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Terry L. Foxhoven
Burns Bio-Tec Laboratories

Ardyce B. Welch
University of Nebraska-Lincoln

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TERRY L. FOXHOVEN*
ARDYCE B. WELCH

University of Nebraska–Lincoln
Lincoln, Nebraska 68588

Aedes triseriatus mosquitoes were trapped and processed for viral isolation. Swiss white mice and BHK-21 cell cultures were used for propagation of virus. A sucrose-acetone extracted antigen was produced from infected mouse brains. Anti-serum was produced in Swiss white mice as hyperimmune ascitic fluid using Sarcoma 180/TG. The isolate was identified using hemagglutination, hemagglutination-inhibition, and serum neutralization techniques. The virus was identified as California group arbovirus, type Trivittatus.

This is the first reported isolation of California group arbovirus, type Trivittatus virus from field samples of *A. triseriatus* mosquito and the first time the virus has been isolated from any species of mosquito in the state of Nebraska.

† † †

INTRODUCTION

Arthropod-borne viruses have been isolated or have been shown to be present by serologic means in nearly all of the midwestern states surrounding Nebraska. A few limited studies of sera from wild and domestic fowl and mammals including rodents and humans have suggested that there is arbovirus activity in Nebraska.

During an outbreak of equine encephalitis in 1943, sera from domestic and wild animals in eastern Nebraska were tested for antibody to Western equine encephalitis (WEE), Eastern equine encephalitis (EEE), and St. Louis encephalitis (SLE) viruses (Hammon, et al., 1945). At that time no antibodies were detected to WEE and EEE viruses. However, a few sera were found to have antibody to SLE virus. Trainer and Hanson (1969) found antibody to WEE and California encephalitis (CE) virus in sera of white-tailed deer in Nebraska. Since that time there have been several recorded human cases of WEE, SLE, and CE in Nebraska (Center for Disease Control, 1963-71).

This investigation was conducted to determine which arboviruses were harbored by *A. triseriatus* mosquitoes in Nebraska.

MATERIALS AND METHODS

Mosquito Trapping: Mosquitoes were trapped using Center for Disease Control (CDC) miniature light traps modified by the addition of a vial of 1*M* lysine and a dry ice container as a source of CO_2, attached to the trap cylinder (Chamberlain and Newhouse, 1966). Trapping was done within a twelve-mile radius of Lincoln, Nebraska, in swampy brush areas containing well established trees. In order to recover a high percentage of *A. triseriatus,* areas were chosen which had a substantial growth of trees, since this mosquito is a tree hole type, feeding primarily on birds. The pool of mosquitoes selected for this work was trapped in the Wilderness Park area on the west edge of Lincoln. Eighty percent of the mosquitoes trapped in this area were *A. triseriatus.*

Trapping was done during the months of August and September, since these months have been found by CDC to show the highest number of cases of viral encephalitis during the year (CDC, 1963-71). Traps were set one hour before sunset and recovered one hour before sunrise. In order to recover mosquito-borne viruses it is essential that trapped mosquitoes be processed before dehydration and death occur due to heat. This recovery of viruses was accomplished by use of ice coolers containing dry ice. Further procedures were carried out at 4 C.

Preliminary separation of mosquitoes was based on commonly accepted characteristics as described by Pratt and Stojanovich (1963) and Carpenter and La Casse (1955). Differentiation between *A. triseriatus* and *A. hendersonnii* was based on the presence of a front dorsal claw of *A. triseriatus.* After identification, the imago female mosquitoes were stored at -70 C.

*Burns Bio-Tec Laboratories, 220th and West Center Road, Omaha, Nebraska.

Virus Source: One hundred *A. triseriatus* mosquitoes were ground according to the method of Sudia and Chamberlain (1967) and 0.02 ml of supernatant fluid was injected intracerebrally into 3- or 4-day-old Swiss white mice for viral isolation and propagation (Hammon and Sather, 1969). The grinding diluent consisted of Eagle's minimum medium prepared in Hanks balanced salt solution (HME), 25 percent fetal calf serum, and 50 ug/ml gentamicin.

Antigen: A suitable antigen for hemagglutination (HA) and hemagglutination inhibition (HAI) tests was produced using the sucrose-acetone method of Clarke and Casals (1958). Control antigens of WEE, VEE, and EEE viruses used in comparative studies were purchased from the CDC.

Antisera: Immune antisera used for HA, HAI, and serum neutralization (SN) tests were obtained as ascitic fluid from adult Swiss white mice carrying large tumors induced by Sarcoma 180/TG (Sartorelli, et al., 1966). Mice were inoculated intraperitoneally with 0.5 ml of sucrose-acetone extracted antigen and seven days later inoculated intraperitoneally with 0.5 ml of Sarcoma 180/TG to produce the tumors. Immune ascitic fluid was harvested approximately ten days after the Sarcoma 180/TG injection.

WEE and SLE antisera were obtained from the CDC. Antisera to CE type LaCrosse was obtained from Beckman Diagnostics, Fullerton, California. Antisera to CE type Trivittatus was obtained from R. E. Shope, Yale University, New Haven, Connecticut.

HA and HAI Tests: HA and HAI tests were performed as outlined by Clarke and Casals (1958) with two modifications by Ardoine and Clarke (1967). Immediately prior to use, antigen was mildly sonicated in a cold water bath for two to three minutes using an L and R Ultrasonic sonicator, diluted 1:10 in Eagle's minimum essential medium prepared in Earles balanced salt solution (MEME) and allowed to stand for one hour at 4 C to allow dispersion of the antigen.

Positive controls consisted of antisera to WEE and SLE viruses *vs* their respective antigens. Viral antigen alone, viral antiserum alone, and antiserum to SLE *vs* WEE antigen were used as negative controls.

SN Test: The SN tests were performed by the constant virus-varying serum method using BHK-21 cells. Equal amounts of virus suspension, 100 Cell Culture Infective Doses ($CCID_{50}$) / ml, and the antisera to be tested at various dilutions were mixed and allowed to incubate at room temperature for one hour. The growth medium was removed from the BHK-21 monolayers and 0.2 ml of the virus-antisera mixture was added to each of six tubes of cell culture and allowed to absorb for two to three hours at 37 C. The virus-antisera suspension was then removed, and MEME containing 2 percent heat inactivated fetal calf serum and antibiotics was added. Cell cultures were observed for cytopathic effects (CPE) and inhibition of CPE for several days. WEE virus was used as a positive control and WEE virus antiserum as a negative control.

RESULTS

Upon initial intracerebral injection of mosquito grindings, 3- to 4-day-old Swiss white mice exhibited nervous convulsions, paralysis, and death within six to seven days. Subsequent mouse injections using brain suspensions from the previously infected mice resulted in a consistent period of five days for the above symptoms and death to occur. After six additional passages in mice, 60 mouse brains weighing a total of 20 gms were ground and utilized in the production of a sucrose-acetone antigen. This antigen was tested by HA and found to hemagglutinate goose erythrocytes in a pH range of 5.8 to 6.0 at 25 C (Table I). These results also showed the HA titer to be 1:160. A pH range of 5.8 to 6.0 is indicative of the California group arboviruses.

Results of HAI tests (Table II) demonstrated that antisera to WEE, SLE, and CE type La Crosse did not inhibit hemagglutination of goose erthrocytes by the viral antigen. Antisera to CE Trivittatus completely inhibited hemagglutination of goose erthrocytes by the viral antigen to a dilution of 1:40 and partially to and including a dilution of 1:160. This indicated tht the virus isolated was CE type Trivittatus.

BHK-21 cells infected with the viral isolate exhibited 75 percent CPE in three to five days, whereupon the virus was harvested. CPE consisted of rounding of cells, granulation of cytoplasm, and ultimate detachment from the glass surface.

In the SN test (Table III) the virus was neutralized by CE type Trivittatus antiserum and the immune ascitic fluid to viral isolate with 50 percent neutralizing endpoints of 1:100 and 1:38, respectively. Antisera to WEE, SLE, and CE type La Crosse did not neutralize the virus. In the serum neutralization test, a titer of 1:10 or less is considered insignificant, since there is slight cross reaction throughout the arboviruses, and since serum itself contains various components which can also have slight neutralizing effects.

DISCUSSION

Results of this investigation have shown that the virus isolated from *A. triseriatus* mosquitoes was a California group arbovirus, type Trivittatus. The hemagglutination test tentatively placed the agent within the California group arboviruses, since the pH range of 5.8 to 6.0 is indicative of the California group arboviruses. Hemagglutination-inhibition and serum neutralization tests demonstrated the virus to be Trivittatus, a member of the California group arboviruses.

TABLE I

Hemagglutination Test

pH	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Control
5.5	+	+	+	±	-	-	-	-	-
5.6	+	+	+	±	-	-	-	-	-
5.7	+	+	+	+	±	-	-	-	-
5.8	+	+	+	+	+	±	-	-	-
6.0	+	+	+	+	+	±	-	-	-
6.2	+	+	+	±	±	-	-	-	-
6.4	+	-	-	-	-	-	-	-	-
6.6	-	-	-	-	-	-	-	-	-

Negative Control = goose erythrocytes and buffer

+ = Complete Hemagglutination

± = Partial Hemagglutination

- = No Hemagglutination

TABLE II

Hemagglutination-Inhibition Test

Type of Antisera	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
CE								
Trivittatus	-	-	-	±	±	+	+	+
LaCrosse	±	+	+	+	+	+	+	+
WEE	+	+	+	+	+	+	+	+
SLE	+	+	+	+	+	+	+	+

Negative Controls

Trivittatus antiserum alone with goose erythrocytes = no hemagglutination.
Sucrose-Acetone viral isolate antigen alone with goose erythrocytes = hemagglutination to 1:160.
SLE antiserum plus WEE antigen with goose erythrocytes-inhibition of hemagglutination to 1:10.

Positive Controls

SLE antiserum plus SLE antigen with goose erythrocytes-inhibition of hemagglutination to 1:320.
WEE antiserum plus WEE antigen with goose erythrocytes-inhibition of hemagglutination to 1:160.

+ = Complete Hemagglutination

± = Partial Hemagglutination

- = No Hemagglutination

TABLE II

Serum Neutralization Test
(Using Mouse Brain Suspension of Isolated Virus)

Type of Antisera	50% Neutralizing End Points*
Immune Ascitic Fluid to Viral Isolate	1:38
CE	
Trivittatus	1:100
LaCrosse	1:2
WEE	1:10
SLE	1:10

*50% Neutralizing End Points were calculated according to the Reed and Muench Method.

To date there have been no reports in the literature citing isolation of Trivittatus virus from naturally or artificially infected female imago *A. triseriatus* mosquitoes. This, we believe, is the first reported isolation of Trivittatus virus from *A. triseriatus.* It is, furthermore, the first time Trivittatus has been isolated from any species of mosquito in Nebraska. Isolation of Trivittatus virus from this natural vector is in agreement with other laboratory studies which demonstrated the ability of *A. triseriatus* mosquitoes to transmit many arboviruses (TenBroeck and Merrill, 1935; Davis, 1940; Hammon and Reeves, 1943; Kissling, et al., 1956; and Watts, et al., 1973). We have now demonstrated the ability of *A. triseriatus* to harbor Trivittatus virus.

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REFERENCES

Ardoine, P., and D. Clarke. 1967. The use of sonication and calcium phosphate chromatography for preparation of Group C arbovirus hemagglutinins. *American Journal of Medicine and Hygiene,* 16:357-363.

Carpenter, S. J., and W. J. La Casse. 1955. *The mosquitoes of North America (north of Mexico).* Berkeley, University of California Press.

Center for Disease Control. 1963-71. Neurotropic viral diseases surveillance-encephalitis. *Annual encephalitis summary.* Atlanta, Georgia, Public Health Service, United States Department of Health, Education and Welfare.

Chamberlain, R. W., and V. F. Newhouse. 1966. Use of dry ice to increase mosquito catches of the CDC miniature light trap. *Mosquito News,* 26:30-35.

Clarke, D. H., and J. Casals. 1958. Techniques for hemagglutination and hemagglutination inhibition with arthropod-borne viruses. *American Journal of Tropical Medicine and Hygiene,* 7:561-573.

Davis, W. A. 1940. A study of birds and mosquitoes as hosts for the virus of eastern equine encephalomyelitis. *American Journal of Hygiene,* 32:45-59.

Hammon, W. M., and W. C. Reeves. 1943. Laboratory transmission of St. Louis encephalitis virus by three genera of mosquitoes. *Journal of Experimental Medicine,* 78: 241-253.

Hammon, W. M., W. C. Reeves, and P. Galindo. 1945. Epizoology of western equine type encephalomyelitis, eastern Nebraska field survey of 1943 with isolations of the virus from mosquitoes. *American Journal of Veterinary Research,* 6:145-148.

Hammon, S. M., and G. E. Sather. 1969. Arboviruses. *In* E. H. Lennette and N. J. Schmidt (eds.), *Diagnostic procedures for virual and rickettsial infections.* New York, American Public Health Association: 227-280.

Kissling, R. E., R. W. Chamberlain, D. B. Nelson, and D. D. Stamm. 1956. Venezuelan equine encephalomyelitis in horses. *American Journal of Hygiene,* 63:274-287.

Pratt, H. D., and C. J. Stojanovich. 1963. Pictoral key to some common female mosquitoes of the United States. Washington, D.C., United States Department of Health, Education and Welfare.

Sartorelli, A. C., D. S. Fisher, and W. G. Downs. 1966. Use of sarcoma 180/TG to prepare hyperimmune ascitic fluid in the mouse. *Journal of Immunology,* 96:675-682.

Sudia, W. D., and R. W. Chamberlain. 1967. Collection and processing of medically important arthropods for arbovirus isolation. Atlanta, Georgia, United States Department of Health, Education and Welfare.

TenBroeck, C., and M. Merrill. 1935. Transmission of equine encephalomyelitis by mosquitoes. *American Journal of Pathology,* 11:847.

Trainer, D. O., and R. P. Hanson. 1969. Serologic evidence of arbovirus infection in wild ruminants. *American Journal of Epidemiology*, 90:354-358.

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 (4117):1140-1141.