EVIDENCE FOR TRANSOVARIAL TRANSMISSION OF JAMESTOWN CANYON VIRUS IN OHIO

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ABSTRACT. The first isolation of Jamestown Canyon virus (California encephalitis group) in Ohio was obtained from *Aedes triseriatus* (Say) mosquitoes collected as eggs between 21 July–12 August, 1975. The eggs were collected in ovitraps in the village of Gambier, Knox county. This is the first report of evidence for transovarial transmission of Jamestown Canyon virus.

INTRODUCTION. California encephalitis group viruses (CEV), LaCrosse (LAC) and trivittatus (TVT), have previously been reported in Ohio (Masterson et al. 1971). Previous studies on the epidemiology of LAC virus in an endemic area, Gambier, Ohio, established *Aedes triseriatus*(Say) as the major vector of the virus in that area (Berry et al. 1975).

As part of a continuing survey of LAC virus and its relationship to *Ae. triseriatus* in the Gambier area, mosquitoes were collected and tested for virus.

This paper reports virus isolation studies and the identification of Jamestown Canyon (JC) virus. Further, evidence of transovarial transmission of JC virus is presented.

METHODS. During 1975, oviposition cans, hereafter called "ovitraps," were set in scattered locations in the village of Gambier. The ovitraps were 1-gallon metal cans, painted dark green inside and out, to which water to a depth of 5–8 cm was added, along with a few dead, dry leaves. A piece of muslin cloth about 10 x 20 cm was hung over the side of the can with one end in the water and the other attached to the can rim with a paper clip. At approximately 4-week intervals, the muslin strips with mosquito eggs were collected and brought to the Columbus laboratory in 1-pint paper ice cream cartons. Eggs were hatched in the laboratory after incubation for 10 days at 100% RH and 75° C, and larvae were reared according to methods essentially as described by Hayes and Morlan (1957). At the 3rd and 4th larval stages, *Ae. triseriatus* and *Ae. hendersoni* Cockrell were separated, and the *triseriatus* were later pooled according to collection site, frozen in the larval, pupal, or adult stage, and stored at −70°C prior to testing for virus. *Ae. hendersoni* specimens were not tested.

The mosquitoes were triturated, and suspensions were prepared for inoculation into suckling mice (SM) by methods described by Sudia and Chamberlain (1967). Individual pools were inoculated by the intracranial route into litters of 6 SM, which were then observed for signs of illness for 14 days after inoculation. Brains of sick or dead SM were inoculated into an additional litter of SM as 10% suspensions in 25% normal rabbit serum diluent.

A single virus isolate was obtained from 676 mosquitoes tested. The isolate was identified as to group with a standard micro-complement fixation test which employs a crude (10% SM) brain antigen in borate saline (pH 9.0) and a battery of reference immune preparations for arbovirus groups A, B, and California obtained from CDC, Ft. Collins (Casey 1965). A sample of the virus was sent to CDC, Ft. Collins, for specific identification.

Subtyping was accomplished by using a serum dilution plaque reduction neu-

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tralization test (Lindsey et al. 1976). Briefly, Vero cells were grown to monolayers in 6-well Linbro[3] plates. Twofold serum dilutions were mixed with an equal 0.1 ml volume of virus containing 200 plaque forming units. After 1 hr incubation at 37°C, 0.1 ml of each mixture was dropped onto the center of a separate well, allowed to absorb at 35°C for 45 min, and overlaid with agar containing no stain. On the third day after inoculation, a second agar overlay, this one containing 1:30,000 neutral red, was added and the plates were reincubated for 24 hrs. Cultures were examined daily thereafter with neutralization recorded as ≥90% reduction in plaque numbers as compared with controls.

Results and Discussion. One SM died 4 days after inoculation with mosquito pool No. 75–1891. Inoculation of a second litter with brain material from the dead SM resulted in 100% mortality 2–3 days after inoculation. The isolate was identified as a California group virus by CF tests. Strain 75–1891 was identified to subtype as Jamestown Canyon virus (Table 1). The original isolate was obtained from 43 adult *Ae. triseriatus* reared from eggs collected between 21 July and 12 August, 1975.

This is the first record of the occurrence of JC virus in Ohio. Prior to 1971, JC virus was only known from California, Colorado, Texas, Utah, Wisconsin, and Alberta (Sudia et al. 1971). During 1971 and 1972, JC virus was isolated from *Ae. canadensis* (Theobald) mosquitoes collected in Maryland (Le Duc et al. 1975). During 1971–1974, JC virus was isolated several times from *Aedes* and *Coquillettidia* mosquitoes in New York (Woodall, J. P., New York State Department of Health, personal communication with C. H. Calisher).

Table 1. Neutralization tests with isolate 75–1891 and four selected California group viruses.

	Antibody to:[a]			
Virus[a]	JC	LAC	SSH	TVT
75–1891	160[b]	<20	<20	<20
JC	320	80	40	40
LAC	<20	320	40	<20
SSH	40	80	320	20
TVT	<20	80	40	160

[a] Jamestown Canyon (JC), LaCrosse (LAC), Snowshoe Hare (SSH), Trivittatus (TVT).
[b] Results given as reciprocal titers.

Jamestown Canyon virus has been isolated from species of *Aedes, Psorophora, Anopheles, Culiseta* and *Coquillettidia* mosquitoes, and also from deer flies and horse flies (Parkin et al. 1972, Woodall, personal communication). This is the first record of its isolation from *Ae. triseriatus*. Because of these isolation records, there is considerable question as to which species is the major vector of JC virus, and transmission studies are needed.

Deer, frequently implicated as the vertebrate reservoir host of JC virus (Issel, 1973, 1974; Le Duc et al, 1975), are common in the Gambier area. However, other vetebrate hosts might also be important in the natural cycle of this virus.

The fact that this isolate was from mosquitoes reared from ovitrap-collected eggs is evidence that it is transovarially transmitted in nature, at least by *Ae. triseriatus*. Although these collections were made during the summer months, it seems reasonable that over-wintering of JC virus may take place in the egg stage of some mosquito species, as has been demonstrated for LaCrosse virus (Pantuwatana et al. 1974; Berry et al. 1974).

Ovitraps have been used extensively in surveys of vector mosquitoes, especially *Ae. triseriatus* and *Ae. aegypti* (L.) (Gauld et al. 1974, Jakob and Bevier 1969). Balfour et al. (1975) used ovitraps to collect eggs for arbovirus testing with negative results. We believe our isolate is the first reported from eggs collected in ovitraps in an arbovirus survey. The simplicity and low cost of materials used in this method may make it an attractive procedure for those involved in arbovirus surveillance. This was the only virus isolate obtained from the

1975 ovitrap collections in Gambier; however, it is noted that since 1972, residents of Gambier have engaged in an extensive anti *Ae. triseriatus* campaign by filling tree holes and removing all discarded water-holding containers to prevent breeding.

Whether or not JC virus is transmitted to man in Gambier or other areas in Ohio is unknown. However, higher CF titers were found in positive human sera with strain 75–1891 than with LAC virus as antigen (D. Keiper, Ohio Dept. Health, personal communication). Perhaps JC virus infections have heretofore gone undetected. Further investigation is needed to determine the significance for man of JC virus in Ohio.

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References Cited

Balfour, H. H., Jr., C. K. Edelman, F. E. Cook, W. I. Barton, A. W. Buzicky, R. A. Siem and H. Bauer. 1975. Isolates of California encephalitis (LaCrosse) virus from field collected eggs and larvae of *Aedes triseriatus*: Identification of the overwintering site of California encephalitis. J. Infect. Dis. 131:712–716.

Berry, R. L., B. J. LaLonde H. W. Stegmiller, M. A. Parsons and G. T. Bear. 1974. Isolation of LaCrosse virus (California encephalitis group) from field collected *Aedes triseriatus* (Say) larvae in Ohio (Diptera: Culicidae). Mosquito News 34:454–457.

Berry, R. L., M. A. Parsons, B. J. LaLonde, H. W. Stegmiller, J. Lebio, M. Jalil and R. A. Masterson. 1975. Studies on the epidemiology of California encephalitis in an endemic area in Ohio in 1971. Amer. J. Trop. Med. Hyg. 23:992–998.

Casey, H. L. 1965. Standardized diagnostic complement fixation method and adaptation to microtest, Part II. Public Health Monograph No. 74.

Gauld, L. W., R. P. Hanson, W. H. Thompson and S. K. Sinha. 1974. Observation on a natural cycle of LaCrosse virus (California group) in southwestern Wisconsin. Amer. J. Trop. Med. Hyg. 23:983–992.

Hayes, R. O. and H. B. Morlan. 1957. Notes on *Aedes triseriatus* egg incubation and colonization. Mosquito News 17:33–36.

Issel, Charles J. 1973. Isolation of Jamestown Canyon virus (a California group virus) from white tailed deer. Amer. J. Trop. Med. Hyg. 22:414–417.

Issel, Charles J. 1974. Maternal antibody to Jamestown Canyon virus in white-tailed deer. Amer. J. Trop. Med. Hyg. 23:242–245.

Jakob, W. L. and G. A. Bevier. 1969. Application of ovitraps in the U.S. *Aedes aegypti* eradication program. Mosquito News 29:55–62.

Le Duc, J. W., W. Suyemoto, J. J. Keefe, J. F. Burger, G. F. Eldridge and P. K. Russel. 1975. Ecology of California encephalitis viruses on the Del Mar Va. Peninsula I. Virus isolations from mosquitoes. Amer. J. Trop. Med. Hyg. 24:118–123.

Lindsey, H. S., C. H. Calisher and J. H. Mathews. 1976. A serum dilution neutralization test for California group virus identification and serology. J. Clin. Microbiol. 4:503–510.

Masterson, R. A., H. W. Stegmiller, M. A. Parsons, C. C. Croft and C. B. Spencer. 1971. California encephalitis—an endemic puzzle in Ohio. Health Lab. Sci. 8:89–96.

Pantuwatana, S., W. J. Thompson, D. M. Watts, T. M. Yuill and R. P. Hanson. 1974. Isolation of LaCrosse virus from field collected *Aedes triseriatus* larvae. Amer. J. Trop. Med. Hyg. 23:246–250.

Parkin. W. E., W. McD. Hammon and G. Sather. 1972. Review of current epidemiological literature on viruses of the California arbovirus group. Amer. J. Trop. Med. Hyg. 21:964–978.

Sudia, W. D. and R. W. Chamberlain. 1967. Collection and processing of medically important arthropods for arbovirus isolation. Public Health Service, Center for Disease Control, Atlanta, Georgia 29 pp.

Sudia, W. D., W. F. Newhouse, C. H. Calisher and R. W. Chamberlain. 1971. California group arboviruses: Isolations from mosquitoes of North America. Mosquito News 31:576–600.