Jamestown Canyon Virus: Vector-host Studies in Northern Indiana

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Introduction

Jamestown Canyon virus (JCV), a subtype of Melao virus of the California serogroup (Bunyaviridae: *Bunyavirus*), was first isolated from *Culiseta inornata* mosquitoes in Colorado in 1961 (16). Extensive studies conducted in Wisconsin and Maryland subsequently have implicated the white-tailed deer (*Odocoileus virginianus*) as the primary, and perhaps exclusive, vertebrate host of JCV (9, 10, 11, 12, 19). More recently, JCV has been implicated as an etiologic agent capable of producing meningitis and encephalitis in humans (3, 5).

Serological surveys have indicated foci of JCV infection in Indiana residents, particularily in the northern third of the state (4, 6). These investigations have prompted an extensive investigation of the ecology of JCV including that of the vertebrate host and any hematophagous arthropods potentially capable of transmitting JCV between deer and/or humans. Our primary study site is the Kingsbury State Fish and Wildlife Area in LaPorte County, Indiana. An earlier report (1) detailed the seasonal succession of hematophagous Diptera collected there in 1982 and the methods used to collect these arthropods. This report summarizes the 1983 mosquito and tabanid collections and two years of serological surveys of the Kingsbury deer herd.

Materials and Methods

Study Site: The Kingsbury State Fish and Wildlife Area, regulated by the Indiana Department of Natural Resources (IDNR), is a site with extensive areas of oak woods, open prairies, and marshlands that provides ideal habitats for a variety of hematophagous Diptera. This area also is an excellent habitat for the deer herd estimated to number 2000 head (7). During the annual deer hunting season, hunters are required to check in and out of the IDNR headquarters located on the property. This requirement greatly aided in obtaining samples for our serological surveys.

Deer Blood Sample Collection: Blood samples were collected in 1981 and 1982 both from pooled blood in the body cavity of field-dressed deer at the Kingsbury check station and by hunters at the kill site. Deer hunters entering Kingsbury were issued 7 cc blood collection tubes and participated in the serosurvey by collecting fresh blood as they field-dressed their deer. Blood samples were collected daily in this manner and refrigerated several days until transported to the laboratory for serological analysis. Blood samples were centrifuged in a refrigerated centrifuge at 4 °C for 15-30 minutes at 2000 x G; supernatant sera were aliquoted into individually labeled 1 dram glass screw cap vials. These sera were held at 4 ° C until serologically tested several days later.

Deer Age and Sex: Age of harvested deer was determined by Kingsbury IDNR personnel at the check station using standard tooth eruption and wear techniques (17). Three age groups were established: fawn deer (0.5yr) included only those animals determined to be less than 1 year old, 1.5 yr animals included only those determined to be older

than one year but less than two years, while the ≥ 1.5 yr group included all adult deer. Sex was readily determined by the IDNR personnel (17).

Viruses: Three reference viral antigens were used in the serological tests: JCV and trivittatus virus (TVTV) were obtained from the reagents bank of the Vector-Borne Diseases Division of the Centers for Disease Control at Fort Collins, Colorado. The La Crosse virus (LACV) used was an Indiana isolate (15) that is very closely related to the prototype LACV as determined by the oligonucleotide fingerprint technique (13).

Mouse Hyperimmune Ascitic Fluids: Hyperimmune ascitic fluids (HAFs) were prepared in 6 week old albino mice[1] using the JCV, LACV, and TVTV stock antigens according to the method of Tikasingh *et al.* (18). The HAFs were used as known positive controls in all serological (neutralization) tests and as reference typing antisera for virus isolations.

Cell Culture: A continuous cell line of African Green Monkey kidney (Vero) was used for all neutralization tests. Cells were cultured in Medium 199 supplemented with Earle's salts, 10% (heat-inactivated) fetal bovine serum, and antibiotics (200 Units of potassium penicillin G, 200 mg of streptomycin sulfate and 250 mcg of Fungizone per ml of media) at 37 °C in a 5% CO_2 atmosphere. This diluent was used throughout the study.

Neutralization Tests: All deer sera were initially diluted 1:8 in M-199 diluent and heat-inactivated at 56 °C for 1 hour. Antibody titers were determined using the microtiter serum dilution neutralization test (SDNT) and the three viral antigens in a manner previously described (6, 14). A virus test dose of 100 median tissue culture infectious doses (100 $TCID_{50}$) per 0.025 ml was used as well as a Vero cell suspension of 30,000 cells per ml for all antibody determinations. All SDNTs were done in 96-well flat-bottomed LINBRO® plastic trays[2]. Antibody titers were reported as the highest dilution showing less than 50% cytopathic effects after a 5 day incubation at 37 °C in a 5% CO_2 atmosphere. If the serum neutralized more than one virus but failed to neutralize another by a 4-fold or greater titer difference, then that serum sample was considered as evidence of a California group infection only.

Hematophagous Diptera Collections: Collections in 1983 were made in a manner similar to that described for the 1982 seasonal succession study (1) with several exceptions. In 1983 the resting boxes were not used nor were human-bait collections made. We did include a new type of trap, a 10′ x 12′ screen house of the type used to cover a picnic table, baited it with 10 pounds of dry ice and erected two plywood life-sized deer silhouettes inside. All hematophagous Diptera were handled and processed for virus isolation as described earlier (1).

Results

A total of 229 deer sera were serologically tested by SDNT in 1981 and 1982 (Table 1). Fawn deer had an average 4.8% antibody prevalence to JCV while the 1.5

TABLE 1. *Antibody prevalence to Jamestown Canyon virus in white-tailed deer at Kingsbury State Fish and Wildlife Area in 1981 and 1982.*

Year	Number Tested	Percent Antibody Prevalence by age group			
		0.5 yr	1.5 yr	≥ 1.5 yr	All ages
1981	109	4	81	82	49
1982	120	5	62	68	37

[1]ICR strain, Harlan Industries, Indianapolis, IN

[2]LINBRO® , Flow Laboratories, McLean, VA

yr old group averaged 70.1%; all adult deer averaged 75.6%. The rate of seroconversion in the 1981 fawn cohort between 1981 and 1982 was 58%. While there was no significant difference in antibody prevalence between fawn deer sampled in 1981 vs. 1982, there *was* a significant difference ($p < 0.05$) between the 1.5 yr groups sampled both years and between all adult animals both years ($p < 0.05$).

Collections of hematophagous Diptera, particularly the early spring (snow-melt) mosquitoes *Aedes stimulans* and *Aedes sticticus*, varied from 1982 to 1983 as did *Aedes vexans* (Table 2). Many more *Aedes cinereus* and *Aedes canadensis*, two other early spring species, were also taken in 1983 than in 1982, however, their numbers were much less than those of *Ae. stimulans* and *Ae. sticticus*. Collections of tabanids (deerflies and horseflies) were also greater in 1983 than in 1982 (Table 3).

Discussion

The results of the two year serological survey of white-tailed deer harvested at Kingsbury demonstrate a high level of deer exposure to JCV in that area of northern

TABLE 2. Record of mosquitoes collected during seasonal succession studies at Kingsbury State Fish and Wildlife Area in 1982 and 1983.

Species	Number of specimens collected	
	1982	1983
Aedes		
abserratus	6	
canadensis	3	119
cinereus	25	326
excrucians		1
sticticus		2509
stimulans	143	10104
triseriatus/hendersoni	46	72
trivittatus	5507	2083
vexans	6216	38091
species*	147	28
Anopheles		
punctipennis	24	54
quadrimaculatus	55	116
walkeri	19	1
species	3	
Mansonia		
perturbans	1029	1567
Culex		
pipiens/restuans	658	369
salinarius		1
tarsalis		2
territans	281	221
species	50	
Culiseta		
inornata	2	3
morsitans	5	
minnesotae	7	
species	1	
Psorophora		
ciliata	1	5
ferox		16
varipes		1
Uranotaenia		
sapphirina	113	16
Totals:	14,341	55,705

* Identification to species was not possible.

TABLE 3. Collections of Tabanidae at Kingsbury State Fish and Wildlife Area, LaPorte County, Indiana in 1982 and 1983.

Species	Total Number Collected	
	1982	1983
Chrysops		
callidus	1	12
cincticornis	4	16
cuclux	1	
frigidus	1	
indus	2	2
niger	2	90
univittatus	4	15
vittatus	1	17
species*		12
Hybomitra		
epistates	22	47
lasiophthalma	89	66
species	1	
Tabanus		
americanus		3
atratus	3	7
limbatinevris		4
lineola	65	961
marginalis	2	2
pumilus	10	13
quinquevittatus	9	17
similis	41	178
trimaculatus	4	19
vivax	129	768
species	25	
TOTALS:	416	2,249

* Identification to species was not possible.

Indiana. Earlier studies elsewhere had indicated that deer are sensitive indicators for JCV infection and that they may be important amplifiers or reservoir hosts for that virus (8, 10, 11, 12, 19). This study supports those earlier findings and helps elucidate an additional key step in the natural cycle of JCV, that of providing susceptible vertebrate hosts each season.

DeFoliart *et al.* (2) isolated JCV from naturally infected late spring and early summer *Aedes* mosquitoes and tabanids at a time that virus apparently was circulating in deer in Wisconsin (8). Additionally, Wright and DeFoliart showed that a variety of these late spring and early summer mosquitoes fed on fawns (20). Thus it seems reasonable to assume that fawn deer would also be fed on by these and other infected mosquitoes (and tabanids?) that apparently transmit virus to adult deer.

However, Issel *et al.* reported lower antibody prevalence rates in fawn deer in Wisconsin and speculated on three possible explanations (11): 1) a sampling error based on low numbers of fawns sampled, 2) the fawns were not exposed to the virus, and 3) the fawns were protected by maternal antibody against infection. Since less than 10% of their sample (50/587) were fawn deer, a sampling error seemed plausible. However, 45% (103/299) of the deer in this study were fawns and similar results were seen at Kingsbury both years. This study thus confirms the third alternative, that fawns are protected from infection, presumably by maternal antibody. Maternal antibody that is colostral in origin is known to persist in fawns for 8 to 23 weeks (a mean

of 19 weeks) and can protect them from a primary JCV infection (9). These fawns would then contribute to the pool of susceptible vertebrate hosts the following season. The high antibody prevalence rates in adult does provides a high rate of protection the following year to their new-born fawns and explains the low rate in fawns. Thus the natural cycle of JCV in northern Indiana, and probably elsewhere, is assured numerous susceptible vertebrate hosts each season.

The significantly lower rate of antibody prevalence in the adult animals in 1982 vs. 1981 is perhaps explained by the climatological conditions and the resulting mosquito populations. Populations of spring *Aedes*, including *Ae. stimulans* and *Ae. sticticus* and others, were abundant in the spring of 1981. The floodwaters resulting from snowmelt assured suitable aquatic habitats and the pools of water persisted for some time. However, in 1982, the snow melted rapidly, pools filled with water and these spring species hatched. Then a period of unseasonably cool and dry weather set in, the pools dried up and very few adult mosquitoes emerged. It is presumed that these early spring *Aedes* are the primary vector(s) of JCV and a reduction in their spring populations then might result in a significant decrease in the seroconversion rate between 1981 and 1982 deer cohorts, and indeed this is what we observed. With the very large populations of these mosquitoes collected in 1983, it will be interesting to see if the antibody prevalence rate in the 1.5 yr cohort increases significantly over that seen for 1982; if so, it will confirm in part this hypothesis.

Collections of tabanids were much greater in 1983 than in 1982 due primarily to the large numbers of *Tabanus lineola* and *Tabanus vivax* trapped (Table 3). While collection numbers of other species increased or decreased between 1982 and 1983, it appears that the large numbers of *T. vivax* we collected probably resulted from use of the tent trap as 98.9% of the total were obtained with that method and only a few specimens were taken in the DeFoliart-Morris trap.

A single isolate of TVTV was made in 1982 from a pool of 50 *Aedes trivittatus* female mosquitos collected in August. This is only the fourth time that this virus has been isolated in Indiana (15). Previously, a single yearling deer was found to be seropositive for antibodies to LACV at Kingsbury (4). Thus all three California serogroup viruses are apparently circulating in the Kingsbury area; however, JCV is by far the most important from the standpoint of vertebrate infection.

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