Ecology of Vectors of Jamestown Canyon Virus: Seasonal Succession of Hematophagous Diptera at Kingsbury State Fish and Wildlife Area, Laporte County, Indiana 1982

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Introduction

This paper is the initial report of arthropod collections made as a part of a long-term investigation into the ecology of vectors of Jamestown Canyon virus (JCV) in the upper Midwest. A primary goal is to identify the arthropod vector(s) of JCV in the natural disease cycle.

Jamestown Canyon is a serotype of Melao virus (Family Bunyaviridae) of the California Serogroup, and has recently been shown to cause viral encephalitis in humans (4). White-tailed deer are considered to be the primary host of the virus (6). Despite the fact that virus isolations have been made from many areas of the United States (4), little is really known about the natural disease cycle of JCV. Before any vector can be identified, we need to know which arthropods are present at the study site, in what numbers, and at what time of the season they are present. An earlier serosurvey of Indiana residents (5) had identified a number of JCV foci of infection in northern Indiana, especially in the Kingsbury area. We anticipated that collections from that region would furnish hematophagous arthropods for virus isolation. The collection of bloodfed specimens would also enable us to identify bloodmeal sources and establish host-vector relationships. This report will describe the seasonal succession of numerous species of hematophagous Diptera collected at Kingsbury in the summer of 1982.

Materials and Methods

Study site

Kingsbury State Fish and Wildlife Area is an Indiana Department of Natural Resources (IDNR) regulated site that has extensive areas of oak woods, open prairies, marshlands, and a tamarack lake. This site is ecologically and geologically characteristic of the Northwestern Prairie and Wetlands Division, one of eight natural divisions described for Indiana (8). Kingsbury is the IDNR area in closest proximity to the most intense focus of human infection by JCV in Indiana, based on an earlier serosurvey (5). An earlier serosurvey of deer from Kingsbury detected numerous animals with neutralizing antibody to JCV (1). Thus, Kingsbury appears to be an ideal site for this study.

Collection sites

Collection sites were chosen for their ecological differences and their spatial distribution within the study site. The six collection sites were: 1) along the Kankakee River in a low lying wooded area on the southern edge of Kingsbury that floods in the spring and periodically during the summer, 2) in the periphery of a heavily wooded oak forest in the interior of Kingsbury, 3) by the tamarack lake in the eastern reaches of the study site, 4) near a large drainage canal in a shrubby, open area in the central Kingsbury site, 5) in a damp, low lying area near an oak-cherry forest, and 6) by a permanent marshy area near small oak and pine woodlots in an open prairie near the headquarters.

Collection methods

Hematophagous Diptera were collected using seven different methods: 1) CO_2-baited miniture light traps,[1] 2) a lightweight battery powered aspirator (10), 3) a CO_2-baited cone trap (2), 4) resting boxes (3), 5) oviposition traps (7), 6) a Malaise trap,[1] and 7) human-baited mosquito biting collections. The lightweight battery powered aspirator was not used at the tamarack lake collection site since the ground cover was inadequate to shelter resting mosquitoes. Human-baited mosquito biting collections were not conducted at the tamarack lake because of the frequently windy conditions that made mosquito flight impossible. The CO_2-baited cone trap and Malaise trap were used only at site 2. These traps are of a permanent nature and could not be moved easily.

All seven collection methods were used at the other collection sites. Light trap collections were made once a week from the last week in May until the end of the collecting season in mid-September. The CO_2-baited cone trap and aspiration collections were initiated in early June. Mosquitoes were aspirated from shrubby ground cover in the immediate vicinity of the light traps. Resting boxes and oviposition traps were set up in mid-July. The Malaise trap was put into operation beginning in early August. Human-baited mosquito biting collections did not begin until mid-August. Biting mosquitoes were captured in 8-dram vials, returned to the laboratory and identified (12) to species.

Specimen handling

All specimens were brought back to the laboratory and the non-hematophagous arthropods were sorted out and discarded. Live tabanid specimens from each day's collection were grouped together and frozen at -70°C until identified at a later time. The tabanids were identified (11) on ice prior to storage to preserve potential virus activity and refrozen at -70°C for future virus isolation. Dead specimens were identified and used for voucher specimens. Live mosquitoes were killed by freezing, held on ice for identification (12), then pooled in groups of 50 or fewer specimens. Mosquito pools were based on collection day and method of collection; these pools were also frozen at -70°C for future virus isolation. Dead mosquitoes were identified (12) and used as voucher specimens. *Simulum vittatum* and *Simulum tuberosum* larvae and pupae were collected from the central drainage canal in Kingsbury in early September. Specimens were placed in 70% ethanol and identified (9) in the laboratory. Simuliid specimens were not pooled and frozen for virus isolation since so few specimens were collected.

Results

The 15,340 specimens collected at the 6 sites during the spring and summer of 1982 included 11 genera representing 38 species of Diptera belonging to three families.

Light traps

The five species of mosquitoes listed in Table 1 represented 98% of the specimens collected with the CO_2-baited light traps. *Aedes vexans* and *Aedes trivittatus* were the most numerous mosquito species collected followed by *Coquillettidia perturbans* and the *Culex pipiens/restuans* complex. *Aedes vexans* was the most numerous species in light trap collections from late May through mid-July.

[1]Bioquip Products, Santa Monica, California 90406, U.S.A.

TABLE 1. *Mosquito light trap collections at Kingsbury State Fish and Wildlife Area in 1982.*

Species Collected	May	June				July				August				September		TOTAL
	4*	1	2	3	4	1	2	3	4	1	2	3	4	1	2	
Aedes vexans	12	448	99	283	84	419	1155	212	340	97	514	567	194	106	38	4155
trivittatus	3	163	83	253	188	382	983	540	673	531	415	189	251	186	96	4936
stimulans	4	22		1	4	9	4									44
Coquillettidia perturbans		50	27	44	18	169	133	16	51	34	29	113	121	134	24	963
Culex pipiens/restuans	2	4					29	13	30	50	218	111	31	32	15	535
TOTAL	26	687	209	581	294	979	2304	781	1094	712	1176	567	597	458	173	10633

*week of the month

TABLE 2. *Mosquito aspiration collections at Kingsbury State Fish and Wildlife Area in 1982.*

Species Collected	June			July				August				September		TOTAL
	1*	2	3	1	2	3	4	1	2	3	4	1	2	
Aedes vexans	20	107	86	150	68	556	254	185	309	114	70	27	19	1965
trivittatus		64	35	40	15	154	63	52	26	19	8	7	2	485
stimulans	61	9	3	12	4	6	4							99
Culex territans	13	8	11	17	14	30	17	53	21	45	15	18	8	279
pipiens/restuans	9	6	4	5		12	5	1	62	14	1	3	4	123
Uranotaenia sapphirina							4		8	13	33	26	28	113
Coquillettidia perturbans	7	9	4	6	1	4	2	3	7	5	6	3	2	59
TOTAL	110	203	143	230	102	762	349	294	433	210	133	84	60	3123

*week of the month

Aedes trivittatus was the most numerous species collected from late July until the end of the collecting season. *Aedes stimulans* persisted through mid-July. *Coquillettidia perturbans* were present throughout the season with peak numbers obtained in early July and late August. *Culex pipiens/restuans* were collected in light traps early in the season, disappeared, and then reappeared in mid-July and peaked in mid-August.

Other mosquito species collected were: *Aedes abserratus*, *Aedes canadensis*, *Anopheles punctipennis*, *Anopheles quadrimaculatus*, *Anopheles walkeri*, and *Uranotaenia sapphirina*. There were no bloodfed mosquitoes collected in light traps.

Aspiration collections

The seven species of mosquitoes listed in Table 2 represented 95% of all specimens collected in this manner. The numbers of males and females of each species were combined to give the totals listed in Table 2. Of the specimens collected, 30% were male and there were 560 bloodfed mosquitoes collected. *Aedes vexans* was the most numerous species collected except in the early and late parts of the collecting season. There were relatively few *Ae. trivittatus* collected compared to the numbers of *Ae. vexans*. The total number of *Ae.* vexans was lower than that obtained in the light traps; numbers of *Ae. trivittatus* and *Coq. perturbans* were greatly reduced from light trap collections. However, this was the only method of collecting *Culex territans* and *Uranotaenia sapphirina*, in numbers. Other mosquito species collected were: *Aedes abserratus*, *Aedes canadensis*, *Aedes cinereus*, *Anopheles punctipennis*, *Anopheles quadrimaculatus*, and *Culiseta inornata*.

Co_2-baited cone trap

The CO_2-baited cone trap collected large numbers of tabanid species but collected only a few mosquitoes and no other hematophagous Diptera. Tabanids in the genus *Tabanus* were the most numerous followed by *Hybomitra* and *Chrysops*. The 18 species of Tabanidae collected are listed in Table 3. The tabanids collected were seasonal in distribution. Only *Tabanus lineola* and *Tabanus similis* were collected throughout the summer. However, there were relatively few tabanids collected in the month of July because the original trap site was rototilled by IDNR personnel, thereby, destroying all foilage in the immediate area. The trap was moved to several other locations within the collection site until good collections were obtained.

Resting boxes

There were only 50 mosquitoes collected in six resting boxes. The four species of mosquitoes collected in this manner are listed in Table 4. This method was best for collecting *Anopheles quadrimaculatus*.

Ovipositions traps

A total of 40 oviposition traps were set up at all collection sites. Only 12 of the oviposition trap paddles containing eggs were collected. The remainder of the oviposition paddles were lost from the oviposition traps due to unknown natural causes. Presence of eggs on the balsawood paddles indicated the presence of *Aedes triseriatus* complex members, either *Aedes triseriatus*, *Aedes hendersoni*, or both.

Malaise trap

The Malaise trap was not effective in catching hematophagous Diptera. Future

TABLE 3. *Tabanid collections at Kingsbury State Fish and Wildlife Area in 1982.*

Species collected	Month Collected				Total
	June	July	August	September*	
Hybomitra					
lasiophthalma	88	1			89
epistates	19	3			22
spp.			1		1
Tabanus					
vivax		1	125		129
lineloa	14	19	31	1	65
similis	24	4	7	6	41
pumilus	10				10
quinquevittatus			7	2	9
trimaculatus	3	1			4
atratus	3				3
marginalis		1	1		2
spp.			25		25
Chrysops					
cincticornis	3	1			4
univittatus		1	3		4
niger	2				2
indus	1				1
callidus	1				1
cuclux	1				1
frigidus	1				1
vittatus			1		1
TOTAL	169	31	204	12	416

*two weeks only

modifications to the trap will include CO_2-baiting and or changing the color of the trap from its present white color to varying shades of green to make the trap less noticeable to the flying insects.

Human-bait collections

The results of the human-bait collections are listed in Table 5. A total of 200 mosquitoes were collected at four different collection sites on four different days.

TABLE 4. *Resting box collections at Kingsbury State Fish and Wildlife Area in 1982.*

Species Collected	Per Cent Collected		
	male	female	TOTAL
Anopheles quadrimaculatus	52	12	64
Aedes trivittatus	24		24
Aedes vexans	8		8
Anopheles punctipennis	2	2	4

TABLE 5. *Human-bait collections at Kingsbury State Fish and Wildlife Area in 1982.*

Species Collected	Per Cent of Total
Aedes vexans	49
Aedes trivittatus	39
Aedes triseriatus	6
Coquillettidia perturbans	4
Anopheles quadrimaculatus	2

Discussion

Collectons that are representative of the field populations are needed for the accurate determination of the seasonal succession of hematophagous Diptera. The different collection methods used in this study clearly demonstrated that not all collection methods yielded the same results and that species numbers varied considerably among the trapping techniques. Thus, a variety of collection methods are essential to minimize collection bias and provide representative collections.

A seasonal succession of hematophagous Diptera was noted in the spring and summer of 1982. No single species predominated at any one trap site all season. The absence of early spring (snow melt) *Aedes* species is striking in our collections. Unfortunately, the early rapid snow-melt was followed by a cool dry period that resulted in vernal pools drying before adult emergence. We were not sure of the effect this might have had on JCV transmission at Kingsbury in the summer of 1982, since earlier studies in Wisconsin (6) indicated JCV was transmitted in the late spring to white-tailed deer. However, our analyses of deer bloods collected in October and November of 1982, indicated approximately 60% of the deer that were now two summers old, had antibodies to JCV. A 1982 serosurvey indicated that 4% of yearling deer had antibody. Thus, it appears that approximately 56% of the deer that spent their second summer in the northwestern area of Indiana and were harvested this fall at Kingsbury, seroconverted to JCV. Virus isolation studies and analyses of bloodmeals are in progress to identify potential vector species and establish host-vector relationships.

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Literature Cited

1. BOROMISA, R.D., unpublished data.
2. DEFOLIART, G.R., and C.D. MORRIS. 1967. A dry ice-baited trap for the collection and field storage of hematophagous diptera. J. Med. Entromol., 4: 363-373.
3. EDMAN, J.D., F.D.S. EVANS, and J.A. WILLIAMS. 1968. Development of a diurnal resting box to collect *Culiseta melanura* (Coquillett). Am. J. Trop. Med. Hyg., 17: 451-456.

4. GRIMSTAD, P.R., C.L. SHABINO, C.H. CALISHER, and R.J. WALDMAN. 1982. A case of encephalitis in a human associated with a serologic rise to Jamestown Canyon virus. Am. J. Trop. Med. Hyg., 31: 1238-1244.
5. GRIMSTAD, P.R., C.L. BARRETT, R.L. HUMPHREY, and M.J. SINSKO. 1983. Serological evidence for widespread infection with La Crosse and St. Louis encephalitis viruses in the Indiana human population. Am. J. Epidemiol., (in press).
6. ISSEL, C.J. 1973. Isolation of Jamestown Canyon virus (a California group arbovirus) from a white-tailed deer. Am. J. Trop. Med. Hyg., 22: 414-417.
7. LEISER L. 1981. Distribution of *Aedes triseriatus* (say) in an urban area: Comparison of two survey methods. Proc. Ind. Acad. Sci., 80: 248-253.
8. LINDSEY, A.A., D.V. SCHMELZ, and S.A. NICHOLS. 1969. Natural areas in Indiana and their preservation. Ind. Nat. Areas Survey, Purdue University, Lafayette, Ind. 594 p.
9. MERRITT, R.W. D.H. ROSS, and B.V. PETERSON. 1978. Larval ecology of some lower Michigan blackflies (Diptera: Simuliidae) with keys to the immature stages. Great Lake Entomol., 11: 177-208.
10. NASCI, R.S. 1981. A lightweight battery-powered aspirator for collecting resting mosquitoes in the field. Mosquito News 41: 808-811.
11. PECHUMAN, L.L. 1981. The horse files and deer files of New York (Diptera: Tabanidae). Cornell Univ. Agr. Exp. Sta. No. 18. Ithaca, N.Y. 68 p.
12. SIVERLY, R.E. 1972. The mosquitoes of Indiana. Indiana State Board of Health, Indianapolis, Ind. 56 p.