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INFECTION OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) IN MICHIGAN WITH JAMESTOWN CANYON VIRUS (CALIFORNIA SEROGROUP) AND THE IMPORTANCE OF MATERNAL ANTIBODY IN VIRAL MAINTENANCE

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ABSTRACT: Sera collected from a captive population of white-tailed deer (*Odocoileus virginianus*) penned in the lower peninsula of Michigan were assayed over a 29-mo period for neutralizing antibody to California serogroup viruses. In all, 130 individual white-tailed deer were bled one to 22 times between June 1983 and November 1985. Of the 130 sampled after active transmission had ceased, or passage of maternal antibody in colostrum had occurred, only one (0.8%), a newborn fawn, had no serum neutralizing antibody to California group viruses. All 18 1-yr-old does sampled acquired specific neutralizing antibody to Jamestown Canyon (JC) virus within a 6-wk period in 1984 and within a 10-wk period in 1985 indicating the prevalence of infection in this nonimmune age group was 100% for 2 successive yr. All 32 2- to 7-yr-old adult does and eight bucks sampled between June 1983 and June 1985 had specific neutralizing antibody to JC virus. No white-tailed deer had specific neutralizing antibody to trivittatus or La Crosse/snowshoe hare viruses at this study site. In 1984 and 1985, 78% and 63% of the adult does respectively exhibited significant anamnestic responses; all 19 adult does sampled over two seasons (between October 1983 and June 1985) showed a significant anamnestic response during at least 1 of the 2 yr. One-third of adult does with significant springtime antibody titer increases apparently experienced reexposure prior to the emergence of aedine mosquitoes, suggesting an alternate vector may overwinter at this site and transmit viruses in early spring. Specific neutralizing antibody was detected in 98% (66/67) of nursing fawns bled within 5 wk of birth in May–June 1984 and 1985, including three of three nursing fawns bled within 24–96 hr of birth. Of the 66 newborn fawns with specific neutralizing antibody to JC virus in June 1984 and 1985, 95% (54/57) of the surviving fawns lost maternal antibody and had no measurable titer when sampled 20–24 wk after birth, however. Serum antibody titers in 25 newborn (1984-cohort) fawns and their mothers and titers in 38 newborn (1985-cohort) fawns and their mothers were significantly correlated at the 5% and 1% levels respectively, suggesting that maternal antibody rather than a naturally acquired infection was the source of immunity in these suckling fawns. Passive immunity provided to the fawns in this natural focus of JC virus assures a sizeable cohort of susceptible 1-yr-old deer every spring for potential virus amplification and dissemination; maternal antibody is characterized as an important component of the natural cycle of JC virus in the upper midwestern United States.

INTRODUCTION

Jamestown Canyon (JC) virus, a subtype of Melao virus of the California group of Bunyaviruses, has emerged recently as an important human neuropathogen (Grimstad et al., 1982; Grimstad, 1983; Srihongse et al., 1984). While 21 species of mosquitoes and three tabanids have yielded viral isolates (Grimstad, 1983, 1987), a vertebrate isolate has come only from a single white-tailed deer (*Odocoileus virginianus*) (Issel, 1973). Subsequent studies by workers in Wisconsin and Maryland suggested that the white-tailed deer was the primary vertebrate host for JC virus (Issel et al., 1972a, b; Watts et al., 1979, 1982). Antibody to JC virus has been detected in many other species of wild vertebrates (McFarlane et al., 1982; Grim-

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stad, 1983; Zarnke et al., 1983; Grimstad et al., 1986b), however, these animals either had a limited geographic distribution, or reduced population, compared to white-tailed deer, or populations have been found generally with only low prevalences of antibody to JC virus.

Issel and coworkers speculated that their detection of a low prevalence of antibody to JC virus in 0.5-yr-old fawns harvested in the fall in Wisconsin (Issel et al., 1972a) was due either to 1) a small sample size of fawns (e.g., 10% of their total sample), 2) the lack of feeding on fawns by arthropod vectors, or 3) the presence of maternal antibody initially protecting the newborn fawns from infection. Their observation of a reduced prevalence of antibody to JC virus in 0.5-yr-old fawns and the subsequent demonstration of maternal antibody in the colostrum of a subcutaneously inoculated doe (Issel, 1974) suggested that perhaps newborn fawns might be protected during their first summer of life from a natural infection.

We here report our use of a penned deer herd located in a natural focus of JC virus in Michigan (Grimstad and Mandracchia, 1985), in an attempt to 1) measure the annual prevalence of natural infection of white-tailed deer and their subsequent neutralizing antibody acquisition (seroconversion), or anamnestic responses (≥four-fold specific neutralizing antibody titer increase to JC virus) in previously infected animals, and 2) to determine the importance of maternal antibody in the natural transmission cycle of JC virus.

MATERIALS AND METHODS

Study site: Deer utilized in this study were maintained at the Porter Ranch, Houghton Lake Wildlife Research Area, Missaukee County, in the northern third of Michigan's Lower Peninsula (Mitchell, 1978). All animals were housed outdoors in 12.2 m × 12.2 m dirt floor pens and were provided water and a pelleted diet daily (Ullrey et al., 1980).

Population and sampling scheme: The population of surviving newborn fawns sampled ranged from 25 to 38 male and female animals each spring (1983–1985). Adult (>1.5- to 8-yr-old) breeding does ranged in number from 19 to 31; in 1983 there were 31 does (born 1976–1982), in 1984 there were 23 does (born 1976–1982), and in 1985 there were 19 does (born 1978–1982) that were sampled. At any time approximately a dozen breeding bucks were housed in a large 1.2 ha pen, except when paired with does; these bucks were sampled only once, in September 1983. The sampling interval varied over the 29-mo period reported here. Adult does were sampled a minimum of one to a maximum of five times/yr—after the birth of their fawns in 1984 and 1985 (doe and fawn bled the same day in mid to late June), and in fall, midwinter, early spring, and again after the birth of fawns the following year in 1983 and 1984 (see tabular data below for month of sampling). Doe and buck fawns were bled 1 day to 5 wk after birth, in fall, winter, early spring, and then weekly/biweekly through the period of virus transmission. Once a 1-yr-old animal was found with specific neutralizing antibody, it was not resampled until fall (see tabular data for ages at sampling intervals). Two-yr-old does were bled in the fall of 1983, winter and early spring of 1984, and then every 2–3 wk from late May 1984, through late June 1985 to determine how rapid antibody decline might be. Adult breeding bucks were bled only once as noted earlier because of difficulty in handling.

We chose to bleed primarily does for two reasons. First, even young (≥1-yr-old) bucks were impossible to run through the chute without damaging their antlers; however, on one occasion when eight adult bucks were anesthetized (for other studies) we obtained individual blood samples. Second, most bucks were used either in breeding (and held in a 1.2 ha pen during the non-breeding season so that sampling would require frequent immobilization by darting) or in nutritional studies conducted by other investigators in which the yearling or adult bucks were killed. Since frequent and/or long-term sampling could not be assured with bucks, we did not sample them beyond 0.5 to 1.0 yr of age.

Serum collection: Individual blood samples were drawn from 0.5-yr-old to adult (≥1.5-yr-old) does restrained in a squeeze chute (Schmitt et al., 1983); all newborn fawns were hand-held while bled. A 7–10 ml blood sample was removed from the jugular vein of 0.5-yr or older animals and ≤2 ml from newborn fawns; samples were allowed to clot at room temperature for 1 hr, then refrigerated on wet ice until re-

TABLE 1. Temporal patterns of neutralizing antibody titer increases and decreases in eight female white-tailed deer over a 2-yr period in Michigan.

Month bled	Doe number and reciprocal titer*							
	7828	7829	7852	7853	7883	7964	8002	8151
June 1983	64	128	32	32	8	16	32	32
October 1983	16	64	16	16	4	16	8	8
January 1984	4	32	16	8	4	4	4	4
April 1984	16	32	16	8	4	16	8	32
June 1984	16	128	32	32	16	64	16	32
November 1984	16	64	4	16	4	16	16	8
April 1985	32	32	8	16	16	32	16	32
June 1985	64	64	64	32	32	64	16	32

* The first two digits of the doe number indicate the year of birth; titers are expressed as the reciprocal of the 50% end-point dilution obtained with the serum dilution neutralization (SDN) test. All sera from each animal were tested in parallel on the same date.

turned to the laboratory. Serum was harvested and frozen at −70 C until assayed for neutralizing antibody.

Serologic testing: Sera were screened initially for neutralizing antibody to JC virus at a single (1:2) dilution; sera neutralizing a test dose (100 median tissue culture infectious doses; 100 $TCID_{50}$) of JC virus in African Green Monkey kidney (Vero) cells were further tested by serum dilution neutralization (SDN) in microtiter as previously described (Grimstad et al., 1984). We used three California serogroup viruses in SDN tests: the prototype JC virus (61V-2235; in suckling mouse (sm) passage 5), the prototype trivittatus (TVT) virus (4124; in sm passage 11), and a 1978 Indiana isolate of La Crosse (LAC) virus (INLAC-78; in sm passage 8) (Pinger et al., 1983) that shows a high degree of oligonucleotide homology with the prototype LAC virus (Klimas et al., 1981). Since snowshoe hare (SSH) virus is a variety of LAC, we assumed that any sera with neutralizing antibody to SSH virus would show cross-reactive neutralization with LAC virus also and thus be detected readily.

We established three criteria for considering a single serum to have specific neutralizing antibody to JC virus: 1) Sera that neutralized a test dose of JC virus at a dilution of 1:4 in the SDN test and showed no cross-reactivity to TVT and LAC/SSH viruses were considered seropositive; 2) sera that neutralized JC virus at only a 1:2 dilution were considered seropositive if a subsequent sample taken from the same host within 1 to 3 wk showed a ≥ two-fold increase in specific antibody titer; or 3) sera with a titer >1:4 to JC virus that showed a ≥ four-fold higher titer to JC virus than to cross-reacting TVT and LAC viruses were considered to have specific neutralizing antibody to JC virus. Serologic results reported below met these criteria.

In addition we routinely assayed individual serum samples collected from each adult doe over a 4- to 17-mo period in parallel in the same 96-well plate to assure that differences in titer among the serum samples drawn weeks to months apart were real and not due to slight variations in tests otherwise performed months apart. The significant antibody titer increases and decreases reported herein were based on this parallel testing procedure. Does whose early sera showed specific neutralizing antibody to JC virus, then showed a ≥ four-fold titer decrease or stable titer over time were assumed to have experienced an anamnestic response on reexposure to JC virus when subsequent sera demonstrated a ≥ four-fold antibody titer increase.

Statistical analysis: A Spearman rank correlation coefficient (r_s) (Snedecor and Cochran, 1967) was calculated to compare the serum antibody titer of the 1984- and 1985-born fawn cohorts and the serum antibody titer of each fawn's mother. This was done to establish if the serum antibody detected in fawns might be maternal (colostral) in origin (significant correlation) or due to a naturally acquired infection (no significant correlation).

RESULTS

Eight adult does bled eight times between June 1983 and June 1985 showed evidence of an anamnestic response to JC virus (Table 1). These eight were the only animals that were available consistently

over this 24-mo period for bleeding (data that include these eight and 15 additional adult does bled less often are summarized below). Five patterns were evident among these eight does: 1) does #7964 and #8151 both showed a ≥ four-fold decrease followed by a ≥ four-fold increase in antibody titer, suggesting an anamnestic response presumably on reexposure to JC virus in 1984 and 1985, 2) doe #7828 initially had a 16-fold antibody titer decrease after June 1983 with an apparent anamnestic response in 1984—this doe's antibody titer remained stable until she experienced a second anamnestic response in 1985, 3) does #7829, #7853, and #8002 initially showed a titer decrease with an anamnestic response in 1984 only and their antibody titers remained stable for the next year, 4) doe #7883 initially had a stable titer until spring 1984 when she had an anamnestic response—a subsequent titer decrease was followed by a second anamnestic response in 1985, and 5) doe #7852 had a stable titer from June 1983 until spring 1985 when she showed an anamnestic response (Table 1). Since none of the 15 additional does was available for bleeding more than 20 mo we could not fit them into these patterns, however, trends in their immune responses suggest that all ultimately would fit one of these five patterns.

Four adult does born in 1976–1981 were sampled five times between 13 June 1983, and 14 June 1984 in addition to the eight listed on Table 1, and were sampled also through June 1985; nine showed significant antibody titer decreases subsequent to June 1983 followed by significant titer increases (anamnestic responses) by mid-June 1984. Of these nine, six showed anamnestic responses between April and June, and three between January and April. The June 1983 data cannot be counted as indicative of exposure in that year since the 12 does sampled at that time were all born 2–5 yr earlier and undoubtedly were infected prior to June 1983. Some of the 12 does most likely were exposed to JC virus in 1983, however, based on the frequency of infection in 1984 and 1985. Eleven adult does born in 1978–1982 were sampled four times between 25 October 1983, and 14 June 1984; nine showed significant antibody titer decreases subsequent to late October 1983, followed by anamnestic responses by mid-June 1984. Of these nine, six showed anamnestic responses between April and June, and three between late January and early April. All 11 were sampled through late June 1985 as well (none is listed on Table 1). In summary, in 1984, 78% (18/23) of these two groups of adult does showed a significant anamnestic response, 67% (12/18) coincident with the 1984 seroconversion in the 1-yr-old 1983 cohort (Table 2, Fig. 1), and 33% (6/18) in the early spring (e.g., between 26 January and 6 April). Of the five showing stable titers (and thus no anamnestic response) two died without being resampled and all of the remaining three showed anamnestic responses in the late spring of 1985.

Eleven adult does born in 1978 through 1982 were sampled four times between 12 June 1984, and 26 June 1985 in addition to the eight listed on Table 1. Of these, 9/19 showed significant antibody titer decreases followed by significant titer increases subsequent to June 1985; 3/19 additional does showed stable titers between mid-June 1984 and early November 1984, but showed a significant titer increase between November and late June 1985. Of these 19, 65% (12/19) showed significant anamnestic responses in 1985, eight between early April and late June coincident with the seroconversion of the 1984 cohort (Fig. 1), and four (33%) between November 1984 and April 1985. Of the seven showing stable titers (and thus no anamnestic response in 1985) all had shown anamnestic responses in the spring of 1984. In summary, 37% (7/19) of adult does

TABLE 2. Proportion of the white-tailed deer population born at Porter Ranch, Michigan in 1983–1985 and found with specific neutralizing antibody to Jamestown Canyon virus: Temporal patterns of seroconversion and loss of maternal antibody.

Cohort year	Date bled	Age when bled	Number with antibody/number tested		Total seropositive/total tested
			Female	Male	
1983	10/26/83	0.4 yr	0/9	0/16	0/25
	1/26/84	0.7 yr	0/9	0/16	0/25
	4/6/84	0.9 yr	0/9	0/16	0/25
	5/23/84	1.0 yr	0/9	0/16	0/25
	6/13/84	1.0 yr	4/9	11/16	15/25
	7/3/84	1.1 yr	9/9	na[a]	9/9
	11/7/84	1.5 yr	9/9	na	9/9
	4/9/85	1.9 yr	9/9	na	9/9
	6/25/85	2.1 yr	9/9	na	9/9
1984	6/13/84	Newborn	13/14[b]	16/16	29/30
	10/23/84	0.4 yr	1/10	2/11[c]	3/21
	2/19/85	0.8 yr	0/9	na	0/9
	4/9/85	0.9 yr	1/9	na	1/9
	5/15/85	1.0 yr	4/9	na	4/9
	6/25/85	1.1 yr	9/9	na	9/9
1985	6/25/85	Newborn	14/14	23/24[d]	37/38
	11/7/85	0.5 yr	0/14	0/22	0/36

[a] na = not available for sampling; bucks were used extensively in nutritional studies where the analyses frequently involved whole-body vitamin/fat content determinations. Since bucks were killed routinely we relied primarily on long-term assay of individual does. In this particular instance the 1-yr-old bucks were removed for a separate study and could not be sampled beyond 13 June 1984. In 1984 the buck-fawns were not available in the winter or following spring.

[b] The single seronegative newborn doe fawn (#8441) did not suckle her mother and thus did not acquire maternal antibody; her sibling did suckle and had a titer of 1:64 72 hr after birth (see text).

[c] One doe-fawn (#8419) and one buck-fawn (#8425) each had a two-fold increase in antibody titer (1:32 to 1:64) between June and October 1984, suggesting an early loss of maternal antibody and a subsequent natural infection in both animals in late summer of 1984. A second buck (#8431) had a 1:64 titer in June which decreased to ≤1:4 by late October 1984 (20 wk after birth)—this presumably was persistent maternal antibody as was described in an earlier study where maternal antibody was found to persist in fawns for a mean of 19 wk, range of 8–23 wk (Issel, 1974).

[d] The single seronegative buck-fawn's mother had demonstrated an anamnestic response (four-fold rise in serum titer between the January and April samples) presumably due to an early-season infection. The doe has been seropositive since first sampled 10/83, however, she has consistently shown only low titers, which may have resulted in her fawn's lack of detectable antibody.

sampled both years (1983–1984, 1984–1985) showed an anamnestic response in 1984 only, 16% (3/9) in 1985 only, and 47% (9/19) in both years. Of the three does sampled both years that did not show an anamnestic response in 1984, all had a significant titer increase in 1985. Thus, every adult doe apparently experienced an anamnestic response to JC virus reexposure at least every other year at Porter Ranch, and almost half (47%) showed significant titer increases every year.

A comparison of the rate of antibody titer decrease subsequent to primary infection vs. anamnestic response(s) in individual does indicated that the decrease in antibody titer after secondary responses was more gradual than after the primary response—a characteristic of true memory immune responses.

On 26 September 1983, eight bucks (born 1980–1982) were bled; all had specific neutralizing antibody to JC virus. Their titers ranged from 1:16 to 1:256 with a geometric mean titer of 1:83, suggesting the recent exposure of most to JC virus.

Six additional adult does (aged 2–7 yr) were bled only once each; all had significant neutralizing antibody titers to JC virus.

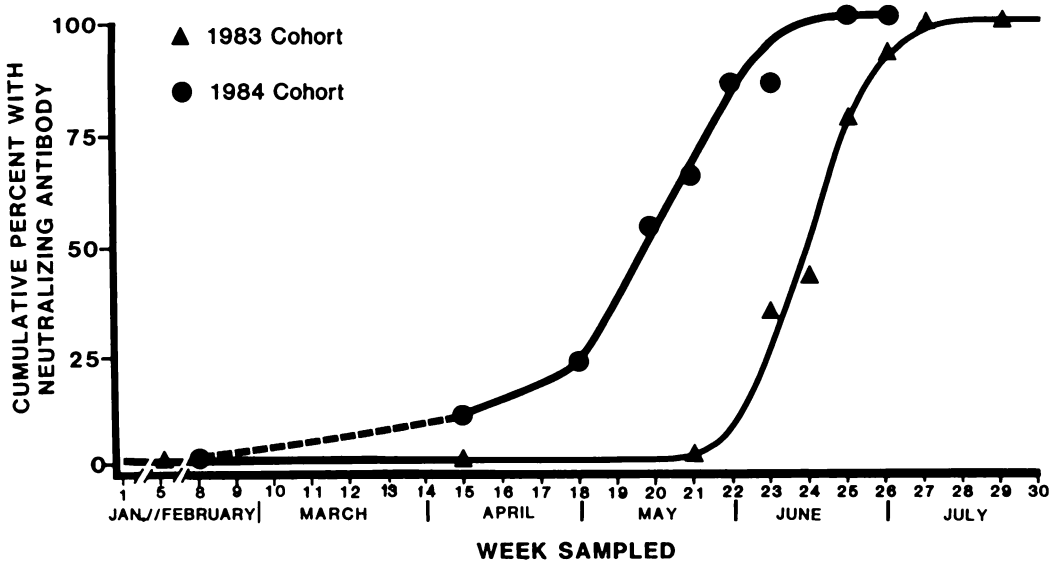

FIGURE 1. Seasonal seroconversion of two cohorts of white-tailed deer (18 does) in Michigan to Jamestown Canyon virus in 1984 and 1985. The figure depicts the week of the year when the yr-old deer born in 1983 seroconverted to Jamestown Canyon virus in 1984 and when the yr-old deer born in 1984 seroconverted to Jamestown Canyon virus in 1985. The cohort represented here each year consisted of nine 1-yr-old does. One 1984-cohort doe acquired a JC virus infection between the 8th and 15th week in 1985; we have represented that event with a dotted line since a more precise time of infection could not be determined as we were able to do later in 1985 for the eight remaining does.

Three doe fawn cohorts (1983-, 1984-, and 1985-born) were sampled between October 1983 and November 1985 (Table 2); the time of seroconversion for the 1983 and 1984 cohorts varied considerably. Seroconversion in the 1983 doe cohort occurred over a 6-wk period (weeks 21–27) in 1984 compared to a 10-wk (weeks 15–25) period in 1985 for eight of the nine 1984-cohort does (Fig. 1). In 1985, one 1984-cohort doe that was seronegative in mid-February had an antibody titer of 1:2 when sampled on 6 April 1985. Her titer remained at 1:2 for 7 wk when it rose sharply to 1:512 (during the 21st wk; Fig. 1). Since exposure to JC virus occurred apparently at some point between mid-February and early April, we have represented this on Figure 1 with a dotted line. As noted above, 4/12 adult does showing anamnestic responses in 1985 had elevated titers by early April also. All 36 surviving 1985-cohort fawns lost maternal antibody by November 1985 (Table 2), and presumably will seroconvert in the late spring and early summer of 1986 in a pattern similar to that seen with the two previous cohorts.

Fifteen of the 18 1-yr-old (1983- and 1984-cohort) does sampled in 1984 and 1985 respectively (Fig. 1) developed serum neutralizing titers of >1:64 within 2 wk; two took 3 wk to acquire similar titers and one acquired a titer of 1:32 within 1 wk that dropped to 1:16 1 wk later and remained at that titer for 19 mo. The annual prevalence of infection in the 1983 and 1984 cohorts was 100% (Fig. 1).

The correlation between the antibody titers of the 38 fawns born in May–June 1985, and that of their mothers was significant at the 1% level ($r_s = 0.511$; 36 df) in the Spearman rank correlation analysis; the correlation between the titers of the 29 fawns born in May–June 1984 and their mothers was significant at the 5% level

(r_s = 0.391; 27 df). The correlation between the titers of the 10 (1985-cohort) fawns with the highest titers and the 10 fawns with the lowest titers and their respective mothers also was significantly correlated (r_s = 0.934). In addition, 72% (21/29) of the 1984 fawn-doe paired sera and 47% (18/38) of the 1985 fawn-doe paired sera had antibody titers that differed by ≤ two-fold. The antibody titers in the fawns decreased to undetectable concentrations by late fall whereas the titers of their mothers remained detectable (either declining or remaining stable by late fall). Given these results and the results of earlier studies of maternal antibody by Issel (1974), we concluded that the serum antibodies detected in both newborn fawn cohorts were maternal (colostral) in origin.

All of the surviving 1984 cohort showed a loss of maternal antibody between June and October 1984 (except for two fawns: doe-fawn #8419 and buck-fawn #8425), and evidence of a subsequent natural infection with the acquisition of specific neutralizing antibody to JC virus in the spring of 1985 (Table 2). Doe-fawn #8419 acquired an antibody titer of 1:32 the 24th week of 1984, but still had a titer of 1:64 the 43rd week of 1984. Buck-fawn #8425 also acquired an antibody titer of 1:64 in the 24th wk of 1984; the antibody titer had not decreased significantly when this buck-fawn was resampled (along with #8419) 19 wk later. These were the only fawns from that cohort that did not show a loss of antibody by the fall of 1984. All 63 0.5-yr-old fawns in the 1983 and 1985 cohorts lost neutralizing antibody by November of the respective years (Table 2).

We had an opportunity to indirectly detect passage of maternal antibody in the colostrum of a nursing doe in the spring of 1984; twin fawns (#8440 and #8441) born to a young (1982-cohort) doe on 23 June were bled on the 26th. The buck-fawn #8440 had suckled and the doe had cleaned the meconium; this fawn had an antibody titer of 1:64 as did its mother. However, on the 26th the doe-fawn #8441 was very weak and could not stand, the meconium had not been cleaned and the fawn had not been observed suckling the doe since birth. Her antibody titer was <1:2, and she died later that day presumably of starvation; since these fawns were part of another study on fawn nutrition/survival, she could not be rescued by bottle feeding.

All sera with California serogroup antibodies were assayed with JC, TVT, and LAC viruses and showed specific neutralizing antibody only to JC virus. Some degree of cross-reactive neutralization of LAC viruses was seen in 54% (19/35) of adult deer and cross-reactive neutralization of TVT virus in 83% (29/35) of adult deer. The pattern seen in 34/35 of these SDN titer comparisons was JC > TVT > LAC; the mean titer reduction between JC and TVT viruses was 32-fold and 64-fold between JC and LAC viruses; however, titer differences were as low as four-fold in three sera for JC/LAC and in two sera for JC/TVT viruses. This is consistent with our observations in other California group serologic studies (Grimstad et al., 1984, 1986a, b).

DISCUSSION

Our results demonstrate the rather rapid (6–10 wk) rate of seroconversion of a deer population to JC virus in a natural focus in midwestern United States (Table 2, Fig. 1). Collections of hematophagous arthropods made coincident with deer bleeding both years (1984 and 1985) indicated that, with one exception, seroconversion in the 1-yr-old cohort began only with the emergence of spring aedine mosquitoes (Grimstad and Mandracchia, 1985), as did anamnestic responses in 67% (20/30) of adult does. This suggests that JC virus probably was transmitted at Porter Ranch primarily by snow-melt pool

breeding aedine mosquitoes as it is in northern Indiana (Boromisa, 1985). At Porter Ranch the aedine mosquito that emerged first, moved out of forest larval breeding sites and was collected in light traps placed among the deer pens as seroconversion began, and has been implicated as a potential vector in Connecticut (Main et al., 1979) was *Aedes abserratus*. However, no isolate of JC virus has been made from this or other species of mosquitoes and tabanids at Porter Ranch to date; the very dry weather in the springs of 1984 and 1985 precluded the emergence of large populations, and collections of this mosquito totaled <1,500 in both years. In years when the mosquito populations are "normal" the period of seroconversion may be less than the 6 wk we observed in 1984.

Interestingly, a significant anamnestic response occurred in six of 18 and four of 12 adult does prior to the April/May emergence of aedine mosquitoes in 1984 and 1985 respectively. The ≥ four-fold titer increases detected between the January/February and April serum samples were probably not due to latent virus. Evidence is emerging from mosquito isolation studies in Ohio (Berry, pers. comm., cited in Grimstad, 1987) and from human case studies (Grimstad, 1987; Grimstad et al., 1986a) that a late season mosquito vector apparently transmits the virus to humans in August–October; evidence suggests this vector may be an anopheline, perhaps *Anopheles punctipennis* or *Anopheles quadrimaculatus* (Grimstad, 1987), both of which overwinter as bloodfed adults and both of which occur at this study site. Indeed, one of two 1983-cohort does repeatedly bled over 24 mo showed an anamnestic response in late September 1984 (titer elevation from 1:32 to 1:128), at a time well beyond the disappearance of spring aedine populations, but coincident with anopheline species bloodfeeding and entering overwintering sites. Early spring mosquito biting activity has been described by workers at Porter Ranch following warm spells in late February and late March in 1984 and 1985 respectively, with mosquitoes attempting to feed on workers (Nellist, pers. comm.). The apparent early-season anamnestic responses in adult does may be the result of reexposure to JC virus transmitted by an overwintering mosquito. If this is true, then spring infection occurs by two mechanisms: transmission by overwintering adult mosquitoes and transmission by newly-emerged transovarially-infected aedine mosquitoes.

We have attempted to isolate virus from the blood of the 18 1-yr-old deer as they became infected and seroconverted during the spring of 1984 and 1985. However, no isolate has been made perhaps in part due to our being able to sample only once every 7–10 days. Alternatively the concentrations of viruses in naturally infected deer may be quite low; Issel (1973) noted that on initial inoculation of suckling mice with blood from a deer, only 2/30 suckling mice became sick—these two eventually yielded the sole vertebrate isolate of JC virus reported to date. We have been using a Vero cell plaque technique for virus isolation that readily detects individually infected mosquitoes in pools with 49 uninfected mosquitoes; perhaps it is not sufficiently sensitive to detect a JC viremia in white-tailed deer. Inoculation of large amounts of high-titered JC virus (2 ml of 3.4 to 6.0 $\log_{10}$SMICLD$_{50}$/0.02 ml) in two studies (Issel et al., 1972; Watts et al., 1982) did not result in detectable viremia in all animals that developed neutralizing antibody. While viremia in those studies had a duration of 3 to 5 days, it may be that the duration of viremia in mosquito-infected white-tailed deer in the field is considerably less than that seen with experimental inoculation of large doses; viremia titers may also be generally low given the small amount of virus in-

oculated by a feeding mosquito. Arthropod-transmitted JC virus, appears to be quite immunogenic in white-tailed deer, however. The large number of mosquitoes and tabanid flies (deer- and horse-flies) that feed on deer daily might also facilitate mechanical transmission of JC virus as well. Three isolations of JC virus have been made from field-collected tabanids in Wisconsin (DeFoliart et al., 1969; Sudia et al., 1971); however, tabanids are not considered to be involved in biological transmission of viruses, but in mechanical transmission (Krinsky, 1976).

Every yearling and adult white-tailed deer sampled 1 yr or later after birth at Porter Ranch has been seropositive for JC virus. The several adult does that did not show significant rises in antibody titer one season (Table 1) might have been exposed, but showed no anamnestic response. All 1-yr-old white-tailed deer sampled showed evidence of recent infection (100% annual prevalence of infection) and 47% of adult does showed evidence of an annual anamnestic response. Thus at Porter Ranch, the vast majority of white-tailed deer potentially experience an annual exposure to JC virus. This high rate of exposure is compatible with antibody prevalences we have measured in three other populations: a) the 94% (49/52) prevalence in ≥1.5-yr-old white-tailed deer from suburban Chicago, Illinois, b) the 91% (51/56) prevalence in ≥1.5-yr-old deer at Cusino Wildlife Research Station in the Upper Peninsula of Michigan, c) the 72% (46/64) prevalence in ≥1.5-yr-old deer harvested in the fall of 1985 in east-central Michigan (Saginaw-Bay-Midland County area), and with the 52–90% prevalence reported for ≥1.5-yr-old deer throughout northern Indiana (Boromisa and Grimstad, 1987). We have further detected a 27% and a 38% prevalence of antibody in equine and bovine populations respectively in Michigan's Lower Peninsula (Grimstad, unpubl. data). Additionally, the report of a high prevalence of specific neutralizing antibody to JC virus in the Michigan human population (30–35% in the northern regions of the Lower Peninsula near Porter Ranch) (Grimstad et al., 1986a) was not unexpected given the high antibody prevalence at Porter Ranch and other populations of white-tailed deer in Michigan.

Our data corroborate the earlier findings of Issel and coworkers regarding maternal antibody and its important role in protecting fawns during their first year (Issel et al., 1972a; Issel, 1974). At Porter Ranch all pregnant does had acquired an infection at least 1 yr prior to giving birth to their first fawn. Fawns that were born at Porter Ranch and suckled these immune does acquired protective maternal antibody for a period of time sufficient to protect most of them their first summer of life. Issel reported that maternal antibody persisted for as short as 8 to as long as 23 wk (mean of 19 wk) in Wisconsin fawns (Issel, 1974). In our study, only fawns #8419 and #8425 (Table 2) apparently lost their passive immunity early and acquired a natural infection in the summer of 1984, since their sequential antibody profile was similar to that of older naturally immune does (Table 1). Maternal antibody to JC virus thus assures a sizeable cohort of susceptible 1-yr-old deer every spring for viral amplification and dissemination, and is a very important component of the natural cycle of JC virus in the midwestern United States and probably throughout its North American range.

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