Kappa = 0.93). In 35 raccoons with SNA by the RFFIT, six individuals had immunoglobulin G binding activity by the IFA test. These results provided serologic evidence of exposure of raccoons to rabies virus in an area free of enzootic raccoon rabies.

Key words: Rabies virus, raccoons, *Procyon lotor*, serologic survey, prevalence, epidemiology, field study.

INTRODUCTION

Since 1982, two epizootics of raccoon rabies in the southeastern and mid-Atlantic areas of the United States have made the raccoon the second most commonly reported rabid wildlife species (Baer et al., 1990). Reported cases of raccoon rabies in the United States increased from 62 cases in 1962 to 1,820 cases in 1983 (Centers for Disease Control, 1984; National Communicable Disease Center, 1964). Although the number of reported cases dropped to 1,463 in 1988 (Centers for Disease Control, 1989), areas considered to be enzootic for raccoon rabies have continued to enlarge outwardly from initial foci of infection (Jenkins et al., 1988). Presently, the majority of rabies cases in raccoons in the United States is limited to these two enzootic areas. In 1988, raccoons from these areas accounted for 99% of all cases of raccoon rabies in the United States (Centers for Disease Control, 1989). Outside of raccoon rabies enzootic areas, raccoons are believed to be alternate hosts and not involved in the transmission and maintenance of the disease in wildlife (Smith et al., 1986).

Rabies has only been sporadically reported in raccoons in Iowa. Only 70 confirmed cases have been reported since 1950 (Iowa Department of Public Health, 1951–89). In contrast, a serologic survey in Iowa in 1971 and 1972 indicated that 7.6% of the raccoons had serum neutralizing antibodies (SNA) (Niemeyer, 1973). The presence of SNA in raccoon populations in other areas of the country free of enzootic raccoon rabies has also been reported (McLean, 1975). In addition, it has been suggested that nonspecific virus-neutralizing factors in raccoon sera with low levels of SNA may confuse interpretation (Barton and Campbell, 1988). Jenkins et al. (1988) suggested that only SN antibody titers ≥25.0 were considered significant. These reports raised questions about the nature of infection, and the interpretation of SN antibody titers in raccoons in areas without the enzootic form. The purpose of this study was to investigate the prevalence of virus-specific antibody, and associated host

and seasonal factors in free-ranging raccoons in two counties in Iowa. The results of three different antibody tests were compared.

MATERIALS AND METHODS

Raccoon samples

Raccoons from Guthrie County (41°36′ to 41°39′N, 94°19′ to 94°28′W) were trapped over the five year period of 1984-1988 as part of a long term study of raccoon population dynamics (Hasbrouck, 1990). Trapping occurred in two 10 wk periods each year starting in March and August. Raccoons were captured using Tomahawk (Tomahawk, Wisconsin 54487, USA) live traps, and were immobilized with an intramuscular injection of approximately 20 mg/kg ketamine hydrochloride (Ketaset, Bristol Laboratories, Syracuse, New York 13220, USA). Blood samples were collected by cardiac puncture and the animal's sex, weight and estimated age were recorded. Definitive ages were determined by tooth extraction, sectioning and cementum annuli analysis (Klevezal and Kleinenberg, 1967). Raccoons from Cerro Gordo County (43°07′N, 93°27′W) were trapped over a 5 mo period (March to July) during each of the years 1984 to 1988 by the Iowa Department of Natural Resources as part of a long term study of the epidemiology and transmission of rabies in skunks and raccoons. Samples and data similar to that for Guthrie County were collected. Serum samples were stored frozen (−20 C) until tested. All sera were heat inactivated (56 C for 30 min) and kaolin treated with an equal volume of a 25% kaolin preparation for 30 min to absorb non-specific inhibitors (Smith et al., 1973).

Serum neutralizing antibody titer determinations

Sera were analyzed by the rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1973) and the mouse serum neutralization (MSN) test (Atanasiu, 1973) for the presence of antibodies against rabies virus. Serial two-fold dilutions were made and titers were expressed as reciprocals of the highest dilution capable of reducing the number of rabies infected baby hamster kidney (BHK) cells by 50%, or producing 50% mortality in mice as determined by the Reed-Muench method (Reed and Muench, 1938). Test controls for the RFFIT included uninfected cell controls, back titration of rabies virus challenge dose, negative antirabies sera and titration of positive raccoon antirabies sera. Sera were tested in BHK-21(C-13) cells (Cells originated from the Centers for Disease Control and were supplied by the National Veterinary Services Laboratories, Ames, Iowa 50013, USA) using Challenge Virus Standard (CVS-11) virus (Rabies virus, Centers for Disease Control, Atlanta, Georgia 30333, USA). Test controls for the MSN test included positive and negative raccoon antirabies sera and 25 to 50 mouse intracerebral lethal dose$_{50}$ (MICLD$_{50}$) of CVS rabies virus (Rabies virus, National Veterinary Services Laboratories, Ames, Iowa 50013, USA). Five 13 to 15 g female mice (Sprague Dawley CF-1 mice, Harlan Sprague Dawley, Inc., Indianapolis, Indiana 46229, USA) per test serum dilution were used. Ten mice per dilution were used for the challenge titration.

Indirect fluorescent antibody determination

An indirect fluorescent antibody (IFA) test was performed using an adaptation of a previously described test (Johnson and Emmons, 1980). One-tenth milliliter of a dilution of Street Alabama Dufferin (SAD) virus (Rabies virus, National Veterinary Services Laboratories, Ames, Iowa 50013, USA) was added to 0.4 milliliters of a cell suspension of Madin Darby canine kidney (MDCK) cells (Cells originated from the ATCC and were supplied by the National Veterinary Services Laboratories, Ames, Iowa 50013, USA) in eight chamber Lab-Tek cell culture slides (Miles Scientific, Division of Miles Laboratories, Inc., Naperville, Illinois 60566, USA). Slides were incubated (37 C) in a humid chamber with 3–5% CO_2. After 72 hr, the supernatant was removed and the cells fixed with cold acetone (4 C) for 10 min, air dried and stored frozen until use. The cell sheet consisted of approximately 30% rabies infected cells. Following a phosphate buffered saline (PBS) rinse, 50 microliters of dilutions of raccoon sera were added to each chamber and incubated (37 C) for 30 min in a humid chamber. Slides were washed for 10 min in PBS. Each well was filled with 50 microliters of fluorescein isothiocyanate conjugated goat origin antiraccoon immunoglobulin G (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Maryland 20879, USA) and incubated (37 C) for 30 min. Positive cell control wells were incubated with fluorescein isothiocyanate conjugated equine or bovine origin antirabies globulin (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, Maryland 21030, USA). Following a 10 min wash in PBS, the slides were covered with a 50/50 (V/V) glycerine-saline solution, overlaid with coverslips and examined with a fluorescent microscope. Titers were expressed as the reciprocal of the highest serum dilution still exhibiting specific fluorescence. Positive reaction was determined by the observation of fluorescing irregular intracytoplasmic inclusions. Test controls included uninfected and infected cell controls,

negative antirabies sera from farm-raised raccoons (RFFIT antibody titer <3.0), and positive antirabies sera (RFFIT antibody titer of 8.0 and 160.0) from raccoons vaccinated with a killed virus vaccine (Rabguard-TC, Norden Laboratories, Lincoln, Nebraska 68501, USA). Optimal dilutions of the reagents were previously determined by checkerboard titration.

Statewide rabies surveillance

A retrospective analysis of confirmed cases of rabies in Iowa was performed using annual rabies surveillance reports for the years 1950 to 1988 (Iowa Department of Public Health, 1951–1989). Rabies cases described in these reports were diagnosed by fluorescent antibody staining of acetone-fixed impression smears of brain tissue (Dean and Abelseth, 1973), mouse inoculation (Koprowski, 1973), or the Sellers' technique (Sellers, 1927) at the Iowa State Veterinary Diagnostic Laboratory and Hygienic Laboratory.

Analysis of data

Data were categorized to study the relationship between factors such as season, sex, weight and age on presence of SNA in the population. Statistical analyses were performed by comparison of proportions of two independent samples or the Chi-Square test (Snedecor and Cochran, 1989).

RESULTS

Raccoon samples

Nine hundred fifty-one blood samples were collected from 891 wild-trapped Guthrie County raccoons. All animals appeared clinically normal when trapped. Fifty-two animals were sampled more than once, eight of which were re-trapped in the same season of the same year. The number of samples collected each year were 181 (1984), 212 (1985), 192 (1986), 207 (1987), and 159 (1988). Sample size on a yearly basis was statistically balanced ($P < 0.05$). The majority (625 (66%)) of the samples were collected during the summer/fall trapping period. Distribution of samples by age group was similar; 504 (53%) were from adults and 447 (47%) from juveniles less than 1-yr-old. The majority (433 (97%)) of the juveniles were collected during the second trapping. The sex distribution of animals trapped over the 5 yr period was 516 (54%) male and 435 (46%) female. A larger percentage of the males (38%) was trapped during the spring than females (30%). The distribution of age groups within sexes was similar.

Ninety-seven blood samples were collected from 94 wild-trapped Cerro Gordo County raccoons. All animals appeared clinically normal when trapped. Three raccoons were sampled twice. Sample size on a yearly basis ranged from a low of 12 in 1984 to 28 in 1985. The largest number of collections was in May and June with 31 (32%) and 24 (25%) of the samples collected during these months. All of the raccoons sampled were adults and 67 (69%) were male.

Serum neutralizing antibody analyses

A total of 1,048 serum samples from 985 wild-trapped raccoons were tested by the RFFIT test for SNA. Fifty-one of 985 raccoons (5%) had SN antibody titers >3.0; antibody titers ranged from 3.2 to 24.2. Thirty-six samples (24 with RFFIT antibody titer >3.0, and 12 <3.0) were subjected to the MSN test and correlation analysis. An insufficient amount of sera prevented all of those with RFFIT antibody titers >3.0 from being tested by the MSN test. Twenty-three of the 24 RFFIT positive samples were positive by the MSN test with antibody titers ranging from 3.2 to 17.9. The RFFIT test identified one more as positive than the MSN test (RFFIT antibody titer 4.8). Results from the two tests correlated well ($r = 0.86$, $P < 0.01$, Kappa = 0.93). Regression analysis showed that overall the RFFIT antibody titers were 1.28 times higher that the MSN antibody titers.

Forty-eight of 891 (5%) Guthrie County raccoons had SN antibody titer >3.0 by the RFFIT. Of the 52 animals which were captured and sampled more than once, 47 animals had no measurable SNA, four animals had SNA in the final sample, and one had a measurable antibody titer on two trapping dates (6/10/87 and 9/27/87). Seroprevalence rates varied among the years, seasons, sexes and age groups. Rates by year were 4% in 1984, 2% in 1985, 2%

TABLE 1. Percentage of Guthrie County raccoons with serum neutralization antibody titers >3.0 as determined by the rapid fluorescent focus inhibition test (RFFIT) for the years 1984–88.

Trapping period[a]	Adult		Juvenile		Total
	Male	Female	Male	Female	
March–July	9.8 (18/183)[b]	7.1 (9/127)	0.0 (0/11)	0.0 (0/3)	8.3 (27/324)[c]
August–October	3.8 (4/103)	9.3 (8/86)	2.8 (6/217)	1.9 (4/213)	3.6 (22/619)
Total by sex	7.7 (22/286)	8.0 (17/213)	2.6 (6/228)	1.9 (4/216)	
Total by age group	7.8 (39/499)[c]		2.3(10/444)		

[a] Eight animals trapped more than once in the same season are not included in these data.
[b] Number of animals in each category shown in parentheses.
[c] Significantly different ($P < 0.01$) from corresponding trapping or age group category.

in 1986, 10% in 1987 and 7% in 1988. The rate in 1987 was significantly higher ($P < 0.01$) than the previous years. The seroprevalence rates for 1987 and 1988 were not significantly different ($P < 0.05$). On a seasonal basis, significantly more ($P < 0.01$) of the samples from the spring trapping period had SNA (8%) than from the fall trapping period (4%) (Table 1). The rate of SNA among adults (8%) was significantly higher ($P < 0.01$) than among juveniles (2%). Seropositive adults ranged in age from 1 to 5 yr. No differences in seroprevalence rates were observed among chronologic age categories for adult animals; rates by chronologic age were 8% (1-yr-old), 10% (2-yr-old), 5% (3-yr-old), 10% (4-yr-old), 9% (5-yr-old). The seropositivity rate for adult animals of unknown age was 5%. Seropositivity rates were similar among males and females (5%). When comparing rates among season and sex groups for adults, adult males trapped during the spring/early summer trapping showed the highest rate (10%), with adult males sampled during the late summer/fall showing the lowest rate (4%). Seasonal rates among adults were not significantly different. All seropositive juveniles were found during the fall; however, the spring sample size was very small.

Ninety-one raccoons were trapped in Cerro Gordo County. Three were captured and sampled twice. Three of the samples had SN antibody titer >3.0 by the RFFIT, representing an overall prevalence of 3%. The three samples with SNA came from two adult males trapped in June 1988 and from one adult female trapped in April 1986. The overall seroprevalence rate for Cerro Gordo County was not significantly different ($P < 0.01$) than the rate for Guthrie County.

Indirect fluorescent antibody analysis

Sera from 81 raccoons from both counties were tested by the IFA test (35 with RFFIT antibody titer >3.0, and 46 <3.0). An insufficient amount of sera prevented all of those with RFFIT antibody titer from being tested by the IFA test. Among the sera tested, six animals with RFFIT antibody titer >3.0 were positive for antirabies immunoglobulin G by the IFA test at the 1:10 dilution and three of these at the 1:25 dilution. All of the positive samples were from adult raccoons in Guthrie County. Sex, seasonal distribution, and RFFIT and MSN antibody titers of those animals positive by the IFA test are shown in Table 2. None of the sera with RFFIT antibody titer <3.0 were positive by the IFA test.

Retrospective analysis of confirmed rabies cases in Iowa

In Iowa, there were 939 confirmed cases of animal rabies during the period from 1984 to 1988 from a total of 9,388 animals of all species submitted for testing. Numbers of cases (cases/total tested) on a yearly basis were 150/1,849 (1984), 150/1,886

TABLE 2. Guthrie County raccoons with rabies-specific immunoglobulin G as detected by an indirect fluorescent antibody (IFA) technique.

Trapping date	Sex	Age in years	Weight in kg	RFFIT[a] titer	MSN[b] titer	IFA[c]
08/18/84	M	1	6.8	4.3	ND[d]	Positive
05/30/87	M	1	5.9	10.8	5.3	Positive
06/07/87	M	3	7.2	5.3	5.2	Positive
08/31/87	F	1	5.4	13.3	6.0	Positive
09/10/87	F	4	6.8	6.4	ND	Positive
05/07/88	M	2	6.1	17.6	ND	Positive

[a] Serum neutralizing antibody titer as determined by the rapid fluorescent focus inhibition test.
[b] Serum neutralizing antibody titer as determined by the mouse serum neutralization test.
[c] Indirect fluorescent antibody test results at serum dilution of 1:10.
[d] ND = Not done.

(1985), 192/1,944 (1986), 272/1,978 (1987), and 175/11,731 (1988). In 1987, the number of cases was significantly higher ($P < 0.01$) than in previous years. The striped skunk (*Mephitis/mephitis*), the principal rabies reservoir in Iowa, accounted for 542 (58%) of the total number of cases and 98% of the reported cases in terrestrial wildlife between 1984 and 1988. Of all skunks submitted for testing, there was an overall test-positive rate of 79% which was significantly higher ($P < 0.01$) than for all other species. Domestic animals accounted for 335 (36%) of the cases, bats accounted for 48 (5%), and other wildlife species accounted for 12 (2%).

In Guthrie County, 115 animals of all species were tested during the study period and 8 (7%) were found positive. In Cerro Gordo County, 150 animals were tested with 15 (10%) positive. The peak year for laboratory confirmed rabies in both counties was also 1987. Fluctuations in the year by year number of test-positive animals for these two counties paralleled changes in the statewide number of test-positive animals.

Two (0.3%) rabies cases in raccoons were reported in the state between 1984 and 1988, from a total of 737 raccoons submitted for testing. In each year except 1987, more raccoons were submitted for testing than skunks. No confirmed cases of rabies in raccoons were reported from either of the two counties involved in the study during the sampling period.

DISCUSSION

In this study, three different tests were used to assess the presence of rabies antibodies in raccoons in an area free of enzootic raccoon rabies. Positive results from all three tests demonstrated the presence of virus-specific antibodies in the sera of free-ranging raccoons.

The RFFIT and MSN tests are based on the measurement of the ability of factors in the serum to inhibit the growth of challenge virus in cell cultures as detected by fluorescent antibody staining or the pathogenesis of the virus in mice. The mouse test has been the accepted standard for comparing other antibody detection tests (Smith, 1991). The neutralizing ability of serum samples, as measured by the RFFIT and MSN test, is due to the presence of virus-specific neutralizing antibodies directed against the surface glycoprotein (Cox, 1982; Nathanson and Gonzalez-Scarano, 1991). Either through natural exposure or vaccination, the surface glycoprotein of the virus is responsible for induction of virus-neutralizing antibodies (Tordo and Poch, 1988). There is little evidence of false positive reactions with the MSN test. There have been reports of false positive levels of 0.8% in studies with prevaccination and post-vaccination sera from people (Larsh, 1965; Thomas et al., 1963). Considering the past performance of the MSN test, the most likely conclusion is that the SN antibody titers observed in this study

represent specific virus neutralizing antibodies.

The third test used to assess the presence of rabies antibodies was an IFA technique to detect immunoglobulin G. The number of positive samples by the IFA was too small to allow for detailed analysis of prevalence rates among different years, seasons, sexes, or age groups. Four of the six positive samples were from raccoons trapped in 1987, the peak year for rabies in each county and the state. No animals which were positive by the IFA test were negative by the RFFIT or MSN test.

There are several factors which may account for more animals positive by the neutralization tests than the IFA test. The IFA test measures antibody binding rather than specific virus neutralizing antibody. The neutralization tests and IFA test do not measure identical spectrums of antibodies; the binding in the IFA test is with the nucleocapsid proteins rather than with glycoprotein as in the neutralization tests (Campbell and Barton, 1988; Schneider et al., 1973). Discrepancies between fluorescent antibody based tests and other antigen-antibody binding tests in sera with low SN antibody titers have been reported (Barton and Campbell, 1988). Inconsistent results or poor correlation of the IFA test with the MSN test have been reported (Grandien, 1977; Grandien and Espmark, 1974; Thomas et al., 1963), as have false negative rates of 14.3% in sera with low level SN antibody titers (Peck, 1966). In contrast, 100% accord between the IFA and the RFFIT test in recognizing positive and negative reactors has been reported when using sera from raccoons with known exposure to rabies antigen (Hill, 1990). Correlation analysis showed the tests to be well correlated when the IFA test was read at the 1:10 dilution ($r = 0.76$, $P < 0.01$, $n = 31$). The detection of antibodies to both internal nucleocapsid proteins (IFA test), and surface glycoprotein (SN antibody tests), demonstrated that the wild-trapped raccoons in this study had been exposed to rabies antigen.

RFFIT seropositivity rates for raccoons with SNA in this sample population varied among age groups, season and year. Finding significantly more seropositive adults than juveniles is consistent with length of time in the population being correlated with risk of exposure. Although the study design restricted collection of samples to limited times of the year, the significant decrease in seroprevalence rate from the spring to the fall trapping coincided with the large number of seronegative juveniles entering the population during the second trapping period. In raccoon rabies enzootic areas it is believed that rises in prevalence of SNA in the spring are associated with animal contact during breeding activities and increased movements and activity patterns (Bigler et al., 1973).

The significant rise in the seropositivity rate in raccoons in 1987 correlated with the increase in the number of confirmed laboratory cases in the skunk in the state and in each study county. Although the rate of confirmed positive skunk cases reported in the state in 1988 dropped significantly from 1987, the seropositivity rate for raccoons in 1988 was similar to the seropositivity rate in 1987. Seropositive raccoons in 1988 were equally balanced between the sexes, but more adults were positive. Persistence of SNA in raccoons exposed the previous year may have accounted for the seropositivity rate remaining high. The one raccoon that was retrapped and remained positive 109 days after the first positive sample also provided evidence for the extended survival of raccoons with SNA in the wild. Previous studies have shown that re-trapped wild raccoons in raccoon rabies enzootic areas maintained SNA for 37 mo (Bigler et al., 1983). Since these were animals living in the wild, the persistence of antibody could have been due to re-exposure to the virus.

Conclusions about the prevalence rate of SNA in this study come mainly from the Guthrie County data because of the larger sample size. However, because seroprevalence rates between counties were not

significantly different the results could be extrapolated to Cerro Gordo County. We must be cautious however in interpreting too much from a population with such a low percentage of animals with SNA. It is uncertain if naturally occurring rabies-specific SNA provide protection from lethal challenge. The true reason for the low prevalence of animals with SNA has yet to be determined.

The retrospective analysis of the specimens submitted to state testing laboratories for rabies examination helped confirm the role of the skunk as primary reservoir host in this state. The rate of rabies positive skunks per number of samples submitted was significantly higher ($P < 0.01$) than for any other species and on a yearly basis, numbers of cases in other animals paralleled numbers of cases in skunks. Cases in other species were considered to have resulted from spill-over from the primary host.

The total number of positive rabies cases in all species in the state were not significantly different from 1984 to 1986, but did increase in 1987. A similar trend was seen in the two study counties, demonstrating that on a smaller scale, these two counties were representative of the occurrence of rabies in the state. The small percentage (0.3%) of confirmed cases of rabies in raccoons in the state during the study period provided evidence that rabies was not maintained in raccoons. It is generally accepted that Public Health surveillance data from rabies testing laboratories is biased and not representative of rabies exposure in nature (Carey, 1985). This serologic survey provided evidence that the frequency of exposure of raccoons to rabies in these study counties was greater than the estimated prevalence of the disease in raccoons based on data from state testing laboratories.

It appeared from this study that there can be small numbers of raccoons with rabies-specific antibodies in the wild in areas without enzootic raccoon rabies. If a positive SN antibody titer represents post-exposure response, it appears that inapparent infections in the wild are possible. The raccoons may have been in the process of developing clinical rabies although they appeared clinically normal. There is evidence that raccoons are less susceptible to experimental challenge with a skunk rabies isolate (Hill and Beran, 1992).

The combined positive RFFIT, MSN and IFA test results in animals with low SN antibody titers demonstrated that some animals with low levels of neutralizing antibodies have been exposed to rabies antigen. This indicated that exclusion of all animals in serologic studies that have low SN antibody titers was not always justified. Such information could be helpful in the interpretation of prevalence estimates in future serologic surveys. These results emphasize that we must be cautious in interpreting the results of serologic studies in wildlife and in extrapolating to make definitive statements about the prevalence of rabies in wildlife species.

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LITERATURE CITED

ATANASIU, P. 1973. Quantitative assay and potency test of antirabies serum and immunoglobulin. *In* Laboratory techniques in rabies, 3rd ed., M. M. Kaplan and H. Koprowski (eds.). World Health Organization, Geneva, Switzerland, pp. 314–318.

BAER, G. M., W. J. BELLINI, AND D. B. FISHBEIN. 1990. Rhabdoviruses. *In* Virology, 2nd ed., B. N. Fields and D. M. Knipe (eds.). Raven Press, Ltd., New York, New York, pp. 883–930.

BARTON, L. D., AND J. B. CAMPBELL. 1988. Measurement of rabies-specific antibodies in carnivores by an enzyme-linked immunosorbent assay. Journal of Wildlife Diseases 24: 246–258.

BIGLER, W. J., R. G. MCLEAN, AND H. A. TREVINO. 1973. Epizootiologic aspects of raccoon rabies in Florida. American Journal of Epidemiology 98: 326–335.

———, G. L. HOFF, J. S. SMITH, R. G. MCLEAN, H. A. TREVINO, AND J. INGWERSEN. 1983. Persistence of rabies antibody in free-ranging raccoons. Journal of Infectious Diseases 148: 610.

CAMPBELL, J. B., AND L. D. BARTON. 1988. Serodiagnosis of rabies: Antibody tests. *In* Rabies, J. B. Campbell and K. M. Charlton (eds.). Kluwer Academic Publishers, Boston, Massachusetts, pp. 223–242.

CAREY, A. B. 1985. Multispecies rabies in the eastern United States. *In* Population dynamics of rabies in wildlife, P. J. Bacon (ed.). Academic Press, Orlando, Florida, pp. 23–41.

CENTERS FOR DISEASE CONTROL. 1984. Rabies prevention—United States, 1984. Morbidity and Mortality Weekly Report 33: 393–402.

———. 1989. CDC surveillance summaries, rabies surveillance, United States, 1988. Morbidity and Mortality Weekly Report 38(SS-1): 1–21.

COX, J. H. 1982. The structural proteins of rabies virus. Comparative Immunology, Microbiology and Infectious Diseases 5: 21–25.

DEAN, D. J., AND M. K. ABELSETH. 1973. The fluorescent antibody test. *In* Laboratory techniques in rabies, 3rd ed., M. M. Kaplan and H. Koprowski (eds.). World Health Organization, Geneva, pp. 73–84.

GRANDIEN, M. 1977. Evaluation of tests for rabies antibody and analysis of serum responses after administration of three different types of rabies vaccines. Journal of Clinical Microbiology 5: 263–267.

———, AND A. ESPMARK. 1974. Follow-up of antibody responses after post-exposure rabies immunization. Symposia Series in Immunobiological Standardization 21: 243–252.

HASBROUCK, J. J. 1990. Demographic responses of raccoons to varying exploitation rates. Ph.D. Dissertation. Iowa State University, Ames, Iowa, 136 pp.

HILL, R. E. 1990. Assessment of rabies antibody activity in serum of normal raccoons. M.S. Thesis. Iowa State University, Ames, Iowa, 116 pp.

———, AND G. W. BERAN. 1992. Experimental inoculation of raccoons (*Procyon lotor*) with rabies virus of skunk origin. Journal of Wildlife Diseases 28: 51–56.

IOWA DEPARTMENT OF PUBLIC HEALTH. 1951–1990. Animal rabies annual summaries, 1950–1989. Veterinary Public Health Section, Des Moines, Iowa, 1 pp.

JENKINS, S. R., B. D. PERRY, AND W. G. WINKLER. 1988. Ecology and epidemiology of raccoon rabies. Reviews of Infectious Diseases 10: S620–625.

JOHNSON, H. N., AND R. W. EMMONS. 1980. Rabies virus. *In* Manual of clinical microbiology, 3rd ed., E. H. Lennette (ed.). American Society for Microbiology, Washington, D.C., pp. 875–883.

KLEVEZAL, G. A., AND S. E. KLEINENBERG. 1967. Age determination of mammals from annual layers in teeth and bones. USSR Academy of Science Severtsov Institute of Animal Morphology. Clearing House for Federal Science, U.S. Department of Commerce, Springfield, Virginia, 128 pp.

KOPROWSKI, H. 1973. The mouse inoculation test. *In* Laboratory techniques in rabies, 3rd ed., M. M. Kaplan and H. Koprowski (eds.). World Health Organization, Geneva, Switzerland, pp. 85–93.

LARSH, S. E. 1965. Indirect fluorescent antibody and serum neutralization response in pre-exposure prophylaxis against rabies. Annals of Internal Medicine 63: 955–964.

MCLEAN, R. G. 1975. Raccoon rabies. *In* The natural history of rabies, Vol. II, 1st ed., G. M. Baer (ed.). Academic Press, New York, New York, pp. 53–78.

NATHANSON, N., AND F. GONZALEZ-SCARANO. 1991. *In* The natural history of rabies, 2nd ed., G. M. Baer (ed.). CRC Press, Boca Raton, Florida, pp. 145–161.

NATIONAL COMMUNICABLE DISEASE CENTER. 1964. Zoonosis surveillance: Annual rabies summary, 1963. U.S. Dept. of Health, Education, and Welfare, Public Health Service, Atlanta, Georgia, 19 pp.

NIEMEYER, C. C. 1973. An ecological and serological survey of rabies in some central Iowa mammals. M.S. Thesis, Iowa State University, Ames, Iowa, 53 pp.

PECK, F. B. 1966. The detection of human rabies antibody by the indirect fluorescence test. Symposia Series in Immunobiological Standardization 1: 201–206.

REED, L. J., AND H. MUENCH. 1938. A simple method of estimating fifty percent endpoints. Journal of Hygiene 27: 493–497.

SCHNEIDER, L. G., B. DIETZSCHOLD, R. E. DIERKS, W. MATTHAEUS, P. J. ENZMANN, AND K. STROHMAIER. Rabies group-specific ribonucleoprotein antigen and a test system for grouping and typing of rhabdoviruses. Journal of Virology 11: 748–755.

SELLERS, T. F. 1927. A new method for staining Negri bodies of rabies. American Journal of Public Health 17: 1080–1081.

SMITH, J. S., P. A. YAGER, AND G. M. BAER. 1973. A rapid tissue culture test for determining rabies neutralizing antibody. *In* Laboratory techniques in rabies, 3rd ed., M. M. Kaplan and H. Koprowski (eds.). World Health Organization, Geneva, Switzerland, pp. 354–357.

———, F. L. REID-SANDEN, L. F. ROUMILLAT, C. TRIMARCHI, K. CLARK, G. M. BAER, AND W. G. WINKLER. 1986. Demonstration of antigenic variation among rabies virus isolates by using monoclonal antibodies to nucleocapsid proteins. Journal of Clinical Microbiology 24: 573–580.

———. 1991. Rabies serology. *In* The natural history of rabies, 2nd ed., G. M. Baer (ed.). CRC Press, Boca Raton, Florida, pp. 236–252.

SNEDECOR, G. W., AND W. G. COCHRAN. 1989. Statistical methods, 8th ed., Iowa State University Press, Ames, Iowa, 503 pp.

THOMAS, J. B., R. K. SIKES, AND A. S. RICKER. 1963. Evaluation of indirect fluorescent antibody technique for detection of rabies antibody in human sera. Journal of Immunology 91: 721–723.

TORDO, T., AND W. POCH. 1988. Structure of rabies virus. *In* Rabies, J. B. Campbell and K. M. Charlton (eds.). Kluwer Academic Publishers, Boston, Massachusetts, pp. 25–46.

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