

SEROLOGIC SURVEY FOR SELECTED INFECTIOUS DISEASE AGENTS IN RACCOONS FROM ILLINOIS

Authors: Mitchell, M. A., Hungerford, L. L., Nixon, C., Esker, T., Sullivan, J., et al.

Source: Journal of Wildlife Diseases, 35(2) : 347-355

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-35.2.347>

The BioOne Digital Library (<https://bioone.org/>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<https://bioone.org/subscribe>), the BioOne Complete Archive (<https://bioone.org/archive>), and the BioOne eBooks program offerings ESA eBook Collection (<https://bioone.org/esa-ebooks>) and CSIRO Publishing BioSelect Collection (<https://bioone.org/csiro-ebooks>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

SEROLOGIC SURVEY FOR SELECTED INFECTIOUS DISEASE AGENTS IN RACCOONS FROM ILLINOIS

M. A. Mitchell,^{1,4} L. L. Hungerford,^{1,2} C. Nixon,² T. Esker,² J. Sullivan,² R. Koerkenmeier,² and J. P. Dubey³

¹ Department of Veterinary Pathobiology, University of Illinois, College of Veterinary Medicine, Urbana, Illinois 61801, USA

² Center for Wildlife Ecology, Illinois Natural History Survey, Urbana, Illinois 61801, USA

³ Parasite Biology and Epidemiology Laboratory, Livestock and Poultry Sciences Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705, USA

⁴ Present address of corresponding author: Department of Veterinary Clinical Sciences, Louisiana State University, School of Veterinary Medicine, Baton Rouge, Louisiana 70803, USA

ABSTRACT: The determination of serologic titers to infectious organisms is a valuable tool for quantitating exposure to disease organisms. Raccoons (*Procyon lotor*) were live-trapped from September 1989 to October 1993 and samples collected from two distinct locations in west-central Illinois (USA); a state recreational facility (Park) and privately owned farming property (Farm). Sera were submitted for testing *Leptospira interrogans* (serovars *bratislava*, *canicola*, *grippotyphosa*, *hardjo*, *icterohemorrhagiae*, and *pomona*), canine distemper virus (CDV), pseudorabies virus (PV), and *Toxoplasma gondii*. Two-hundred and twenty-two (48%) of 459 raccoons were seropositive for *L. interrogans*. Eighty-five (23%) out of 368 raccoons were seropositive for canine distemper virus. Eighty-two (17%) of 479 raccoons were seropositive for pseudorabies virus. One hundred and eighty-four (49%) of 379 raccoons were seropositive for *T. gondii*. A significant difference ($P < 0.05$) in seroprevalence for *L. interrogans* between the park (43%) and farm (52%) areas was found. A correlation between increasing age and seroprevalence was found for *L. interrogans*, CDV, PV, and *T. gondii*. Furthermore, there was a significant difference in seroprevalence for *T. gondii* during the spring trapping seasons (73%), when compared with the fall (33%). This type of information on exposure to infectious agents is important for developing control programs to manage raccoon-human and raccoon-domestic animals interactions.

Key words: Canine distemper virus, *Leptospira interrogans*, *Procyon lotor*, pseudorabies virus, raccoon, serological survey, *Toxoplasma gondii*.

INTRODUCTION

The raccoon (*Procyon lotor*) is a highly adaptive mammal ubiquitous throughout the United States (Kaufmann, 1982). Human expansion into rural areas, outdoor recreational activities, and raccoon expansion into urban areas have increased human contact with raccoons and led to greater potential for zoonotic disease transmission. The adaptability of raccoons to human inhabited areas also presents opportunities for exposure of domestic animals to raccoons, with resultant illness, decreased productivity and monetary loss from infectious diseases.

Raccoons may carry a number of infectious agents transmissible to humans and their domestic animals (Acha and Szyfres, 1987). Although rabies has been one of the most widely recognized, canine distemper virus (CDV) also can substantially impact

the health of raccoons and wild and domestic canids (Roscoe, 1993). It also has been implicated in deaths of captive exotic felids (Appel et al., 1994). Raccoons may also serve as reservoirs or provide a mechanism for translocation of other agents. Raccoons are considered the natural reservoir for the human and animal pathogen, *Leptospira interrogans* serotype *grippotyphosa*, and may be infected with other serovars (Prescott et al., 1991; Shotts et al., 1975). Experimentally, infected raccoons are able to transmit pseudorabies virus (PV) to swine (Kirkpatrick et al., 1980). Raccoons may serve as a source of food-borne exposure to *Toxoplasma gondii* or may be sentinels of environmental exposure potential for humans and domestic animals (Dubey et al., 1993).

In this study, seroprevalence patterns of four infectious agents, *L. interrogans*, CDV, PV, and *T. gondii*, were examined in

raccoons from a state park and from a nearby farmed area in west-central Illinois (USA). We examined differences in seroprevalence among raccoons with different age, sex, seasonal or geographical profiles to provide more information on the epidemiology of these zoonotic diseases.

MATERIALS AND METHODS

The study was conducted from September 1989 to October 1993 in Brown county (Illinois, USA). Trapping seasons were divided into spring (March–June) and fall (August–October). Two distinct study areas were used; these included a state recreational facility (Park) and privately owned farming property (Farm). The park study area was a 644 ha state park (39°53'N, 90°56'W) with a substantial raccoon population, estimated to be at least 13.5 raccoons/km² (Nixon et al., 1994). Land cover was 75% oak-hickory forest, 6% row crops and 11% pasture/forage. Public attendance at this park was approximately 205,000 visits per year. Campers that visited the park reported that raccoons ate food and garbage from campsites and accepted food from campers' hands. A section of the park was designated for individuals to camp and trailer horses and the park had trails for horse-back riding. Dogs or cats were brought along as travelling companions by some campers.

The farm study area was a 2,310 ha forested and extensively farmed area (39°57'N, 90°53'W) with a raccoon population estimated to be at least 4.5 raccoons/km² (Nixon et al., 1994). Land cover was 59% row crops, 15% pasture and forage and 25% shrub forest. Human contact occurred as a result of removing nuisance raccoons living in farm buildings and hunting and trapping of raccoons. Livestock (cattle, sheep, swine and horses) were maintained within the study area in confinement or on pasture. Free-roaming dogs and cats could be found throughout this study area.

Home-made box traps baited with canned sardines in mustard sauce (Stinson Seafood Company; Prospect Harbor, Maine, USA) were placed in suitable raccoon habitat, left overnight and checked for capture the following morning. Most traps were placed along streams or along drainage areas. Each raccoon was removed from the trap, weighed and sedated using Telazol (Tiletamine HCl and Zolazepam HCl; Fort Dodge Laboratories, Inc.; Fort Dodge, Iowa, USA) at 5mg/kg. After satisfactory sedation was achieved, raccoons were given a thorough physical examination and a blood sample was collected via cardiac puncture. Rac-

coons were ear tagged, allowed to recover in a cool, dark place, and released at the site of capture.

Age was estimated by evaluation of body weight, tooth eruption and general appearance (Nixon et al., 1994). Raccoons were categorized as juveniles, yearlings, or adults. Juveniles were those animals born in the spring of the current trapping year or entering their first fall. Raccoons were classified as yearlings during the subsequent spring and fall seasons (12 to 24 mo). Animals became adults the second spring after their birth (>24 mo). A first premolar was extracted from yearling and adult raccoons and submitted to Matson's Laboratory (Milltown, Montana, USA) where the cementum annuli were used to confirm field age (Grau et al., 1970).

The blood sample was allowed to clot at room temperature and then centrifuged and separated. Serum was removed from each sample and aliquotted into cryovials. Samples were immediately frozen at -10 C until tested. All samples could not be tested with all serologic assays due to insufficient total volume.

Testing for *L. interrogans* was performed by L. Hansen and D. Tripathy (University of Illinois College of Veterinary Medicine, Department of Veterinary Pathobiology, Urbana, Illinois, USA) using the microscopic agglutination microtiter test (Sulzer and Jones, 1973). Serovars that were tested included: *bratislava*, *cannicola*, *grippotyphosa*, *icterohemorrhagiae*, *hardjo*, and *pomona*. A titer >1:80 was considered positive for exposure to *L. interrogans* (Sulzer and Jones, 1973).

Canine distemper virus testing was performed by E. Dubovi (New York State Animal Diagnostic Laboratory, Ithaca, New York, USA) using serum neutralization. Samples were tested for CDV neutralizing antibody against the Onderstepoort strain of CDV adapted to Vero cells (Appel and Robson, 1973). The starting dilution was 1:4. Raccoons were classified as negative if no antibody was detected at the 1:4 dilution. Higher titers indicated the animal was exposed to CDV (Appel and Robson, 1973).

Testing for pseudorabies virus was performed by G. Scherba (University of Illinois College of Veterinary Medicine, Department of Veterinary Pathobiology, Urbana, Illinois, USA) using a slight modification of the standardized PV virus neutralization procedure (Scherba et al., 1991). A titer \geq 1:2 was considered positive for exposure to the pseudorabies virus (Scherba et al., 1991).

Toxoplasma gondii testing was performed at the United States Department of Agriculture Laboratory (Beltsville, Maryland, USA) using a modified direct agglutination test (MAT) as de-

scribed (Dubey and Desmonte, 1987). Serum from each raccoon was screened at 1:25, 1:50, and 1:500 dilutions using formalin-fixed tachyzoites to obtain estimates of titers (Dubey et al., 1993). A titer of 1:25 was considered positive for exposure to *T. gondii* (Dubey et al., 1993).

Urine was obtained via cystocentesis on sedated raccoons. One drop of urine was placed in 5 ml of bovine albumin media, liquid and semi-solid, and stored at room temperature. Samples were shipped to L. Hanson (University of Illinois College of Veterinary Medicine, Department of Veterinary Pathobiology, Urbana, Illinois, USA) where one-tenth of the inoculum was placed into each of three separate tubes and incubated at 30 C. Cultures were checked weekly for organisms, over a period of 6 mo, using dark-field microscopy (Ellinghausen and McCullough, 1965).

Chi-square tests and odds ratios (OR) were used to evaluate year of capture and age class for each of the four serological outcomes (ProcFreq; SAS, 1991). Mantel-Haenszel (MH) chi-square tests and adjusted OR were used to evaluate gender, site, and season, while controlling for age class, for each of the four serological outcomes. Stratum specific and overall adjusted OR were evaluated. Values of $P < 0.05$ were considered statistically significant. Separate logistic regression analyses were used to model risk for each of the four serological outcomes. Age class, gender, site, and season of capture were all included in initial models (Proc Logistic; SAS, 1991). Year of study was also included if it was significant in the univariate testing, but removed first if it did not affect the coefficients of other variables. Main effect variables were removed individually from full models to assess effects on model likelihood ratio statistics, magnitude of coefficients for other variables, and Hosmer and Lemeshow goodness-of-fit statistics (Hosmer and Lemeshow, 1989). Biologically relevant interactions of main effect variables were also evaluated. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Sera were collected from 479 raccoons of which 270 were from the farmed area and 209 were from the park. There were 154 juveniles (98 from the farmed area and 56 from the park), 139 yearlings (86 from the farmed area and 53 from the park), and 186 adults (86 from the farmed area and 100 from the park). There were 249 males (159 from the farmed area and

90 from the park), and 230 females (111 from the farmed area and 119 from the park) captured during the study.

Two hundred and twenty-two (48%) of 459 raccoons were seropositive for *L. interrogans* including *grippotyphosa* ($n = 220$), *canicola* ($n = 1$), *icterohemorrhagiae* ($n = 1$). Seroprevalence was found to increase across the study years 1990 (28%), 1991 (45%), 1992 (57%), and 1993 (65%). Seroprevalence was lower in juveniles (33%) than in older animals (yearlings = 53%, adults = 58%). There was no significant difference in seroprevalence between the sexes (Males = 49%, Females = 47%). Raccoons captured in the farm area (52%) were more likely to be seropositive than raccoons captured in the park (43%). Raccoons captured in the spring season (58%) were more likely to be seropositive than raccoons captured in the fall (44%) but when adjusted for age, which moderated the effect of juveniles which were present only in the fall, seasonal differences disappeared.

In the final logistic regression model for *L. interrogans*, juvenile animals were less likely to be seropositive than yearlings and adults (Odds Ratio = 0.4, 95% Confidence Interval (CI) = 0.2–0.6, $P < 0.05$). Raccoons captured in the farm area were more likely to be seropositive (OR = 1.5, 95% CI = 1.2–1.8, $P < 0.05$). The final regression equation for *L. interrogans* was as follows: Seropositive = 0.4–1.0 (Juvenile) + 0.4 (Farm). The Hosmer and Lemeshow goodness of fit statistic was 0.94. There were no significant interaction terms.

One-hundred thirty-three urine specimens were cultured for *L. interrogans*. Fifty-one samples were overgrown and presence of *L. interrogans* could not be assessed. Five (6%) of 82 remaining urine samples were positive for *L. interrogans* serovar *grippotyphosa*. All five were trapped in the park during the fall season. All five culture positive raccoons were seropositive.

Eighty-five (23%) of 368 raccoons were

seropositive for CDV. There were no significant differences between study years although prevalence was slightly higher in 1991 (28%) than in 1992 (18%) or 1993 (24%). Adult raccoons were more likely to be seropositive (39%) than were juveniles (14%) or yearlings (13%). There was no significant difference in the seroprevalence between males (22%) and females (25%). Raccoons in the farm study (19%) were less likely to be seropositive than raccoons captured in the park (29%), however, this difference was no longer significant when adjusted for age differences between sites. There was no significant difference in seroprevalence between trapping seasons (Spring = 26%, Fall = 21%).

In the final regression model for CDV, adult raccoons were more likely to be seropositive, compared to juveniles and yearlings (OR = 4.3, 95% CI = 2.3–6.5, $P < 0.05$). The final regression equation for CDV was as follows: Seropositive = $-1.99 + 1.35$ (Adult). The Hosmer and Lemeshow goodness-of-fit was 0.97. None of the interaction terms were significant.

Eighty-two (17%) of 479 raccoons were seropositive for the PV. There were 54 raccoons with a titer of 1:2, 4 raccoons with a titer of 1:4, and 24 raccoons with a titer $>1:4$. Seroprevalence was higher in the 1992 trapping year (23%) than in the other trapping years (15% in 1990, 13% in 1991 and 15% in 1993), although differences were not significant. Seroprevalence in juveniles (3%) was lower than in yearlings (15%), while adult seroprevalence (30%) was higher than yearlings. Among adult raccoons, there were no significant differences in seroprevalence as animals aged. There were no significant differences in the seroprevalence between the sexes (Males = 15%, Females = 19%) or study areas (Farm = 17%, Park = 17%). Animals captured in the spring trapping season were more likely to be seropositive (24%) than those captured in the fall (13%), but after adjusting for age class, there were no significant differences be-

tween trapping seasons. Juveniles were only captured during the fall trapping season.

In the final regression model for PV, juveniles were less likely to be seropositive than yearlings (OR = 0.2, 95% CI = 0.1–0.5, $P < 0.01$), while adults were more likely to be seropositive than yearlings (OR = 2.4, 95% CI = 1.4–4.3, $P < 0.01$). The final regression equation for PV was as follows: Seropositive = $-1.7-1.7$ (Juvenile) + 0.9 (Adult). The Hosmer and Lemeshow goodness-of-fit statistic was 0.99. There were no significant interaction terms.

One hundred and eighty-four (49%) of 379 raccoons were seropositive for *T. gondii*. Raccoons captured during the 1991 (55%) and 1992 (60%) trapping years were more likely to be seropositive than those raccoons captured in 1993 (31%), while 1990 (37%) and 1993 were similar. Seroprevalence in juveniles (14%) was lower than in yearlings (53%), while adult seroprevalence (73%) was higher than yearlings. Among adult raccoons, there were no significant differences in seroprevalence with increasing age. There were no significant differences in seroprevalence between sexes (Males = 47%, Females = 50%) or study areas (Farm = 49%, Park = 48%). Raccoons captured in the spring season were more likely to be seropositive (73%) than raccoons in the fall (33%). This effect persisted after adjusting for age class.

In the final logistic regression model for *T. gondii*, juveniles were less likely to be seropositive than yearlings (OR = 0.3, 95% CI = 0.1–0.6, $P < 0.05$), while adults were more likely to be seropositive than yearlings (OR = 2.5, 95% CI = 1.9–3.3, $P < 0.05$). Raccoons captured during the fall season were less likely to be seropositive than those captured in the spring (OR = 0.3, 95% CI = 0.2–0.4, $P < 0.05$). The final regression equation for *T. gondii* was as follows: Seropositive = $0.6-1.2$ (Juvenile) + 0.9 (Adult) -1.1 (Fall). The Hosmer and Lemeshow goodness-of-fit statis-

tic was 0.99. There were no significant interaction terms.

DISCUSSION

The raccoon has been reported to be a natural reservoir for *L. interrogans*, especially *grippotyphosa* (Shotts et al., 1975). In this study, *L. interrogans grippotyphosa* was detected as the primary serovar in all but two of the raccoons; it was the serovar isolated in all 5 urine samples. *Leptospira interrogans grippotyphosa* has been associated with acute febrile disease in humans (Jackson et al., 1993). *Leptospira interrogans* serovars *canicola* and *icterohemorrhagiae* were the other serotypes identified, both in animals from the farm area.

In this study, seroprevalence in juveniles was significantly lower than in yearlings and adults. Juvenile home ranges are smaller than older animals and expected exposure to the pathogen would be lower. When adjusting for trapping location, there were no significant differences in the seroprevalence levels between yearlings and adults or among adults. This pattern suggests that the spirochete continuously cycles in these populations and exposure is probably sporadic, occurring in areas where moisture and temperature are ideal and can sustain the spirochete. Humans and domestic species would be at highest risk during warmer weather and after heavy rains when there is standing water. Acha and Szyfres (1987) reported that the incidence of leptospirosis in humans can be sporadic, as the result of environmental variability.

Although raccoon densities were estimated to be 3 times higher in the park area than the farm area, the seroprevalence for *L. interrogans* was higher in the farm area where the potential for domestic animal-raccoon interaction was most likely. In the farm area, raccoons had access to water sources used by domestic species. The sharing of water, in combination with the findings of active spirochete shedding in urine, could lead to a cycle involving interspecies transmission. Infections in do-

mestic species would increase the risk of exposure to humans working with them. Preventing raccoon access to domestic species, avoiding contact with water sources that have been contaminated with raccoon urine, and practicing strict hygiene when working with domestic animals would be essential in the prevention of zoonotic disease transmission. The high seroprevalence in the park area raises concern for zoonotic exposure of humans during recreational activities. A lake and a number of small creeks were found in the park area. Raccoons spend a great deal of time around water hunting for aquatic food items (Kaufmann, 1982). Raccoons that are actively shedding *L. interrogans* in their urine could contaminate water sources shared by other animals species and humans. Humans that drink, bathe or cook with these water sources could be exposing themselves to the spirochete. Domestic pets that are brought along to the park, such as dogs, cats, and horses, could be exposed to *L. interrogans* when drinking or swimming in contaminated water sources. Many animals will drink from small pools of standing water, which should be prevented because of the risk of exposure to the spirochete.

A study of raccoons from Florida (USA) and Georgia (USA) also detected a high seroprevalence rate for *L. interrogans* (59%; Schotts et al., 1975), but was based on only 17 animals. The findings of high seroprevalence and the shedding of the spirochete in urine, in combination with the raccoon's affinity for water, raises concerns for the potential exposure of humans and domestic animal species to raccoons in both urban and rural environments. Translocation of feral raccoons could pose a significant threat to animal and human populations and is not recommended. Further study to identify possible patterns in animals that are seropositive and actively shedding the spirochete in the urine is necessary in establishing a management protocol to decrease the risk of exposure

to *L. interrogans* in humans and domestic species.

Canine distemper virus can be devastating in raccoons, leading to high mortality and a slow return to normal population size. Serologic surveys in New York (USA) and Maryland (USA) detected seroprevalence for CDV neutralizing antibody titers ranging from 22% to 84% of raccoons (Jamison et al., 1973; Parker et al., 1961). The seroprevalence in raccoons from Florida was 55% (Hoff et al., 1974). Seroprevalence detected in Illinois was at the lower range of these levels.

Raccoon populations in Illinois have increased in recent years as a result of decreased harvests (Nixon et al., 1994). The higher density and adaptability of raccoons to urban environments poses an infection risk for domestic and captive animal species. Canine distemper virus was recently isolated from a black leopard (*Panthera pardus*) that died at the Rock Island Forest Preserve (Naibi Zoo, Coal City, Illinois, USA; Appel et al., 1994). The authors were of the opinion that raccoons were the source of infection. In light of CDV seroprevalence in free-ranging raccoons, vaccination against CDV for domestic canids and control of the potential for exposure in domestic and exotic species is strongly recommended in areas where raccoon populations are high.

Previous reports have described a 4 yr distemper cycle in raccoon populations (Hoff et al., 1974; Roscoe, 1993). These studies found no association between age and CDV infection during or between outbreak years (Hoff et al., 1974; Roscoe, 1993). In our study, positive titers to CDV were detected in all three age groups every year with a significantly higher seroprevalence in adults than subadults. The higher seroprevalence in adults suggests that there is a constant exposure to CDV in these environments. Serologic evidence of exposure, in combination with disease related mortalities reported during the study period (1990 to 1993), suggests that CDV is enzootic in these populations. Our study

found a significant decrease in the seroprevalence between the 1991 and 1992 trapping years. During the summer-fall of 1992, disease related mortality was the leading cause of death in the park study area (Nixon et al., 1994). Population density in the park is estimated to be 3 times higher in the farmed area, however there was no significant difference in the seroprevalence between the study areas (1991 to 1992). The disease related mortalities and decreasing seroprevalence during this time suggests that an epizootic occurred between the 1991–92 trapping years. Adult raccoons that were born in 1988 or earlier had a higher seroprevalence than those adults born after 1988. The higher seroprevalence in the adults >4-yr-old suggests that there may be a 4 yr interval between CDV epidemics in these populations. The epidemics may have resulted from exposure to a more virulent strain of CDV circulating through the populations, causing losses of both immunocompetent and naive animals, and/or the result of the number of unprotected raccoons becoming great enough that exposure could support an outbreak. Extending the time period studied would be required to confirm a 4 yr epidemic cycle in these populations of raccoons.

Pseudorabies virus has been considered an acutely fatal disease in aberrant hosts, such as raccoons (Wright and Thawley, 1980). Previous experimental studies have indicated that raccoons can transmit PV to swine (Kirkpatrick et al., 1980). Although a potential role for raccoons in PV transmission has been postulated, previous studies have had difficulty in identifying free-ranging raccoons with positive serum neutralizing titers (Wright and Thawley, 1980). Platt et al. (1983) reported that 5 of 47 (11%) raccoons trapped in PV endemic areas were positive using serum neutralization, however all titers were <1:4. The authors believed that serum neutralizing titers >1:4 would have been diagnostic for exposure to PV (Platt et al., 1983). In this study there were 24 rac-

coons (29%) with titers $>1:4$. At the start of this study, there was a PV positive swine farm equidistant between the study areas. The farm was a partial confinement operation. The farm utilized testing and depopulation to eradicate PV and was removed from quarantine during the first year of the study. No vaccination program was ever instituted. Most (81%) of the 82 PV seropositive raccoons were born before the PV swine farm was released from quarantine. The majority of those animals born later (14/16) were from the farm area. The increase in seroprevalence during the 1992 trapping year, when compared to the 1991 trapping year, suggests that there may have been another source of PV in these populations. No other farms were placed on PV quarantine in the vicinity of the study areas during this period. Privately-owned family swine herds, that are not routinely tested and are of unknown PV status, could be a source of PV for wildlife. Previous studies have implicated feral swine as a source of PV for wildlife (Glass et al., 1994). Although feral swine are not present in Illinois, free-ranging pigs were commonly seen during the raccoon trapping and observation periods. Young pigs were occasionally caught in the box traps in the farm area. Identification of free-ranging PV seropositive raccoons that survive over time (>6 mo) could indicate a potential for raccoons in maintaining or spreading the pseudorabies virus. In this study, one raccoon, trapped in August 1990, had a serum titer of 1:2. He was again trapped in April 1991 and had a serum titer of $>1:4$. A second raccoon was captured in September of 1990 and 1991 and had a titer of 1:2 on both occasions.

In this study, seroprevalence increased with age. This suggests that raccoons were repeatedly exposed to PV and that a source of PV, other than the identified positive swine herd, existed in these populations. The increase in seroprevalence during the 1991 and 1992 trapping years, when there were no reported PV positive swine farms in the area, supports this con-

clusion. Because testing does not include small farms where slaughter is done privately, the actual number of PV positive swine herds in the area cannot be determined. These findings suggest that the raccoon may act as a sentinel for PV in certain areas. Raccoons have been found to approach swine farms and feed on assorted feedstuffs, swine carcasses, and garbage, in this and other studies (Kirkpatrick et al., 1980; Thawley and Wright, 1982). The identification of seropositive raccoons could be used to help assess the PV status of a geographic region. Further study to identify patterns in these raccoons is necessary and may be useful in detecting sources of PV in these environments. Our findings of survivability of seropositive feral raccoons and an increasing seroprevalence with age should raise concerns about the role of raccoons in PV transmission and methods to prevent contact between raccoons and swine may be warranted.

Previous studies have described a variation in seroprevalence for *T. gondii* based upon the testing procedure used. Dye-test antibodies were detected in the sera of 24% of 77 raccoons from Maryland (Jacobs and Stanley, 1962) and 33% of 67 raccoons from Georgia (Walton and Walls, 1964). Indirect hemagglutination antibodies were detected in 18% of 530 raccoons from Florida (Burridge et al., 1979). In this study the modified direct agglutination test was used. This procedure detects antibodies earlier and in higher titers than the other serologic tests (Dubey et al., 1993).

Seasonal differences in *T. gondii* seroprevalence might be related to differences in diet throughout the year. During the fall, raccoons are more likely to be actively feeding on vegetation and crops in agricultural fields; prior and post harvest. The primary diet in the spring is animal source (Kaufman, 1982). Most feral cats become infected with *T. gondii* by consuming tissue containing infective cysts (Dubey, 1973). The increased consumption of rodents, birds, and carrion containing *T. gon-*

dii cysts, might account for the increased seropositive rate detected in these raccoons during the spring. In the analysis of these populations, age was adjusted to prevent confounding due to the lack of juveniles in the spring.

Dubey et al. (1995) found MAT *T. gondii* antibodies (>1:25) in sera of 67% of 1888 raccoons trapped on 34 swine farms. Infected raccoons were trapped on 72% of farms indicating wide exposure. The slightly lower prevalence of *T. gondii* in this study (48%) than on swine farms (67%) may be due to higher *T. gondii* environmental contamination around farms than in open range; 68% of cats on swine farms had antibodies to *T. gondii* indicating that they had already had shed oocysts in the environment.

As omnivores, raccoons feed on carrion and vegetation, and are considered as good monitors of environmental *T. gondii* contamination. However, despite the higher seroprevalence, raccoons are unlikely to serve as a primary source of infection for humans or other animals because they do not shed *T. gondii* oocysts in feces (Dubey et al., 1993). However, improperly cooked raccoon flesh can serve as a source of infection for humans.

Feral or farm cats were trapped during the study, with a lower percentage caught in the park (5%) than the farm area (95%). Seroprevalence was expected to be higher in the farm area due to the apparent higher cat density, however the seroprevalence in the two study areas were similar (farm 49%, park 48%). Reports that individuals from a surrounding town were releasing cats in the park area were unsubstantiated. Poor trapping rates for cats in the park area could be due to trap placement.

Raccoon hunting and trapping are enjoyed by a number of individuals. Because of the high prevalence of *T. gondii* in raccoons, hunters should wash their hands thoroughly with soap and water after skinning raccoons; tissue stages of *T. gondii* are killed by water. Although *T. gondii* infections in raccoons are usually asymptomat-

ic, concurrent infections of *T. gondii* and immunodepressant infections (CDV) can be fatal in raccoons (Dubey et al., 1992).

Increasing raccoon densities, in a variety of habitats, bring a higher potential for interspecies transmission of infectious agents. Awareness of prevalence levels in wild raccoon populations can allow informed decision-making in management of raccoon-human and raccoon-domestic animal interactions.

ACKNOWLEDGMENTS

This project was supported with funds made available through Federal Aid for Wildlife. Project number W-104-R-4.

LITERATURE CITED

- ACHA, P. N., AND B. SZYFRES. 1987. Zoonoses and communicable diseases common to man and animals. 2nd Edition. Pan American Health Association Publishing, Washington, D.C., 963 pp.
- APPEL, M. J. G., AND D. S. ROBSON. 1973. A micro-neutralization test for canine distemper virus. *American Journal of Veterinary Research* 34: 1459-1463.
- , AND R. A. YATES, G. L. FOLEY, J. J. BERNSTEIN, S. SANTINELLI, L. H. SPELMAN, L. D. MILLER, L. H. ARP, M. ANDERSON, M. ANDERSON, M. BARR, S. PEARCE-KELLING, AND B. A. SUMMERS. 1994. Canine distemper epizootic in lions, tigers, and leopards in North America. *Journal of Veterinary Diagnostic Investigation* 6: 277-288.
- BURRIDGE, M. J., W. J. BIGLER, D. J. FORRESTER, AND J. M. HENNEMANN. 1979. Serologic survey for *Toxoplasma gondii* in wild animals in Florida. *Journal of the American Veterinary Medical Association* 175: 964-967.
- DUBEY, J. P. 1973. Feline toxoplasmosis and coccidiosis: a survey of domiciled and stray cats. *Journal of the American Veterinary Medical Association* 162: 873-877.
- , AND G. DESMONTE. 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. *Equine Veterinary Journal* 19: 337-339.
- , A. N. HAMIR, C. A. HANLON, AND C. E. RUPPRECHT. 1992. Prevalence of *Toxoplasma gondii* infection in raccoons. *Journal of the American Veterinary Medical Association* 200: 534-536.
- , ———, S. K. SHEN, P. THULLIEZ, C. E. RUPPRECHT. 1993. Experimental *Toxoplasma gondii* infection in raccoons. *The Journal of Parasitology* 79: 548-552.
- , R. M. WEIGEL, A. M. SEIGEL, P. THULLIEZ, U. D. KITRON, M. A. MITCHELL, A. MANELLI,

- N. E. MATEUS-PINILLA, S. K. SHEN, O. C. H. KWOK, AND K. S. TODD. 1995. Sources and reservoirs of *Toxoplasma gondii* infection on 47 swine farms in Illinois. *The Journal of Parasitology* 81: 723–729.
- ELLINGHAUSEN, H. C., AND W. G. MCCULLOUGH. 1965. Nutrition of *Leptospira pomona* and growth of 12 other serotypes: frosting of oleic albumin complex and a medium of bovine albumin and polysorbate 80. *American Journal of Veterinary Research* 26: 45–51.
- GLASS, C. M., R. G. MCLEAN, J. B. KATZ, D. S. MAEHR, C. B. CROPP, L. J. KIRK, A. J. MC-KEIRNAN, AND J. F. EVERMANN. 1994. Isolation of pseudorabies (Aujeszky's Disease) virus from a Florida panther. *Journal of Wildlife Diseases* 30: 180–184.
- GRAU, G. A., G. C. SANDERSON, AND J. P. ROGERS. 1970. Age determination of raccoons. *The Journal of Wildlife Management* 34: 364–372.
- HOFF, G. L., W. J. BIGLER, S. J. PROCTOR, AND L. P. STALLINGS. 1974. Epizootic of canine distemper virus infection among urban raccoons and gray foxes. *Journal of Wildlife Diseases* 10: 423–428.
- HOSMER, D. W., AND S. LEMESHOW. 1989. Applied logistic regression. 1st Edition. John Wiley and Sons, New York, New York, 307 pp.
- JACOBS, L., AND A. M. STANLEY. 1962. Prevalence of *Toxoplasma* antibodies in rabbits, squirrels, and raccoons collected in and near the Patuxent Wildlife Research Center. *The Journal of Parasitology* 48: 550.
- JACKSON, L. A., A. F. KAUFMAN, W. G. ADAMS, M. B. PHELPS, C. ANDREASEN, C. W. LANGKOP, B. J. FRANCIS, J. D. WENGER. 1993. Outbreak of leptospirosis associated with swimming. *The Pediatric Infectious Disease Journal* 12: 48–54.
- JAMISON, R. K., E. C. LAZAR, L. N. BINN, AND A. D. ALEXANDER. 1973. Survey for antibodies to canine viruses in selected wild animals. *Journal of Wildlife Diseases* 9: 2–3.
- KAUFMAN, J. H. 1982. Raccoon and allies *In* Wild mammals of North America, 1st Edition. Johns Hopkins University Press, Baltimore, Maryland, 1147 pp.
- KIRKPATRICK, C. M., C. L. KANITZ, AND S. M. MCCROCKLIN. 1980. Possible role of wild mammals in transmission of pseudorabies to swine. *Journal of Wildlife Diseases* 16: 601–614.
- NIXON, C. M., J. B. SULLIVAN, R. KOERKENMEIER, A. A. ROTHERING, J. THOMAS, J. STEVENS, L. L. HUNGERFORD, M. A. MITCHELL, G. F. HUBERT, AND R. D. BLUETT. 1994. Illinois raccoon investigations. Federal Aid in Wildlife Restoration Project Report, W-104-R-5. Illinois Natural History Survey, Urbana, Illinois, 219 pp.
- PARKER, R. L., V. J. CABASSO, D. J. DEAN, AND E. J. CHEATUM. 1961. Serologic evidence of certain virus infections in wild animals. *Journal of the American Veterinary Medical Association* 138: 437–440.
- PLATT, K. B., D. L. GRAHAM, AND R. A. FAABORG. 1983. Pseudorabies: Experimental studies in raccoons with different virus strains. *Journal of Wildlife Diseases* 19: 297–301.
- PRESCOTT, J. F., R. FERRIER, V. M. NICHOLSON, K. M. JOHNSON, AND B. HOFF. 1991. Is canine leptospirosis underdiagnosed in southern Ontario? A case report and serological survey. *Canadian Veterinary Journal* 32: 481–486.
- ROSCOE, D. E. 1993. Epizootiology of canine distemper in New Jersey raccoons. *Journal of Wildlife Diseases* 29: 390–395.
- SCHERBA, G., R. M. WEIGEL, L. JIN, W. HALL, AND F. A. ZUKERMANN. 1991. Sensitivity of the standardized pseudorabies virus neutralization test varies with the test strain used. *Journal of Veterinary Diagnostic Investigation* 3: 306–312.
- SHOTTS, E. B., C. L. ANDREWS, AND T. W. HARVEY. 1975. Leptospirosis in selected wild mammals of the Florida panhandle and southwestern Georgia. *Journal of the American Veterinary Medical Association* 167: 587–589.
- STATISTICAL ANALYSES SYSTEMS INSTITUTE, INC. (SAS). 1991. SAS/STAT Users Guide, version 6, 4th Edition, Vol. 1. SAS Institute Incorporated, Cary, North Carolina, 1686 pp.
- SULZER, C. R., AND W. L. JONES. 1973. Leptospirosis *In* Methods in laboratory diagnosis. Department of Health, Education, and Welfare, Publication #7408275, Atlanta, Georgia, pp. 1–40.
- THAWLEY, D. G., AND J. C. WRIGHT. 1982. Pseudorabies virus infection in raccoons: A review. *Journal of Wildlife Diseases* 18: 113–116.
- WALTON, B. C., AND K. W. WALLS. 1964. Prevalence of toxoplasmosis in wild animals from Fort Stewart, Georgia, as indicated by serological tests and mouse inoculation. *American Journal of Tropical Medicine and Hygiene* 13: 530–533.
- WRIGHT, J. C., AND D. G. THAWLEY. 1980. Role of the raccoon in transmission of pseudorabies: A field and laboratory investigation. *American Journal of Veterinary Research* 41: 581–583.

Received for publication 22 November 1996.