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ISOLATION OF SNOWSHOE HARE VIRUS FROM *AEDES IMPLICATUS* LARVAE IN SASKATCHEWAN[1]

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ABSTRACT. Snowshoe hare virus of the California encephalitis group of arboviruses was isolated from female *Aedes implicatus* Vockeroth reared from larvae collected in the southern edge of the mixed-wood forest near Macdowall, Saskatchewan, Canada. This is the first reported isolation of an arbovirus from *A. implicatus* and suggests that transovarial transmission contributes to the natural maintenance of snowshoe hare virus in Saskatchewan. The advantages of transovarial transmission in the mosquito for survival of a virus whose hosts alternate between a univoltine *Aedes* and rodents or lagomorphs at northern latitudes are briefly discussed. An infection cycle, involving an *Aedes* that takes only one blood meal during its lifetime, is suggested.

INTRODUCTION. The major unsolved problem in the ecology and epidemiology of temperate and subarctic climate arboviruses is the means by which these viruses survive the winter season. The hypotheses put forward to explain winter survival by these viruses were reviewed by Reeves (1959), Hess and Hayes (1967) and Reeves (1974). Prominent among these hypotheses is the possibility that the viruses survive the adverse season in immature stages of mosquito vectors which receive their infections through the mechanism of transovarial transmission of the viruses. Either a primary summertime vector species, or a secondary vector that periodically reintroduces infection to vertebrate hosts which then serve to infect the primary vectors, could function in this role (Reeves 1974). Watts and Eldridge (1975) reviewed field and laboratory evidence for and against transovarial transmission of arboviruses by mosquitoes. For the Flavoviruses and Alphaviruses (Wildy 1971) the evidence to date for transovarial transmission is inconclusive, but for the California encephalitis (CE) group of Bunyavirus supergroup (Theiler and Downs 1973) the evidence is more conclusive. There is no doubt that adult *Aedes triseriatus* are the primary vectors of La Crosse (LAC) virus of the CE group (Sudia et al. 1971; Thompson et al. 1972; Watts et al. 1972; Gauld et al. 1974) and that the eggs of that species, infected by transovarial transmission, serve as overwintering hosts of the virus (Watts et al. 1973, 1974). On the basis of isolation of virus from field-collected larvae, or from adults reared from field-collected larvae, transovarial transmission of LAC virus has also been recognized in *A. triseriatus* in Ohio (Berry et al. 1974) and in Minnesota (Balfour et al., 1975). LeDuc et al. (1975a, 1975b) isolated Keystone (KEY) virus of the CE group from larvae, reared males and reared females of *Aedes atlanticus* collected on the DelMarVa Peninsula thus indicating transovarial transmission of KEY virus in that mosquito. McLean et al. (1975) isolated the snowshoe hare (SSH) virus of the CE group from *Aedes* spp. larvae collected in the Yukon Territory, indicating transovarial transmission of a third CE group virus.

The SSH virus and Jamestown Canyon (JC) viruses of the CE group were isolated from several species of *Aedes* in the mixed-wood forest zone of Saskatchewan (Iversen et al. 1973), an area in which SSH virus is known to be enzootic through

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the utilization of sentinel domestic rabbits during the summers of 1973 and 1974. In addition, isolations of SSH virus have been made in forested regions of the Yukon Territory (McLean et al. 1972), British Columbia (McLean 1970), Alberta (Iversen et al. 1969), Saskatchewan (Iversen et al. 1973) and Ontario (McKiel et al. 1966), in the plains of southern Alberta (Morgante and Shemanchuk 1967) and in the "barrens" of the Keewatin District (Wagner et al. 1975). With such a wide distribution in the boreal forests of North America and extending into the agricultural area to the south and into the subarctic to the north, it would be of considerable interest to know in what mosquito species the SSH virus is passing transovarially. We report here an isolation of SSH virus from *Aedes implicatus* Vockeroth females reared from larvae collected in the Macdowall district of Saskatchewan.

MATERIALS AND METHODS. Mosquito larvae and pupae were collected in the spring from their breeding sites adjacent to the Canadian National Railroad tracks and McFarlane Creek, 3 kilometers south of Macdowall, Saskatchewan (53N, 106W). Macdowall lies within the humid continental climatic region and black-grey organic soils are characteristic of the district. It is located in a southward projection of the mixed-wood forest and is considered to be transitional between the mixed-wood forest and the aspen parkland. Intermixed with the forested areas are lands that have been cleared for agricultural usage. Spruce (*Picea glauca*) and poplar (*Populus* spp.) predominate in the area. Three types of breeding pools were sampled, viz. sphagnum pools, pools with soil substrates and abandoned wells at the edge of the forest and a cleared field.

Larvae and pupae were collected by dipping and concentrated in a gasoline filter funnel. Contents of the funnel were then rinsed on to cotton terry towelling that lined the bottoms of 16"x 9" x 2" enamel trays. After the excess water was drained off, the trays containing the larvae and pupae were transported to the laboratory where the larvae and pupae were washed off the towelling back into the trays with Bates' (1941) "Medium S." Dry sterilized powdered "TetraMin" fish food (Hayes et al. 1974) was added to each tray. The trays were held at room temperature and covered with glass plates to retard evaporation.

Collections were made at approximately weekly intervals from 4 different sites in the Macdowell area. The numbers of larvae and pupae collected, particularly at the beginning of the season, were too numerous to be identified individually but samples of larvae from each collection were reared to adults for identification. Approximately 16,000 *Aedes* spp. larvae were pooled in groups of 100 larvae each; these were all negative when tested for virus.

When mosquito larvae were infected with St. Louis encephalitis virus by Collins (1962, 1963) the resulting adults emerged with somewhat higher titres of virus than the larvae had acquired. Consequently, on the assumption that adults developing from infected larvae would harbor virus at higher titres, we reared to the adult stage all larvae and pupae collected after May 27. Further justification for this was found in the report of a study of transstadial transmisison of LAC virus in *Aedes triseriatus* by Watts et al. (1975) who observed that virus concentration increased during metamorphosis from low levels of "$10^{0.5}$SMLD$_{50}$/0.03 ml or less, to titres of $10^{3.5}$SMLD$_{50}$/0.03 ml in adult mosquitoes." Samples of larvae from most of the collections were reared individually for identification so that we were familiar with the local variants of the 7 species taken in the Macdowall district during the collection period. The adults were segregated by sex and species and pooled in groups of 62 or less. The laboratory in which the mosquitoes were reared and identified had never been used for virus work.

Mosquitoes were assayed for virus in suckling mice following technics described by Iversen et al. (1969). Identification of the viral isolate was done at the first suck-

ling mouse passage level by neutralization tests using SSH virus hyperimmune serum. The neutralization tests were performed in both newborn mice and baby hamster kidney cell culture (BHK_{21} cell line).

RESULTS. A total of 959 adult mosquitoes reared from larvae and pupae and distributed in 41 pools were tested for virus (Table 1). From these, one viral isolate was obtained from a pool of 60 female *Aedes implicatus* reared from larvae collected on June 10, 1975. The specimens were from an abandoned well which simultaneously contained *Aedes communis, Aedes punctor* and *Aedes pionips*. The isolate was identified as SSH virus by neutralization tests performed in newborn mice and BHK_{21} cells. Reisolation of the virus was made by a subsequent inoculation of the original mosquito suspension into newborn mice.

DISCUSSION. Reports of transovarial transmission of LAC virus in *Aedes triseriatus* (Watts et al. 1975; Balfour et al. 1975), of KEY virus in *Aedes atlanticus* (LeDuc et al. 1975a, b), of SSH virus in *Aedes* spp. (McLean et al. 1975) and in *Aedes implicatus* suggest that winter survival of CE group viruses through the mechanism of transovarial transmission might be the rule in North America. *A. implicatus,* like other northern aedines, overwinters in the egg stage. This eliminates the possibility of the virus overwintering in the adult, blood-feeding stage of that mosquito (Reeves 1974). In the northern boreal forests and tundra, with relatively short mosquito seasons of 6 to 12 weeks, transovarial passage of virus could ensure survival of SSH virus in *Aedes* eggs during the long severe winters.

A considerable amount of evidence (Henderson and Coleman 1971; Iversen et al. 1971) supports the hypothesis that the primary infection cycle for some viruses of the CE group is small nonmigratory mammals—mosquitoes—small non-migratory mammals. In Wisconsin, chipmunks and tree squirrels are the major primary vertebrate hosts of LAC virus (Gauld et al. 1974) and in the boreal forest zone of Alberta snowshoe hares are important reservoirs of SSH infection (Hoff et al. 1969). However, even if rodent or lagomorph hosts had chronic relapsing infections that could serve as sources of infection for vectors or other vertebrates (Reeves 1974), at northern latitudes transovarial transmission of SSH virus could also provide for survival of the virus through periods of scarcity in fluctuating rodent or lagomorph populations.

The isolation of SSH virus from *Aedes implicatus* adds another mosquito species to the 14 or more from which SSH virus has already been isolated (Sudia et al. 1971; Iversen et al. 1973). Isolations of the SSH virus from *Aedes* larvae, or from adults reared from larvae collected in the field, indicate that the virus can overwinter in eggs infected by transovarial transmission, but we still know nothing about the vector ability of *A. implicatus*. After acquiring the virus by transovarial transmission, it would still be necessary for infected *A. implicatus* to be able to transmit

Table 1. Preimaginal mosquitoes collected from May 28 to July 11, 1975 in Central Saskatchewan and reared to adults for California encephalitis virus isolation attempts.

Species	Males	Females	Total	Pools	Virus
Aedes canadensis	22	17	39	7	0
Aedes cinereus	0	2	2	1	0
Aedes communis	26	485	511	15	0
Aedes implicatus	0	147	147	5	1
Aedes pionips	0	67	67	5	0
Aedes punctor	0	2	2	1	0
Culiseta inornata	0	3	3	1	0
Aedes spp.	188	0	188	6	0
Total	236	723	959	41	1

the virus by bite to be of significance in the endemicity of the virus. At present the only evidence that a mosquito found infected with the SSH virus in nature is able to transmit the virus by bite comes from the study of McLean et al. (1974) who found that the virus was transmitted to mice by the bites of *Aedes cinereus* 14 and 15 days after intrathoracic injection following incubation at 55°F and by bite of *Culiseta inornata* 17 days after intrathoracic injection following incubation at 80°F. The geographical range of *A. implicatus* extends from about 40°N latitude northward through the forested regions of North America (Carpenter and LaCasse 1955; Carpenter 1968, 1970; Vockeroth 1954). In Canada it is found mainly in the boreal forest with larvae first appearing in late March and early April, and adults are present during June or July (Vockeroth 1954; Happold 1965; Stewart 1974), but it is said to be rare throughout most of its range (Carpenter and LaCasse 1955). The females are vigorous biters (Matheson 1944; Rees and Nielson 1951) and could therefore play a role in the transmission of SSH virus. Little is known of the biology of *A. implicatus* (Barr 1958), and further studies are needed to clarify its role in the spread and maintenance of SSH virus.

McLean (1975) has drawn attention to the probability that virus transmission by boreal *Aedes* and *Culiseta inornata* rarely occurs due to their well-known reluctance to take more than one blood meal during their lifetime. There could therefore be another variation of the vector-host cycles suggested by Reeves (1974), i.e. a primary summertime vector that receives its virus infection through transovarian transmission, infects a vertebrate host when it takes its only blood meal and that vertebrate in turn infects another primary vector *Aedes* which in turn passes the infection to its offspring. This could be called delayed biological transmission in that the extrinsic incubation occurs during the larval and pupal development of the offspring.

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.

Barr, A. R. 1958. The mosquitoes of Minnesota (Diptera: Culicidae: Culicinae). Tech. Bull. 228:1–154. University of Minnesota Agr. Expt. Sta.

Bates, M. 1941. Studies in the technique of raising anopheline larvae. Amer. J. Trop. Med. 21:103–122.

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.

Carpenter, S. J. 1968. Review of recent literature on mosquitoes of North America. Calif. Vector Views 15:71–98.

Carpenter, S. J. 1970. Review of recent literature on mosquitoes of North America, Supplement I. Calif. Vector Views 17:39–66.

Carpenter, S. J. and W. J. LaCasse. 1955. The mosquitoes of North America (north of Mexico). University of California Press, Berkeley. 360 pp.

Collins, W. E. 1962. Trans-stadial passage of St. Louis encephalitis virus in *Aedes aegypti* mosquitoes. Amer. J. Trop. Med. Hyg. 11: 535–538.

Collins, W. E. 1963. Transmission of St. Louis encephalitis virus by larval-infected *Culex quinquefasciatus* mosquitoes. Ann. Entomol. Soc. Amer. 56:237–239.

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

Happold, D. C. D. 1965. Mosquito ecology in Central Alberta. I. The environment, the species, and studies of the larvae. Canad. J. Zool. 43:795–819.

Hayes, R. O., M. Montoya, G. C. Smith, D. B. Francy and W. L. Jakob. 1974. An analysis of *Culex tarsalis* Coquillett laboratory rearing productivity. Mosquito News 34:462–466.

Henderson, B. E. and P. H. Coleman. 1971. The growing importance of California arboviruses in the etiology of human disease. Prog. Med. Virol. 13:404–461.

Hess, A. D. and R. O. Hayes. 1967. Seasonal dynamics of western encephalitis virus. Amer. J. Med. Sci. 253:333–348.

Hoff, G. L., T. M. Yuill, J. O. Iversen and R. P. Hanson. 1969. Snowshoe hares and the California encephalitis virus group in Alberta,

1961–1968. Bull. Wildlife Dis. Assoc. 5:254–259.

Iversen, J. O., R. P. Hanson, O. Papadopoulos, C. V. Morris and G. R. DeFoliart. 1969. Isolation of viruses of the California encephalitis group from boreal *Aedes* mosquitoes. Amer. J. Trop. Med. Hyg. 18:735–742.

Iversen, J. O., G. Seawright and R. P. Hanson. 1971. Serologic survey for arboviruses in Central Alberta. Canad. J. Public Health 62:125–132.

Iversen, J. O., R. J. Wagner, C. deJong and J. McLintock. 1973. California encephalitis virus in Saskatchewan: isolation from boreal *Aedes* mosquitoes. Canad. J. Public Health 64:590–593.

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

LeDuc, J. W., W. Suyemoto, B. F. Eldridge, P. K. Russell and A. R. Barr. 1975b. Ecology of California encephalitis viruses on the Del Mar Va Peninsula. II. Demonstration of transovarial transmission. Amer. J. Trop. Med. Hyg. 24: 124–126.

Matheson, R. 1944. A handbook of the mosquitoes of North America. Comstock Publ. Assoc., Ithaca, N.Y. 314 pp.

McKiel, J. A., R. R. Hall and V. F. Newhouse. 1966. Viruses of the California encephalitis complex in indicator rabbits. Amer. J. Trop. Med. Hyg. 15:98–102.

McLean, D. M. 1970. California encephalitis virus isolation from British Columbia mosquitoes. Mosquito News 30:144–145.

McLean, D. M. 1975. Mosquito-borne arboviruses in Arctic America. Med. Biol. 53:264–270.

McLean, D. M., E. J. Goddard, E. A. Graham, G. J. Hardy and K. W. Purvin-Good. 1972. California encephalitis virus isolations from Yukon mosquitoes, 1971. Amer. J. Epidemiol. 95:347–355.

McLean, D. M., S. K. A. Bergman, E. A. Graham, G. P. Greenfield, J. A. Olden and R. D. Patterson. 1974. California encephalitis virus prevalence in Yukon mosquitoes during 1973. Canad. J. Public Health 65:23–28.

McLean, D. M., S. K. A. Bergman, A. P. Gould, P. N. Grass, M. A. Miller and E. E. Spratt. 1975. California encephalitis virus prevalence throughout the Yukon Territory, 1971–1974. Amer. J. Trop. Med. Hyg. 24:676–684.

Morgante, O. and J. A. Shemanchuk. 1967. Virus of the California encephalitis complex: isolation from *Culiseta inornata*. Science 157: 692–693.

Rees, D. M. and L. T. Nielson. 1951. Four new mosquito records from Utah (Diptera: Culicidae). Pan. Pac. Ent. 27:11–12.

Reeves, W. C. 1959. Problems of overwintering and natural maintenance of mosquito-borne viruses. Proc. 6th Int. Congr. Trop. Med. Malar. 5:48–57.

Reeves, W. C. 1974. Overwintering of arboviruses. Progr. Med. Virol. 17:193–220.

Stewart, W. W. A. 1974. The rate of larval development of *Aedes implicatus* Vockeroth in field and laboratory. Mosquito News 34:283–285.

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

Theiler, M. and W. G. Downs. 1973. The arthropod-borne viruses of vertebrates. Yale Univ. Press, New Haven & London. 578 pp.

Thompson, W. H., R. O. Anslow, R. P. Hanson and G. R. DeFoliart. 1972. LaCrosse virus isolations from mosquitoes in Wisconsin, 1964–1968. Amer. J. Trop. Med. Hyg. 21:90–96.

Vockeroth, J. R. 1954. Notes on the identities and distribution of *Aedes* species of northern Canada, with a key to the females (Diptera: Culicidae). Canad. Entomol. 86:241–255.

Wagner, R. J., C. deJong, M. K. Leung, J. McLintock and J. O. Iversen. 1975. Isolations of California encephalitis virus from tundra mosquitoes. Canad. J. Microbiol. 21:574–576.

Watts, D. M. and B. F. Eldridge. 1975. Transovarial transmission of arboviruses by mosquitoes: A review. Med. Biol. 53:271–278.

Watts, D. M., C. D. Morris, R. E. Wright, G. R. DeFoliart and R. P. Hanson. 1972. Transmission of LaCrosse virus (California encephalitis group) by the mosquito *Aedes triseriatus*. J. Med. Entomol. 9:125–127.

Watts, D. M., S. Pantuwatana, G. R. DeFoliart, T. M. Yuill and W. H. Thompson. 1973. Transovarial transmission of LaCrosse virus (California encephalitis group) in the mosquito, *Aedes triseriatus*. Science 182:1140–1141.

Watts, D. M., W. H. Thompson, T. M. Yuill, G. R. DeFoliart and R. P. Hanson. 1974. Overwintering of LaCrosse virus in *Aedes triseriatus*. Amer. J. Trop. Med. Hyg. 23:694–700.

Watts, D. M., S. Pantuwatana, T. M. Yuill, G. R. DeFoliart, W. H. Thompson and R. P. Hanson. 1975. Transovarial transmission of LaCrosse virus in *Aedes triseriatus*. Ann. New York Acad. Sci. 266:135–143.

Wildy, P. 1971. Classification and nomenclature of viruses. Monographs in Virology, Vol. 5, 81 pp. S. Karger, Basel & New York.