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## THE ROLE OF DEER AS A POSSIBLE RESERVOIR HOST OF POTOSI VIRUS, A NEWLY RECOGNIZED ARBOVIRUS IN THE UNITED STATES

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**ABSTRACT:** Potosi (POT) virus (Bunyaviridae) was isolated from *Aedes albopictus*, an introduced Asian mosquito species, collected at a used tire yard in Potosi, Missouri (USA), in August and September, 1989. In September, 1990, small animals were trapped at the tire yard and six cattle were sampled at an adjacent farm; in November 1990 and 1991, blood samples were collected with filter paper strips from 364 hunter-killed, white-tailed deer (*Odocoileus virginianus*) in the region to determine the possible reservoir hosts of the virus. Deer specimens from Arkansas ( $n = 70$ ), Colorado ( $n = 29$ ), and Iowa ( $n = 763$ ) (USA) were also analyzed. Specimens from 33 small vertebrates captured at the tire yard were negative for viruses. Only one eastern chipmunk (*Tamias striatus*) and none of six cattle had neutralizing (N) antibody against POT virus by the plaque-reduction serum neutralization test in Vero cell culture but 45 (25%) of 178 deer specimens in 1990 and 55 (30%) of 186 in 1991 were antibody positive. The 186 deer sera from 1991 were tested further and 29 (16%) were also N antibody positive to Cache Valley (CV) virus. From the 763 deer specimens tested from Iowa in 1993, 114 (15%) had N antibody to POT virus. Of 70 serum specimens from Arkansas deer in 1990, 33 (47%) had N antibody to POT and 15 (21%) to CV viruses; two (7%) of 29 CV negative serum specimens from Colorado deer in 1981 were serologically positive to POT virus. Three eastern chipmunks were experimentally inoculated with POT virus to determine their reservoir potential; none became viremic but all developed N antibody. Thus we propose that POT virus may be another virus regularly infecting wild deer populations but its impact on the health of these animals is unknown.

**Key words:** Arbovirus, Potosi virus, Cache Valley virus, white-tailed deer, *Odocoileus virginianus*, ectoparasites, small mammals, Missouri.

### INTRODUCTION

A newly described arbovirus, Potosi (POT) virus (*Bunyavirus*, Bunyaviridae), was isolated from *Aedes albopictus*, an introduced Asian mosquito species, found infesting a used tire yard in Potosi, Missouri (USA), in 1989 (Francy et al., 1990a). This virus belongs to the Bunyamwera serogroup of the genus *Bunyavirus* which contains five other known viruses (Cache Valley (CV), Tensaw (TEN), Lokern, Main Drain, and Northway) that occur within the continental United States and Canada (Karabatsos, 1985; Calisher et al., 1986; Campbell et al., 1989); it is distantly related to TEN virus of this serogroup (Francy et al., 1990a). Tensaw virus is found only in the states bordering the Gulf of Mexico;

whereas, CV virus of this serogroup is distributed throughout the central provinces of Canada and most regions of the United States (Calisher et al., 1986). The other three viruses have been reported only from the western United States.

Following the initial isolation, an intensive investigation of the mosquitoes in and around this tire dump was conducted in 1989 and 1990 (Francy et al., 1990a; Mitchell et al., 1990; Savage et al., 1993). Potosi virus was not isolated again at the site in 1990 (Mitchell, et al., 1995) despite the isolation of 16 strains in 1989 and the moderate infection rate of *Ae. albopictus* in September, 1989 (Francy et al., 1990a; Mitchell et al., 1990). This new virus was unlikely to have been introduced into Missouri with the introduction of *Ae. albopictus*.

*tus* mosquito eggs or larvae in imported used tires because vertical transmission to progeny of infected female mosquitoes was not observed experimentally (Mitchell et al., 1990; Heard et al., 1991) and because of the apparent disappearance of the virus from this site in 1990. Therefore, it is more likely that there is a natural transmission cycle involving other mosquito species and vertebrate hosts and this introduced vector species became infected after feeding on a local viremic host. Since this mosquito species prefers mammals, although it does feed on a variety of hosts (Savage et al., 1993), and since the other viruses in this genus have been isolated mostly from medium-sized to large-sized mammals (Calisher et al., 1986), a search for the mammalian species that serve as the natural vertebrate hosts was begun.

Our purpose was to identify possible natural reservoir hosts for Potosi virus and to determine the distribution of the virus in the region surrounding Potosi, Missouri.

#### MATERIALS AND METHODS

The study area where the small animals were captured consisted of about 10 ha of hardwood forest, primarily oak (*Quercus* spp.), hickory (*Carya* spp.), beech (*Fagus grandifolia*), and maple (*Acer* spp.), 2 to 3 ha of tall grass and shrubs (old field habitat); an edge of mostly sumac (*Rhus* spp.), blackberry (*Rubus allegheniensis*), and goldenrod (*Solidago* spp.); and a 1-ha lake all surrounding a 3 to 4 ha site covered with used tires. This area is located about 5 km northeast of Potosi (37°56'N, 90°47'W) in Washington County of east central Missouri.

Field techniques were similar to those described previously (Sudia et al., 1975; McLean et al., 1985). Traps for small mammals were set in transect lines of 20 Sherman traps (8 × 9 × 23 cm, H.B. Sherman Traps, Inc., Tallahassee, Florida, USA) and four small live traps (13 × 13 × 41 cm, Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA), and raccoon-sized live traps (25 × 30 × 80 cm, Tomahawk Live Trap Company) were set at locations likely to capture medium-sized mammals. Small traps were baited with peanut butter and oats and larger traps with peanut butter sandwiches and sardines. Eastern box turtles (*Terrapene carolina*) in the area were captured by hand and cattle (*Bos taurus*) from a nearby farm were restrained in a squeeze chute as necessary.

There were 686 trap nights (TN) for Sherman traps, 193 TN for small live traps, and 127 TN for large live traps for a total of 1,006 TN during the period of 7 to 13 September 1990. Traps were checked twice a day and captured animals were taken to a central location for processing. Small mammals were anesthetized with methoxyflurane (Metofane, Pittman-Moore, Inc., Mundelein, Illinois, USA) and examined for ectoparasites. A blood sample (0.2 ml) was taken from the suborbital sinus with a capillary pipet and was mixed with 0.9 ml of field diluent, consisting of M199 nutrient medium (GibcoBRL, Life Technologies Inc., Grand Island, New York, USA) with antibiotics and 20% heated (56 C for 30 min) fetal bovine serum (Hyclone Laboratories Inc., Logan, Utah, USA) (McLean et al., 1985). Mammals were marked with a monel ear tag and returned to the location of their capture following recovery from anesthesia. Medium-sized mammals were anesthetized with 0.5 to 1.5 ml (depending upon size) of an equal volume mixture of ketamine at 100 mg/ml (Vetalar, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) and xylazine at 20 mg/ml (Rompun, Mobay Corporation, Animal Health Division, Shawnee, Kansas, USA) and were examined for ectoparasites. Blood samples were taken from the femoral vein with a 5 cc syringe and 22 gauge needle. The mammals were marked with a monel ear tag and allowed to recover from anesthesia before being released near the capture location. Blood samples from turtles were obtained by cardiac puncture with a 3 cc syringe and 22 gauge needle and from cattle by venipuncture of the jugular vein with a 10 cc vacutainer and 20 gauge needle. Blood samples were kept on wet ice and centrifuged at 900 × G for 15 min the same day. The serum was removed and stored in labeled vials, and the vials were kept frozen on dry ice until returned to the laboratory where they were stored at -70 C until tested. Ectoparasites were removed from the small and medium-sized mammals with forceps, placed in labeled vials containing 70% ethyl alcohol, and identified to species in the laboratory (Cooley and Kohls, 1945; Clifford et al., 1961; Strickland et al., 1976). Ticks were deposited in the Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro, Georgia, USA, with accession numbers RML 122287 through 122299.

Blood samples from 364 white-tailed deer (*Odocoileus virginianus*) were collected from the carcasses of hunter-killed animals as they were processed at deer check stations (Gill et al., 1993) during the hunting seasons in the east central and southeast regions of Missouri in

November of 1990 and 1991. The age of deer were estimated by the technique of tooth eruption and wear (Severinghaus, 1949). Filter paper strips (Microfiltration Systems, Dublin, California, USA) (Wolff and Hudson, 1974) were used to collect blood from the thoracic cavity, air-dried, and placed in envelopes containing information on the collection location, age, and sex of each animal. Only one strip was used for each animal in 1990; whereas, two strips were collected from each deer in 1991 to obtain a larger volume of each blood sample. Blood collection tubes of 25 ml capacity with instructions and postage-paid return packages were mailed to hunters in Iowa for the December 1993 deer-hunting season (Adrian and Keiss, 1977). Blood samples from venous blood or the thoracic cavity of 763 white-tailed deer were mailed to the Department of Microbiology, University of Osteopathic Medicine and Health Sciences, Des Moines, Iowa, where they were centrifuged. Aliquots of serum were dispensed into label vials and kept frozen at  $-100^{\circ}\text{C}$  until they were shipped frozen on dry ice to the Centers for Disease Control and Prevention (CDC) laboratory in Fort Collins, Colorado (USA). Serum samples were obtained from 70 hunter-killed, white-tailed deer (Adrian and Keiss, 1977) from the northern counties of Arkansas (USA) in 1990 and from 29 hunter-killed white-tailed and mule deer (*Odocoileus hemionus*) from Colorado in 1981 (Webb et al., 1987). The Colorado samples had been tested previously for antibody against CV virus and were all negative (R. G. McLean, unpubl.).

The laboratory procedures are described by McLean et al. (1985, 1987, 1993a). Briefly, the 39 serum specimens from the trapped animals and cattle were thawed and tested for virus by placing a 0.1 ml sample onto a monolayer of Vero cells (American Type Culture Collection, Rockville, Maryland, USA) in 6-well plastic plates. The samples were allowed to absorb for 1 hr at  $37^{\circ}\text{C}$  and were then overlaid with nutrient medium 199 containing 1% Noble agar (Difco laboratories Inc., Detroit, Michigan, USA) and 1:25,000 neutral red (GibcoBRL, Life Technologies Inc.). The cultures were incubated at  $37^{\circ}\text{C}$  in 5%  $\text{CO}_2$  for 10 days or until plaques were observed. Cultures with observable plaques were harvested and the supernatant fluid as well as the original serum specimen were inoculated onto other cell cultures. Virus isolates were initially screened by indirect immunofluorescence against virus grouping antibody preparations (National Institutes of Health, Bethesda, Maryland, USA). Specific identification was determined by serum-dilution plaque-reduction neutralization tests with

known viruses (Francy et al., 1990a; Karabatsos, 1985).

For serology, the blood samples collected from the Missouri deer were eluted from the filter paper strips by placing them overnight in 0.4 ml of 1 M borate buffer (pH 8.0) at  $4^{\circ}\text{C}$  (Wolff and Hudson, 1974) resulting in a 1:10 dilution of serum. Serum specimens heated at  $56^{\circ}\text{C}$  for 30 min were tested for neutralizing (N) antibody against POT virus (strain 89-3470), CV virus (strain M25097), TEN virus (strain A9-171B), eastern equine encephalitis virus (EEE, strain NJO/60), and western equine encephalitis virus (WEE, Fleming strain) (World Health Organization, Center for Reference and Research, Centers for Disease Control and Prevention, Fort Collins, Colorado) by the plaque-reduction neutralization test in Vero cell culture in 6-well plastic plates (McLean et al., 1985, 1987). Equal volumes of serum were mixed with each virus diluted to contain 100 to 200 plaque-forming units. The mixtures were incubated for about 16 hr at  $4^{\circ}\text{C}$  and then 0.1 ml of each was added to Vero cell cultures and allowed to absorb for 1 hr. The inoculated cultures were overlaid with agar medium containing neutral red and held at  $37^{\circ}\text{C}$  in 5%  $\text{CO}_2$  until plaques were counted. A reduction in plaque counts by 80% or more as compared with controls for serum specimens at a 1:20 dilution or higher were considered positive. Any serum specimens that were initially positive at less than a 1:20 dilution but were not positive at this dilution when retested were considered equivocal and counted as negative.

Three adult eastern chipmunks (*Tamias striatus*) captured as immature animals in Madison, Wisconsin, in 1988 were held in captivity along with chipmunks used in another experiment (McLean et al., 1993b) at the CDC laboratory in Fort Collins. They were inoculated subcutaneously with  $3.7 \log_{10}$  plaque-forming units of POT virus (strain 89-3470) to determine their reservoir potential for this virus. Blood samples were collected prior to inoculation, daily for the first 7 days post-inoculation (PI), and on day 30 PI from the orbital sinus with a capillary pipet. The blood samples (0.2 ml) were dispensed into 0.9 ml of field diluent, kept on wet ice, and centrifuged within 1 hr. The diluted serum specimens were removed and stored in labeled vials at  $-70^{\circ}\text{C}$  until tested. Aliquots (0.1 ml) of all of these serum specimens also were tested for virus isolation and for N antibody against POT virus as described. The data were analyzed by the Chi-square and Student's *t* tests (Sokal and Rohlf, 1981).

## RESULTS

Forty-five (25%) of 178 deer from Missouri tested in 1990, and 55 (30%) of 186

TABLE 1. Neutralizing antibody<sup>a</sup> against Potosi and Cache Valley viruses in wild deer populations.<sup>b</sup>

State	Year	Num- ber tested	Potosi % pos- itive	Cache Valley % pos- itive
Missouri	1990	178	25	NT <sup>c</sup>
Missouri	1991	186	30	16
Arkansas	1990	70	47	21
Iowa	1993	763	15	NT
Colorado	1981	29	7	0
Colorado <sup>d</sup>	1981	286	NT	19
Total: Potosi		1,226	20	
Cache Valley		571		18

<sup>a</sup> Neutralizing antibody as determined in the plaque-reduction neutralization test in Vero cell culture.

<sup>b</sup> Deer were collected by hunters during regulated hunting seasons. Deer in all states except Colorado were white-tailed deer (*Odocoileus virginianus*); mule deer (*O. hemionus*) were included in the Colorado samples.

<sup>c</sup> NT, not tested.

<sup>d</sup> R.G. McLean, unpubl.

deer sampled in 1991 were antibody positive to POT virus (Table 1). The 186 deer serum specimens from 1991 were tested further and 29 (16%) were also N-antibody positive to CV virus. Of 70 serum specimens from Arkansas deer in 1990, 33 (47%) had N antibody to POT and 15 (21%) to CV viruses; two (7%) of 29 CV virus antibody negative serum specimens from Colorado deer in 1981 were serologically positive to POT virus (Table 1). In 1993, 114 (15%) of 763 hunter-killed deer from Iowa were positive for N antibody to POT virus (Table 1). There were no significant regional differences in the antibody prevalence in deer within Iowa. There were statistically ( $P < 0.05$ ) significant differences in the POT antibody prevalences between all four states except between Iowa and Colorado; also a north-south cline from lowest in Iowa to highest prevalence in Arkansas was evident.

For the 186 specimens from Missouri white-tailed deer in 1991, there was a significantly ( $\chi^2 = 9.6$ ,  $P < 0.002$ ) higher antibody prevalence to POT virus ( $n = 55$ , 30%) than to CV virus ( $n = 29$ , 16%) and 10 (5%) were antibody positive to both viruses (Table 2). Four (6%) of the 70 spec-

TABLE 2. Comparative neutralizing antibody<sup>a</sup> results for Potosi (POT) and Cache Valley (CV) viruses from Missouri white-tailed deer, 1991.

		POT virus		
		Positive	Negative	Total
CV virus	Positive	10 (5%) <sup>b</sup>	19 (10%)	29 (16%)
	Negative	45 (24%)	112 (60%)	157 (84%)
	Total	55 (30%)	131 (70%)	186

<sup>a</sup> Neutralizing antibody as determined in the plaque-reduction neutralization test in Vero cell culture.

<sup>b</sup> (%), percent positive.

imens from Arkansas in 1990 were seropositive to both (Table 3). There were no significant differences in antibody prevalences of the 1990 and 1991 Missouri specimens to POT virus or the 1991 specimens to CV virus by age or sex. However, the antibody prevalences for adult deer ( $\geq 2\frac{1}{2}$  yr) were higher for both viruses: 83 (30%) of 278 versus 14 (19%) of 73 for POT virus and 24 (17%) of 143 versus three (8%) of 36 for CV virus. The geographic distribution of POT virus activity was not equal between two Missouri counties from which adequate samples were obtained in 1991. There was a significantly ( $\chi^2 = 10.8$ ,  $P < 0.001$ ) higher prevalence of POT antibody in Cape Girardeau County (38 of 93, 41%) than in Washington County (11 of 70, 16%) where the tire

TABLE 3. Comparative neutralizing antibody<sup>a</sup> results for Potosi (POT) and Cache Valley (CV) viruses from Arkansas white-tailed deer, 1990.

		POT virus		
		Positive	Negative	Total
CV virus	Positive	4 (6%) <sup>b</sup>	11 (16%)	15 (21%)
	Negative	29 (41%)	26 (37%)	55 (79%)
	Total	33 (47%)	37 (53%)	70

<sup>a</sup> Neutralizing antibody as determined in the plaque-reduction neutralization test in Vero cell culture.

<sup>b</sup> (%), percent positive.

TABLE 4. Ticks removed from mammals captured in Potosi, Washington County, Missouri in September, 1990.<sup>a</sup>

Animal species	Num- ber cap- tured	Num- ber with ticks	Tick species and stage <sup>b</sup>											
			Aa		Dv		It		Im		Ic		Hlp	
			L	N	L	N	L	N	L	N	L	N	L	N
Opossum	11	4	7	0	0	0	0	0	0	0	0	0	0	0
Raccoon	6	6	120	3	2	0	21	2	0	0	0	4	0	0
Eastern cottontail	2	2	0	0	0	0	0	0	0	0	0	0	9	8
Fox squirrel	1	1	0	0	0	0	0	0	2	0	0	0	0	0

<sup>a</sup> No ticks were collected from an additional two white-footed mice, two eastern chipmunks, and one eastern woodrat.<sup>b</sup> Aa, *Amblyomma americanum*; Dv, *Dermacentor variabilis*; It, *Ixodes texanus*; Im, *Ixodes marxi*; Ic, *Ixodes cooki*; Hlp, *Haemaphysalis leporispalustris*; L, larvae, and N, nymph.

yard near Potosi was located. In addition, the POT virus antibody prevalence in Arkansas deer (47%) in 1990 was significantly higher than the 1990 (25%,  $\chi^2 = 10.8$   $P = 0.001$ ), 1991 (30%,  $\chi^2 = 6.5$   $P = 0.01$ ), or combined (27%,  $\chi^2 = 10.3$   $P = 0.001$ ) prevalences of Missouri deer. There was also a higher prevalence of CV antibody in Cape Girardeau County (19 of 93, 20%) than in Washington County (8 of 70, 11%) but it was not statistically significant. None of the 186 deer from Missouri in 1991 had N antibody to EEE virus and two (0.3%) of 763 deer from Iowa in 1993 had N antibody to WEE virus.

Only six rodents, two white-footed mice (*Peromyscus leucopus*), two eastern chipmunks, one eastern woodrat (*Neotoma floridana*), and one fox squirrel (*Sciurus niger*), and two cottontail rabbits (*Sylvilagus floridanus*) were captured during the sampling period, for a frequency of 0.006 rodents per TN in Sherman traps and 0.02 small mammals per TN in small live traps. In contrast, 18 medium-sized mammals, 11 opossums (*Didelphis virginiana*), six raccoons (*Procyon lotor*), and one domestic cat (*Felis catus*), were captured for a frequency of 0.14 per TN. Seven box turtles were captured by hand. No virus isolations were made from the 33 vertebrate animals captured around the tire yard. Only one eastern chipmunk among the 33 small-sized and medium-sized animals sampled, and none of six cattle had N an-

tibody to POT virus; none of these animals had antibody to CV and TEN viruses.

None of the three eastern chipmunks experimentally inoculated with POT virus became viremic but all developed N antibody. The chipmunks were serologically negative prior to inoculation and N antibody was first detected on day 5 PI in one chipmunk. All three chipmunks were positive on day 30 PI with high antibody titers: mean ( $\pm$ SE) of  $533 \pm 107$  and range of 320 to 640 reciprocal antibody titers. The naturally infected chipmunk captured at the tire dump had a titer of 1:80.

Six species of ticks (Table 4) and four species of fleas were collected from the mammals captured at the tire yard. The mammal species most commonly infested with ticks was the raccoon in which all six animals were infested with four different tick species; in contrast, only one fox squirrel of the four rodent species sampled had ticks. The most common tick found was *Amblyomma americanum*, and the *Ixodes marxi* from the fox squirrel is a new state record for Missouri (L.A. Durden, pers. comm.). The fleas collected were *Ctenocephalides felis*; there was one each on two raccoons and one opossum, and two on a second opossum. One *Ctenophthalmus pseudagyrtis* and three *Orchopeas howardi* were collected on a third opossum and two *Orchopeas sexdentatus* on the eastern woodrat.

## DISCUSSION

The high prevalences of N antibody against POT virus in the deer populations of the region for two successive years and in Iowa in 1993, and the low antibody prevalences in other mammalian species is evidence that deer may be the natural reservoir host for this virus as it is for Jamestown Canyon (JC) virus in the upper midwestern states (Issel et al., 1972; Issel, 1973). It was surprising that there was not a significant increase in the prevalence of POT and CV antibody with age of deer as occurs with enzootic JC virus (Boromisa and Grimstad, 1987). However, insufficient samples of the various age groups were tested in this study to be sure that an increase in antibody prevalence with age did not occur with POT virus as well. The susceptibility and reservoir potential of white-tailed deer to Potosi virus are unknown and can best be determined by experimental infection studies. The impact of POT virus on the health of deer is also unknown, but a closely related virus, CV virus, was isolated from a moribund caribou (*Rangifer tarandus terraenovae*) and a moribund horse (*Equus caballus*) in Wisconsin (Hoff et al., 1970), from an asymptomatic horse in Michigan (McLean et al., 1987), and from sick domestic animals in Texas (Calisher et al., 1986). There is evidence that CV virus infects humans and may produce clinical disease (Calisher and Sever, 1995), but there is little information available on POT virus infections in humans.

The natural mosquito vector of POT virus is also not known although isolations of the virus have been obtained from *Cochillettidia perturbans*, *Culex restuans*, and *Psorophora columbiae* (Mitchell et al., 1995). Even though *Ae. albopictus* mosquitoes were susceptible to and vector-competent for POT virus (Mitchell et al., 1990; Heard et al., 1991), they were probably not an important natural vector for transmission in east central Missouri because the prevalence of POT virus anti-

body in deer was significantly higher in Cape Girardeau County than in Washington County where the tire-yard populations of *Ae. albopictus* were located.

Initially, samples were tested for antibody against all three bunyaviruses that might be present in Missouri. There were no serologic reactors against TEN virus and there was little or no cross reaction between POT and CV viruses in the N test since most of the positive samples for each virus were monotypic (Tables 2, 3). The low prevalence (5 to 6%) of antibody to both viruses in some samples was probably due to dual infections since both viruses occur in Missouri and Arkansas (Calisher et al., 1986; Francy et al., 1990a). Potosi virus may have a broad distribution similar to CV virus (Calisher et al., 1986) since antibody positive deer were detected in four of six counties (only three samples were collected in the two negative counties) in Missouri, throughout Iowa, and in deer from Arkansas and Colorado. In addition, POT virus has been isolated from mosquitoes in other midwestern and southeastern states (Mitchell et al., 1995; Harrison et al., 1995). If the north to south increase in POT antibody prevalence in deer from Iowa to Arkansas is valid, it may be due to latitudinal clines that affect breeding patterns of hosts and vectors; mosquito species composition, distribution, and abundance; and temperature and other environmental factors. Some of these factors could affect the efficiency of POT virus transmission. The current distribution of *Ae. albopictus* could also partially explain the higher prevalences of virus activity farther south since this species has a wider distribution and greater abundance in the southern states of the U.S. (Francy et al., 1990b).

The serum specimens collected with filter paper strips from Missouri deer in 1991 were also tested for N antibody against EEE virus to be sure that nonspecific, false positive reactors were not occurring with this collection method. The reliability of the filter paper method was

strongly supported since all 186 sera were negative for N antibody to EEE virus and these negative data were in agreement with the known distribution of EEE virus activity in the United States (McLean et al., 1985; Moore et al., 1993). Also by using a 1:20 dilution or higher for positive samples, the occurrence of nonspecific serologic reactors also should have been reduced (Sudia et al., 1975). As in previous studies (Ubico et al., 1988), the filter paper strip method proved useful in collecting blood samples for serologic testing and was particularly useful in collecting specimens from carcasses of hunter-killed deer at check stations. The low antibody prevalence (0.3%) against WEE virus in the 763 Iowa deer is evidence that nonspecific serologic reactors did not occur and validates the blood collection procedure used in Iowa. The results are in agreement with the known distribution of WEE virus (Moore et al., 1993).

Surprisingly, few rodents were captured in the woods, old field, and the area around the lake in September when rodents are usually quite abundant. The structure of the various habitats sampled appeared quite adequate to support a large rodent population, but the availability of food was not investigated. More eastern chipmunks were observed but not captured around the periphery of the tire dump site which, in addition to finding one chipmunk serologically positive to POT virus, was another reason that we experimentally infected this species. The chipmunks in this experimental infection study did not develop detectable viremias; however, experimentally-inoculated hamsters (*Mesocricetus auratus*) were moderately susceptible (44 to 50% viremic) and developed low to moderate viremia titers of short duration (Mitchell et al., 1990; Heard et al., 1991). All but one of the 12 hamsters inoculated developed N antibody against POT virus (Heard et al., 1991) and all three of the chipmunks in this study developed high-titered antibody. Even though rodents may not be reservoir-com-

petent for POT virus, they produce adequate antibody responses so that they could still be used as sentinels for this virus (McLean, 1991; Moore et al., 1993).

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