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Source: Journal of Wildlife Diseases, 23(1) : 23-33

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-23.1.23

Journal of Wildlife Diseases, 23(1), 1987, pp. 23–33

SEROCONVERSION RATES TO JAMESTOWN CANYON VIRUS AMONG SIX POPULATIONS OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) IN INDIANA

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ABSTRACT: The annual seroconversion of fawns, yearlings, and adult white-tailed deer (*Odocoileus virginianus*) to Jamestown Canyon virus (California group) was followed at six Indiana sites from 1981 through 1984. In all, sera from 1,642 deer (515 fawns, 618 yearlings, and 509 adults) were tested for neutralizing antibody to three California serogroup viruses: Jamestown Canyon, La Crosse, and trivittatus. Virtually all deer with specific neutralizing antibody showed evidence of a prior infection with Jamestown Canyon virus; only three deer showed evidence of a prior infection with only La Crosse virus and none showed evidence of an infection with only trivittatus virus. While there were no significant differences in antibody prevalence to Jamestown Canyon virus between yearling and adult deer at any site, fawns had significantly lower antibody prevalences than either of the two older age groups. Significant differences in antibody prevalence were found between northern versus southern populations of white-tailed deer in Indiana, however, no significant differences were found among the four northern populations or between the two southern populations. The mean antibody prevalences in the two southern fawn, yearling, and adult populations were 15%, 38%, and 41% respectively, while the prevalences in the four northern fawn, yearling, and adult populations were 5%, 67%, and 67% respectively. These different prevalences (northern vs. southern) correlate with the higher Jamestown Canyon virus antibody prevalence in human residents of northern Indiana (2–15%) compared to residents of southern Indiana (<2%) found in other studies. The significantly lower prevalence of antibody to Jamestown Canyon virus in fawns is attributed to maternal antibody protecting them from a primary infection their first summer. Yearling deer showed high rates of seroconversion following their second summer of life. These results suggest that infection of white-tailed deer in Indiana with Jamestown Canyon virus is a common phenomenon.

INTRODUCTION

Jamestown Canyon (JC) virus, a subtype of Melao virus of the California serogroup (Bunyaviridae: *Bunyavirus*), was first isolated from mosquitoes in Colorado in 1961 (Sather and Hammon, 1967). Subsequent isolations have come from numerous species of mosquitoes and a few tabanids (deer-flies, horse-flies); these extensive records were summarized by Grimstad (1983).

Trainer and Hanson (1969) reported that white-tailed deer, *Odocoileus virginianus*, were sensitive indicators of the presence of many arbovirus infections because of their wide distribution, large populations, non-migratory behavior, and the ease of estimating the age of the animals. Indeed, the sole vertebrate isolation of JC virus reported to date was from a yearling white-tailed deer used as a sentinal animal (Issel, 1973). Subsequent studies by numerous workers have demonstrated that the white-tailed deer was the primary, and perhaps exclusive, vertebrate host in the enzootic cycle of JC virus throughout much of North America (Issel et al., 1972a, b, 1973; Hoff et al., 1973; Issel, 1973, 1974; LeDuc, 1979; Watts et al., 1979, 1982; Grimstad et al., 1987). Data revealed that a high percentage of deer were infected in nature and experimental JC virus infection of deer produced viremias lasting up to five

Received for publication 14 October 1985.
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days and reaching maximum titers of 5.0 $\log_{10}$ median suckling mouse lethal doses per ml prior to the development of neutralizing antibody.

Issel (1974) hypothesized that JC virus maternal antibody in the colostrum of previously infected does might protect white-tailed deer fawns during their first summer of life. This would presumably result in the availability of susceptible yearling deer the following season, and foster annual transmission of JC virus.

The objectives of our research are to describe the natural transmission and maintenance cycle of JC virus by examining the biological relationships among potential arthropod vectors, white-tailed deer (and any other possible vertebrate hosts), and JC virus strains. This report describes one key objective: a multi-year monitoring of Indiana deer populations in JC virus enzootic foci to determine antibody prevalence and seroconversion rates.

MATERIALS AND METHODS

Study sites

Six study sites were chosen for their geographical proximity to areas of Indiana where an earlier study (Grimstad, 1983; Grimstad et al., 1984) showed elevated human antibody prevalence to JC virus (Fig. 1). Lindsey et al. (1969) described eight natural divisions in Indiana based on their geological and biological environments. In the present study, four of the eight natural divisions were represented by the following six study sites (Fig. 1):

(1) The Kingsbury State Fish and Wildlife Area, LaPorte County, encompasses 810 ha of oak woods, open prairies, and marshlands that provide ideal habitats for a variety of potential vector species and supports a deer population estimated at 2,000. This site is ecologically and geologically characteristic of the Northwestern Prairie and Wetlands Division, and also is within a California serogroup virus focal area where elevated human JC virus antibody prevalences had earlier been noted.

(2) Pigeon River State Fish and Wildlife Area, LaGrange County, is typical of the Northeastern Moraine and Kettle Division; its 4,655 ha encompass several lakes, open grassy areas, and dry oak-type upland woods. Pigeon River is situated within a prominent JC virus focal area which extends north into Michigan (Grimstad et al., 1987).

(3) Jasper-Pulaski State Fish and Wildlife Area, in Jasper, Pulaski, and Starke counties, encompasses 3,246 ha of wetlands and upland game habitats; this property is also characteristic of the Northwestern Prairie and Wetlands Division and is within the same JC virus focal area as Kingsbury.

(4) Willow Slough State Fish and Wildlife Area, Newton County, is also characteristic of the Northwestern Prairie and Wetlands Division; it encompasses 3,885 ha of hardwood uplands, open grasslands, and wetlands. Human JC virus antibody prevalence in the Willow Slough area is apparently lower than in the two previous Northwestern Prairie sites.

(5) Crane Naval Ammunition Depot (Daviess, Green, Lawrence, and Martin counties) is characteristic of the South-Central Oak and Mixed Woods Division; it encompasses 25,091 ha of hardwood uplands, numerous creeks, water storage ponds, and a 344 ha reservoir. Crane is located in an area of low human antibody prevalence to JC virus.

(6) Atterbury State Fish and Wildlife Area in Bartholomew, Brown, and Johnson counties, is characteristic of the Tipton Till Plain Beech-Maple Division. Atterbury covers 2,230 ha of upland game habitat, 81 ha of marsh and shallow impoundments, and is also located in an area where the human antibody prevalence to JC virus is low.

Blood collection procedure

Blood samples were collected in the fall of 1981–1984 from the body cavity of field-dressed deer at the check stations in each of the six study sites. Also hunters were utilized at Kingsbury in 1981 and 1982 for making sample collections at the kill sites. Blood samples were collected in 7 cc tubes and refrigerated for 1–7 days at the six sites until transported to the laboratory for serological testing. In the laboratory, blood samples were centrifuged at 4 C for 15–30 min at 2,000 *g* and the sera aliquoted into individually labeled vials. Sera were stored at −70 C until assayed for California serogroup virus antibody at the end of the hunting season each year.

Age of harvested deer was determined by Indiana Department of Natural Resource personnel at the respective check stations using standard tooth eruption and wear techniques (Taber, 1963). Criteria for aging deer were: 1) *fawns* were those animals less than 1 yr old; 2) *yearling* deer were those older than 1 yr but less than 2 yr old (born the previous year); and

3) *adult* deer were deer ≥2 yr of age. The sex of each deer also was recorded for each sample.

Virus neutralization tests

All deer sera were initially diluted 1:8 in Medium-199 with Earle's salts (supplemented with 10% heat-inactivated fetal bovine serum, 200 units of potassium penicillin G, 200 mcg of streptomycin sulfate, and 250 mcg of Amphotericin B per ml of media) and heat inactivated at 56 C for 1 hr. Antibody titers were determined using the previously described microtiter serum dilution neutralization (SDN) test in African Green Monkey (Vero) cells (Lindsey et al., 1976; Grimstad et al., 1984).

Three California virus subtypes, JC, La Crosse (LAC), and trivittatus (TVT) (the only ones known to occur in Indiana) were used in the SDN tests. The prototype strains of JC and TVT viruses were obtained from the reagents bank of the Vector-Borne Viral Diseases Division of the Centers for Disease Control, Ft. Collins, Colorado 80522, USA. The LAC virus was an Indiana isolate (Pinger et al., 1983) that is very closely related to the prototype LAC virus based on the oligonucleotide fingerprint pattern (Klimas et al., 1981). JC, LAC, and TVT mouse hyperimmune ascitic fluids (MHAF) were prepared in 6-wk-old albino mice (ICR strain, Harlan Industries, Indianapolis, Indiana 46229, USA) as described by Tikasingh et al. (1966). A virus test dose of 100 median tissue culture infectious doses (100 $TCID_{50}$) per 0.025 ml was used with each of the three viruses, as was a Vero cell suspension of 30,000 cells per ml for all antibody determinations. Each serum sample was serially diluted (two-fold from 1:8 to 1:512) and tested for antibody specific to each of the three viruses by SDN tests employing 96-well plastic trays (Linbro®, Flow Laboratories, McLean, Virginia 22102, USA). Antibody titers were reported as the highest dilution showing less than 50% cytopathic effects (CPE) after a 5 day incubation period at 37 C in a 5% CO_2 atmosphere. A ≥ four-fold difference in antibody titers was considered evidence of prior infection with either JC, LAC, or TVT virus. If the serum neutralized more than one virus by less than a four-fold difference, we considered that as evidence of a prior California serogroup infection of the individual deer, but a sample for which the etiologic agent(s) could not be identified further.

Statistical evaluations

Calculation of *P* values reported below for the Chi-square contingency table analyses was performed according to the methods of Steel and Torrie (1980). A conservative *P* value was used throughout these analyses to detect statistically significant differences. Exact *P* values were calculated by using the IMSL subroutine-MDCH (Chi-square Probability Distribution Function, IMSL, Inc., Houston, Texas 77036, USA, 1982) on an IBM 370/168 mainframe computer.

RESULTS

Antibody prevalence to JC virus between age groups

The prevalence of neutralizing antibody to JC virus determined for the three age groups of deer sampled from the six sites is summarized in Table 1. Fawns (0.5-yr age group) had a lower antibody prevalence to JC virus than either yearling (1.5-yr age group) or adult (2.5+-yr age group) deer at all sites. These differences were especially noticeable for deer populations at Jasper-Pulaski, Kingsbury, and Pigeon River because of larger sample sizes collected every year. While there was no significant difference in antibody prevalence to JC virus between the 1.5-yr and 2.5+-yr age groups at any site, antibody prevalence was almost always significantly lower for the 0.5-yr age group than for the 1.5-yr and 2.5+-yr age groups by year of collection with several exceptions (Table 2): (1) Sera collected at Atterbury showed no significant differences in JC virus antibody prevalence in the 1982 0.5/1.5-yr and in the 1984 0.5/2.5+-yr age groups; (2) sera collected at Crane in 1983 and in 1984 were not significantly different for the 0.5/1.5-yr or the 0.5/2.5+-yr age groups; and (3) differences in comparisons with the 0.5-yr cohort in the 1982 Jasper-Pulaski and in the 1982 and 1983 Willow Slough sera undoubtedly were affected by the small sample sizes in several cells and calculated *P* values were probably meaningless.

Antibody prevalence to JC virus by site by year

There were no significant differences in the prevalence of JC virus antibody with-

in the three age groups at Jasper-Pulaski and Pigeon River between the years 1982 through 1984 (Table 3). Statistically significant year by year differences in antibody prevalence to JC virus within age groups at other sites only occurred when a year was compared with 1984. This suggests that 1984 may have been a year of changing antibody prevalence to JC virus at sites other than Jasper-Pulaski and Pigeon River, the only populations that did not show a significant difference.

Differences in antibody prevalence by sex within age groups

There was essentially no difference in antibody prevalence to JC virus in bucks vs. does throughout this study ($P > 0.05$); however, there were a few exceptions that were probably the result of either small sample size or a highly skewed sex ratio.

Antibody prevalence to JC virus between the different study sites

In 1982, there were no significant differences in antibody prevalence for deer among the six study sites, except for the 1.5-yr age group comparison between Crane and Pigeon River ($P = 0.0018$). Antibody was detected in 47% of the 1.5-yr age group from Crane and 84% of the same age group from Pigeon River (Table 1). Pigeon River is located in northern Indiana while Crane is in the southern region (Fig. 1).

In 1983 again, the difference in antibody prevalence was between deer from northern and southern study sites. Significant differences in antibody prevalence to JC virus were observed between deer from Crane and three sites—Kingsbury, Pigeon River, and Willow Slough—in the 1.5-yr age groups (Crane vs. Kingsbury, $P = 0.0011$; Crane vs. Pigeon River, $P = 0.0010$; and Crane vs. Willow Slough, $P = 0.0000062$) and between deer from Crane vs. Kingsbury in the 2.5+-age group ($P = 0.000054$). In the 1.5-yr age group, the antibody prevalence was 34% at Crane and 72%, 67%, and 92% at Kingsbury, Pigeon River, and Willow Slough respectively (Table 1). In the 2.5+-age group, 43% of the sera from Crane and 90% of the sera from Kingsbury had antibody to JC virus (Table 1).

In 1984, the same difference in antibody prevalence in northern vs. southern deer populations was observed. In the 1.5-yr age group, antibody prevalences in Jasper-Pulaski deer were significantly different from Crane and Atterbury deer ($P = 0.0011$ and 0.0000027 respectively); Pigeon River deer were also significantly different from Crane and Atterbury deer for the same age group ($P = 0.0089$ and 0.0000017 respectively). Atterbury and Crane sera had antibody prevalences of 31% and 18% respectively, while Jasper-Pulaski and Pigeon River sera had antibody prevalences of 73% and 75% respectively (Table 1). In the 2.5+-yr age group, only Crane and Pigeon River prevalences (11% and 54% respectively) were significantly different ($P = 0.00022$).

Antibody to other California group viruses

Three deer from Atterbury had LAC virus neutralizing antibody (one detected each year, 1982–1984), while no deer at any site had specific antibody only to TVT virus. A number of animals were found with cross-reactive (non-specific) antibody to JC and TVT or JC, TVT and LAC viruses; titers of these sera were < four-fold different for JC vs. TVT virus (rarely JC vs. LAC). The antibody titers fit a pattern of JC > TVT ≥ LAC; this was the pattern in the SDN tests where ≥ four-fold differences were seen for the respective viruses.

DISCUSSION

The results of this 4-yr serological survey of six populations of white-tailed deer in Indiana demonstrated an overall high prevalence of exposure to JC virus. While

TABLE 1. Antibody prevalence to Jamestown Canyon virus in six populations of white-tailed deer in Indiana, 1981–1984.

Site and year of collection	n[a]	Age group			
		0.5 yr	1.5 yr	2.5+ yr[b]	All ages
Atterbury					
1982	56	10 (20)[c]	42 (12)	67 (24)	41
1983	87	18 (22)	57 (35)	57 (30)	47
1984	77	4 (23)	31 (29)	20 (25)	19
Crane					
1982	97	18 (28)	47 (36)	52 (33)	40
1983	115	22 (36)	34 (44)	43 (35)	33
1984	128	19 (31)	18 (49)	11 (48)	18
Jasper-Pulaski					
1982	17	25 (4)	40 (10)	33 (3)	29
1983	63	8 (12)	61 (33)	56 (18)	49
1984	49	0 (14)	73 (30)	60 (5)	51
Kingsbury					
1981	108	4 (46)	82 (27)	82 (35)	49
1982	120	5 (58)	64 (39)	83 (23)	39
1983	91	5 (38)	72 (32)	90 (21)	48
1984	119	2 (41)	45 (51)	52 (27)	32
Pigeon River					
1982	91	16 (37)	84 (31)	83 (23)	56
1983	148	6 (36)	67 (55)	72 (57)	54
1984	123	2 (52)	75 (28)	54 (43)	36
Willow Slough					
1982	28	0 (1)	72 (18)	89 (9)	75
1983	45	33 (3)	92 (24)	78 (18)	82
1984	80	0 (13)	43 (35)	44 (32)	36
Total tested:	1,642	(515)	(618)	(509)	
% of total:		(31.4)	(37.6)	(31.0)	

[a] Number of samples tested.
[b] Deer 2.5 yr of age or older.
[c] Percent with antibody (number of samples assayed).

there was no significant difference in antibody prevalence to JC virus between yearling and adult deer, there were varying degrees of significant differences in antibody prevalence between fawns and the two older age classes. Issel and coworkers reported lower antibody prevalence rates in fawns in Wisconsin and speculated on three possible explanations (Issel et al., 1972b): 1) a sampling error based on low numbers of fawns sampled; 2) the fawns were not exposed to the virus; or 3) the fawns were protected by maternal antibody against infection. Since only 8.5% of their sample (50/587) were fawns, a sampling error seemed plausible.

In our study, 31.4% (515/1,642) of the deer sampled were fawns and we also saw a lower antibody prevalence rate in fawns vs. older age groups with 10 times the number of fawns tested compared to the Wisconsin study. Thus, the low rate in fawns reported by Issel et al. (1972b) was probably not due to sampling bias. DeFoliart and coworkers isolated JC virus from naturally infected late spring and

TABLE 2. Comparison of age groups of white-tailed deer in six Indiana populations for antibody to Jamestown Canyon virus using two by two chi-square contingency table analyses.

Site and year of collection	P value for age group comparisons[a]		
	0.5 yr/1.5 yr	0.5 yr/2.5+ yr	1.5 yr/2.5+ yr
Atterbury			
1982	0.036	0.00014*	0.15
1983	0.0038*	0.0053*	1.00
1984	0.0150*	0.10	0.36
Crane			
1982	0.014*	0.0063*	0.73
1983	0.24	0.063	0.43
1984	0.73	0.75	0.82
Jasper-Pulaski			
1982	0.59	0.81	0.84
1983	0.0024*	0.0086*	0.72
1984	0.66^{-5}*	0.0016*	0.54
Kingsbury			
1981	0.60^{-7}*	0.60^{-7}*	0.89
1982	0.60^{-7}*	0.60^{-7}*	0.12
1983	0.60^{-7}*	0.60^{-7}*	0.10
1984	0.43^{-5}*	0.25^{-5}*	0.57
Pigeon River			
1982	0.60^{-7}*	0.16^{-5}*	0.89
1983	0.60^{-7}*	0.60^{-7}*	0.60
1984	0.60^{-7}*	0.60^{-7}*	0.068
Willow Slough			
1982	0.13	0.035	0.33
1983	0.0070*	0.12	0.20
1984	0.0044*	0.0041*	1.00

[a] *P* values are based on the chi-square 2 × 2 contingency table comparisons between two age classes (fawns vs. yearlings, fawns vs. adults, and yearling vs. adults) by site by year. The exact *P* value for each pairwise comparison was obtained with the IMSL subroutine—MDCH program; *P* values reflect a comparison of the three paired age classes' chi-square values for each site by year and where the significance level is $P < 0.017$ (0.05/3 = 0.017). Significant pairwise comparisons are designated by an asterisk and negative numbers refer to negative powers of 10.

TABLE 3. Comparison of antibody prevalences for Jamestown Canyon virus between years in six populations of white-tailed deer in Indiana using two by two chi-square contingency table analyses.

Site and year comparison	P value for age groups[a]		
	0.5 yr	1.5 yr	2.5+ yr
Atterbury			
1982 vs. 1983	0.70	0.35	0.45
1982 vs. 1984	0.47	0.51	0.00097*
1983 vs. 1984	0.14	0.037	0.0057*
Crane			
1982 vs. 1983	0.66	0.23	0.48
1982 vs. 1984	0.89	0.0043*	0.00086*
1983 vs. 1984	0.76	0.10	0.0085*
Jasper-Pulaski			
1982 vs. 1983	0.55	0.25	0.48
1982 vs. 1984	0.052	0.052	0.46
1983 vs. 1984	0.27	0.29	0.89
Kingsbury			
1981 vs. 1982	0.84	0.13	1.00
1981 vs. 1983	0.84	0.39	0.43
1981 vs. 1984	0.57	0.0020*	0.0086
1982 vs. 1983	0.68	0.48	0.45
1982 vs. 1984	0.49	0.073	0.022
1983 vs. 1984	0.51	0.017	0.0042*
Pigeon River			
1982 vs. 1983	0.14	0.11	0.32
1982 vs. 1984	0.011*	0.40	0.019
1983 vs. 1984	0.33	0.48	0.057
Willow Slough			
1982 vs. 1983	0.51	0.094	0.48
1982 vs. 1984	1.00	0.043	0.016*
1983 vs. 1984	0.033	0.00014*	0.020

[a] A significance level of $P < 0.017$ (0.05/3) was used for all sites except Kingsbury where a value of $P < 0.0083$ (0.05/6) was used. Significant pairwise comparisons are designated by an asterisk.

early summer *Aedes* mosquitoes and tabanids at a time when virus apparently was circulating in white-tailed deer in Wisconsin (DeFoliart et al., 1969). Additionally, Wright and DeFoliart (1970) showed that a variety of these late spring and early summer mosquitoes fed on fawns. Thus, it seems reasonable to assume that fawn deer would also be fed on by these and other potential vectors that apparently transmit JC virus to older susceptible deer, and the second alternative explanation of Issel et al. (1972b) is not likely.

Our study confirms Issel's third alternative, that fawns are protected from infection their first summer of life by maternal antibody. Maternal antibody from colostrum persists in fawns for 8–23 wk (mean of 19 wk) and can protect them

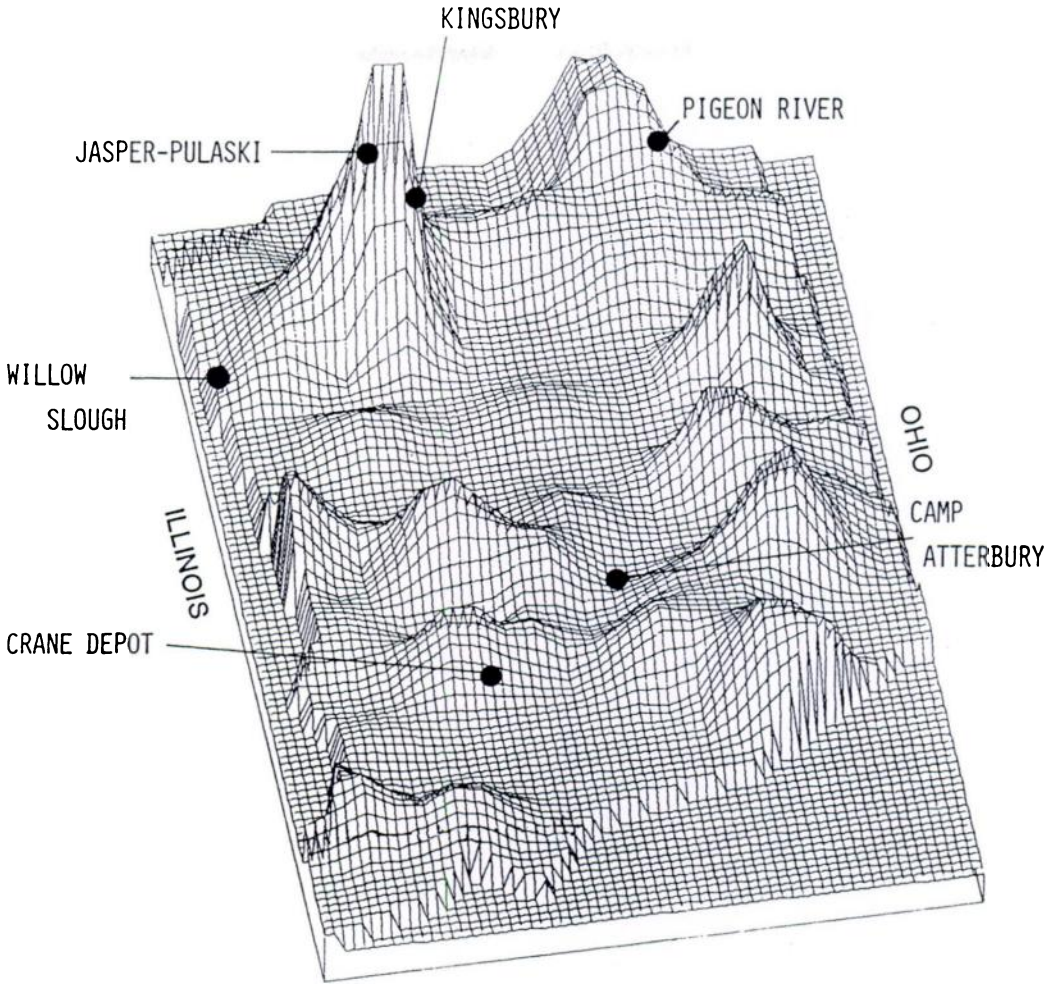

FIGURE 1. Location of the six study sites in Indiana *superimposed on* an ASPEX-computer drawn map (Grimstad et al., 1984) depicting the prevalence of antibodies to Jamestown Canyon virus in the human population.

from an experimental (Issel, 1974) and a natural vector-transmitted (Grimstad et al., 1987) JC virus infection. These fawns then contribute to the pool of susceptible vertebrate hosts, as yearling deer, the following season. A high antibody prevalence to JC virus in adult does provides protection the following spring to their newborn fawns and explains the low antibody prevalence in fawns harvested at least 18 wk after birth in the four northern Indiana sites. In northern Michigan, where 100% of does are seropositive, all fawns are seronegative in the fall (Grimstad et al., 1987) as would be expected from our results here. Thus the continuation of the natural cycle of JC virus in Indiana, Michigan, and probably elsewhere throughout its North American range, is assured by the presence of numerous susceptible yearling deer each season.

There was no significant difference in antibody prevalence to JC virus within the three age groups between the years 1982 and 1984 at Jasper-Pulaski and Pigeon River, and the same observation was made

at all the other four sites (including the 1981–1984 period at Kingsbury) except when comparisons were made with 1984. The significantly lower antibody prevalence in 1984 vs. the other years is perhaps explained by the trend of the 1981–1984 climatological conditions and the resulting marked variation in mosquito populations.

Among the six sites for the 1981–1984 period we have complete climatological and mosquito population records only for Kingsbury. The completeness of mosquito collection records made by local control agencies in close proximity to the other five sites is open to question. Adult populations of spring *Aedes* at Kingsbury (including *Aedes stimulans* and other potential vectors) were abundant in the spring of 1981 (Boromisa, 1985). Floodwaters resulting from snow-melt assured suitable aquatic habitats and, importantly, the pools of water persisted for some time that year. In 1982, the snow melted rapidly, pools filled with water and spring species hatched. Then a period of unseasonably cool weather set in, the vernal pools at Kingsbury dried up before the majority of adult mosquitoes could emerge. In 1983, spring rains filled *Aedes* breeding sites with sufficient water to allow for large adult emergences at Kingsbury. However, by late May a prolonged drought period with "record-breaking heat" (Leiser and Craig, 1984) began that persisted into early fall throughout the upper Midwest. This drought decimated the mosquito population, reducing virtually all populations to the lowest levels seen in years in some areas (Leiser and Craig, 1984). In 1984 at Kingsbury, and in much of northern Indiana, a cool dry spring contributed to a small population of *Ae. stimulans* (Boromisa, 1985); elsewhere other species were "almost rare" (Craig, 1985). Thus, spring mosquito populations in the Kingsbury area of northern Indiana were highest in 1981, markedly declined in 1982, rose in 1983 but to levels much below 1981, then drastically declined in 1984 (Boromisa and Grimstad, 1986).

Although these years of reduced populations of spring mosquitoes were not consecutive at each site (Boromisa, 1985), a statistically significant decrease in the 1984 antibody prevalences relative to the previous years was noticed. At Kingsbury in 1981 with normal population levels of spring *Aedes* mosquitoes, the antibody prevalence to JC virus was 82% for both of the older age groups (Table 1). In 1982, the spring *Aedes* populations (especially *Ae. stimulans*) were low and this presumably resulted in a marked (though not statistically significant; $P = 0.13$, Table 3) reduction in antibody prevalence to 64% from 82% in 1981 for the 1.5-yr age group (Table 1). There was no real difference in antibody prevalence in the 2.5+-yr group between 1981 and 1982 ($P = 1.00$, Table 3), likely due to the fact that the majority of this cohort had already seroconverted to JC virus in previous years and would not continue to rise significantly in antibody prevalence in succeeding years. In 1983, the population of spring *Aedes* was greater than in 1982; this resulted in a marked increase in antibody prevalence to JC virus for the 1.5-yr age group from 64% to 72% ($P = 0.48$, Table 3), and the 2.5+-yr group from 83% to 90% ($P = 0.45$, Table 3) in 1983 at Kingsbury as compared to 1982 (Table 1). In 1984, there was a significant decrease ($P = 0.0042$; Table 3) in antibody prevalence in the 1.5-yr and 2.5+-yr age groups at Kingsbury when compared to 1983 (Table 1) paralleling 10 ten-fold decrease in the population of *Ae. stimulans* in 1984 (Boromisa and Grimstad, 1986).

These mosquito data are relevant because these early spring *Aedes* species include the primary vector(s) of JC virus to deer and a reduction in their spring populations should result in marked decreases in seroconversion rates; while the de-

creases in seroconversion may or may not be statistically significant, the trends have shown a decline in all cases. In addition, population levels of *Ae. stimulans* are important to monitor because we have demonstrated the vector potential of *Ae. stimulans* in the laboratory, including transmission to vertebrates, and evidence for its transovarial transmission of JC virus by that vector (Boromisa and Grimstad, 1986).

These data suggest that JC virus transmission to white-tailed deer is directly correlated with the size of the spring *Ae. stimulans* mosquito population in northern Indiana and the persistence of that population into early summer. As other vectors are specifically identified throughout the North American range of JC virus, we would expect that similar relationships would be noted.

Trainer and Hanson (1969) reported no significant differences in antibody prevalence to JC virus between male and female deer. No significant differences were detected between the sexes in our study except for those few attributable to small sample size and/or a skewed sex ratio.

The deer populations in the four northern Indiana sites were never significantly different from each other in antibody prevalence rates. Also, the two southern Indiana populations also were never significantly different from each other in antibody prevalence to JC virus. However, there were significant differences between the northern vs. the southern sites. This pattern is similar to that seen in the Indiana human population; a higher prevalence of antibody to JC virus has been detected in residents of northern counties (as high as 15%) as compared to southern counties (only as high as 2%; Grimstad, unpubl. data). Thus, in Indiana, the human antibody prevalence and the deer antibody prevalence parallel each other, suggesting a close link between the deer as vertebrate host and human infection. In Michigan, we have documented also the close correlation of prevalence of JC virus antibody in adult residents with the size of the deer population on a regional basis (Grimstad et al., 1986).

The high prevalence of antibody to JC virus in all deer populations examined may in part mask the serologic evidence of LAC and/or TVT virus infection in these animals. Deer sera having cross-reactive neutralization may represent infection with multiple serotypes. Three LAC-infected deer were detected at Atterbury, an area of Indiana where prevalence of antibody to JC virus in people is low, but prevalence of antibody to LAC is high (Grimstad et al., 1984) and numerous sera had TVT virus antibody titers ≤ two-fold different from the JC virus antibody titer. Our work and that of others has shown that *Aedes triseriatus* and *Aedes trivittatus* mosquitoes, the natural vectors of LAC and TVT viruses respectively, feed readily on white-tailed deer (Burkot and DeFoliart, 1982; Nasci, 1985; Boromisa and Grimstad, 1986) and one might expect to find deer that had a prior infection of LAC and/or TVT virus. Alternatively, some may represent repeated exposure to JC virus strains that represented varying antigenic topotypes, thus producing a "group-specific" immune response.

The report by Patrican et al. (1985) of laboratory studies with *Ae. triseriatus* has considerable relevance to the field results we have reported here of both LAC and JC viruses infecting deer from Atterbury. They noted that *Ae. triseriatus* which ingested LAC virus in deer blood drawn from JC virus-immune deer became infected with LAC virus, but were refractory to the transmission of that virus to suckling mice. DeFoliart et al. (1986) have suggested that if this inhibitory effect occurs in LAC virus infected field mosquitoes that feed on JC virus-immune deer, it might explain the "pocket distribution" of LAC virus within the vast midwestern

JC virus enzootic focal area. This inhibitory effect might in part also explain the lack of LAC virus infections of humans in Michigan where the JC virus antibody prevalence in humans ranges from 8 to 42% (Grimstad et al., 1986).

In summary, deer are an important component of the natural cycle of JC virus in the upper Midwest. They serve as the primary vertebrate host and are excellent sentinal animals. Since fawns are passively immunized by maternal antibody in the colostrum within 72 hr after birth (Issel, 1974; Grimstad et al., 1987), they are protected from a natural JC virus infection during their first summer. The fawns lose this passive immunity within 6 mo (Issel, 1974; Grimstad et al., 1987) and are susceptible to JC virus the following spring. Thus, there is always a susceptible yearling cohort that annually becomes infected and presumably aids in virus amplification and perhaps dissemination. Since some potential vector species do not disperse far from their larval habitats, movement of deer with high viremia levels might be important in disseminating JC virus over limited geographic areas. At Kingsbury we found infected mosquitoes at only one of five widely separated sites (Boromisa and Grimstad, 1986), however, deer harvested throughout Kingsbury were seropositive. In other areas, variable prevalences of antibodies to JC virus in deer may be caused by reduced vector populations (such as *Ae. stimulans* at Kingsbury in 1982 and 1984) due to springtime environmental conditions unfavorable to maximum adult emergence, or to low vector competence of the mosquito population due to genetic variation (Grimstad, 1983). These differences in antibody prevalence to JC virus among deer populations may in turn be reflected in the antibody prevalence of the human population.

ACKNOWLEDGMENTS

The authors thank S. M. Schmitt for critically reviewing the manuscript and for helpful comments, the personnel of the Indiana Department of Natural Resources, especially H. Demaree, P. Meyer, R. Haney, and M. Carlisle for assisting in the blood collections, and D. Campbell, J. Coleman, and D. Williams for excellent technical assistance. Supported in part by Grant AI-19679 from the National Institutes of Health, National Institute of Allergy and Infectious Disease (to P. R. Grimstad), a service contract from the Indiana State Board of Health (to P. R. Grimstad), and a student Wildlife Research Grant (to R. D. Boromisa) from the Indiana Department of Natural Resources.

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