tion, and Welfare have agreed to assist us in drafting the exemption regulations.

As you know, even under the "old system" we registered a juvenile hormone analog—ALTOSID—for mosquito control, in both a microencapsulated and briquette form. On the horizon for mosquito control—we are aware of two possible bacterial agents: *Bacillus sphaericus* and *Bacillus thuringiensis* which are currently being field tested.

As we work on the various elements of the biologicals registration program and on new concepts in registration, we need your active participation and input. Our programs need to be in tune with the directions in which you will lead research and development of the mosquito control systems of the future. We look forward to working with you.

PRELIMINARY STUDY OF TRANSMISSION OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS BY LABORATORY INFECTED *AEDES HENDERSONI* COCKERELL[1]

DAVID R. SENOGLE[2] AND STEELE R. LUNT
Department of Biology, University of Nebraska at Omaha, Omaha, Nebraska 68182

ROBERTA J. WHITE
Department of Biology, University of Nebraska at Omaha, Omaha, Nebraska 68182

ABSTRACT. A laboratory experiment was conducted to test the western equine encephalomyelitis (WEE) virus transmission ability of *Aedes hendersoni* Cockerell, a species thought to be closely related to *Aedes triseriatus* (Say), which has been shown in laboratory studies to transmit WEE virus. *Ae. hendersoni* became infected at a rate of 100% following ingestion of blood from viremic baby chicks containing $10^{9.8}$–$10^{11.3}$ suckling mouse LD_{50} of WEE virus. Thirteen days after ingestion of the infectious blood meal 56% of the mosquitoes were able to transmit the virus to normal baby chicks.

INTRODUCTION

Aedes triseriatus (Say) and *Ae. hendersoni* Cockerell are the most widely distributed tree-hole breeding mosquitoes in the United States (Zavortink 1972). They are considered to be sibling species because they are sympatric over much of their geographical range and the reproductive barriers between them sometimes break down (Truman and Craig 1968, Lunt 1969, Grimstad et al. 1974, Lunt and Peters 1976). Sibling species would be expected to have similar abilities to transmit pathogenic organisms. However, *Ae. triseriatus* is a transmitter of the LaCross (LAC) strain of California encephalitis virus in the north-central United States (Thompson et al. 1972, Watts et al. 1972), but *Ae. hendersoni* is not (Watts et al. 1975). *Ae. triseriatus* is a laboratory transmitter of western equine encephalomyelitis (WEE) virus (Chamberlain et al. 1954). WEE virus has been isolated from a pool of *Ae. hendersoni* collected in Colorado (Dr. D. Bruce Francy, pers. commun.); however, nothing was known about its ability to transmit WEE virus under laboratory conditions or in nature. The purpose of the present study was to collect data on the ability of *Ae. hendersoni* to transmit WEE virus under laboratory conditions.

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[2] Present address: 560 11th Ave. N.W., New Brighton, Minnesota 55112.

MATERIALS AND METHODS

Ae. hendersoni was collected from a tree hole at the base of an American elm in south-central Nebraska. Immature stages were placed in 25 × 46 cm white enameled pans half-filled with water from the tree hole. Larvae were reared on a finely ground rat chow and adults were given watersoaked raisins as a source of sugar. Adults were retained in 60 × 60 cm screen cages in an insectary having a constant temperature of 24°C, a relative humidity of 60%, and a 15-hr photoperiod. These same environmental conditions were maintained during the infection, incubation, and transmission phases of this study.

Females were allowed to ingest WEE virus by feeding on viremic 1– to 2–day-old chicks. The virus used was originally isolated from field-collected *Culex tarsalis* and had undergone 4 suckling mouse brain passages. After the mosquitoes had fed, blood samples were taken from the chicks by cardiac puncture. The amount of virus in the blood ingested by the mosquitoes was determined by inoculating 0.03-ml aliquots of serial 10-fold dilutions of chicken blood into the brains of 1-day -old mice. Five mice were inoculated with each virus dilution used.

Virus transmission trials were conducted following 13 days of incubation after ingestion of WEE virus, by allowing 25 randomly selected female mosquitoes to feed on 25 1- to 2-day-old normal chicks (1 mosquito/chick). Immediately after feeding, the mosquitoes were stored at −65°C. The chicks were observed for 12 to 13 days for signs of illness and deaths. Blood specimens from live chicks and brains from dead chicks were stored at −65°C. All normal chicks were tested for antibody to WEE virus before the transmission studies were initiated.

The concentration of virus in the viremic chicks used for blood meals was calculated as the 50% lethal dose (LD_{50}) by the method of Reed and Muench (1938). The WEE virus recovered from mosquito suspensions and from chick blood and brain tissue was identified using techniques described by Sudia and Chamberlain (1967) and Sudia et al. (1970).

In both the infection and transmission studies control groups were used.

RESULTS AND DISCUSSION

Chicks providing the initial blood meal showed symptoms of severe illness 36 to 42 hrs after inoculation and had blood titers of $10^{9.8}$ to $10^{11.3}$ LD_{50}/0.03 ml, 42 to 50.5 hr after inoculation with WEE virus.

Following the incubation period, virus was detectable in 22 of the 25 mosquitoes as evidenced by death in the suckling mice after intracerebral inoculation with the ground mosquito suspension. However, the 3 mosquitoes in which virus was undetectable by mouse inoculation did transmit the virus since it could be isolated from blood of the chicks upon which these infected mosquitoes fed. These results indicate that all of the mosquitoes became infected with WEE virus after feeding on birds with a high titer of the virus in their blood.

Fourteen of the chicks fed upon by these infected mosquitoes developed a viremia indicating at least a 56% transmission rate. Antibody studies on the remaining birds were not done.

These preliminary experiments indicate that *Ae. hendersoni* may be a potential vector for WEE virus. To establish the efficacy of this mosquito as a vector of WEE virus, further studies to determine the virus titer required in the donor to infect the mosquito, the length of the extrinsic incubation period and length of time the virus is maintained in the body of the mosquito and the threshold level of virus necessary for transmission by *Ae. hendersoni* are needed.

Literature Cited

Chamberlain, R. W., R. K. Sikes, D. B. Nelson and W. D. Sudia. 1954. Studies on the North American arthropod-borne encephalitides. VI Quantitative Determinations of Virus-Vector Relationships. Amer. J. Hyg. 60 278–290.

Grimstad, P. R, C. E. Garry and G. R. DeFoliart. 1974. *Aedes hendersoni* and *Aedes triseriatus* (Diptera:Culicidae) in Wisconsin: Characterization of larvae, larval hybrids and comparison of adult and hybrid mesoscutal patterns. Ann. Entomol. Soc. Amer. 67:795–804.

Lunt, S. R. 1969. The occurrence of *Aedes hendersoni* Cockerell in Nebraska (Diptera:Culicidae). Proc. Nebr. Acad. Sci. 79:9–10.

Lunt, S. R. and G. E. Peters. 1976. Distribution and ecology of the tree-hole mosquitoes along the Missouri and Platte Rivers in Iowa, Nebraska, Colorado and Wyoming. Mosquito News. 36:80–84.

Reed, L. J. and H. Muench. 1938. A simple method of estimating fifty percent endpoints. Amer. J. Hyg. 27:493–497.

Sudia, W. D. and R. W. Chamberlain. 1967. Collection and processing of medically important arthropods for arbovirus isolation. p. 16–29. U.S. Department of Health, Education, and Welfare, Public Health Service, Bureau of Disease Prevention and Environmental Control, National Communicable Disease Center, Atlanta, Georgia.

Sudia, W. D., R. D. Lord and R. O Hayes. 1970. Collection and processing of vertebrate specimens for arbovirus studies p. 49–52. U.S. Department of Health, Education, and Welfare, Public Health Service, Bureau of Disease Prevention and Environmental Control, National Communicable Disease Center, Atlanta, Georgia.

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.

Truman, J. W. and G. B. Craig, Jr. 1968. Hybridization between *Aedes hendersoni* and *Aedes triseriatus*. Ann. Entomol. Soc. Amer. 61:1020–25.

Watts, D. M., P. R. Grimstad, G. R. DeFoliart and T. M. Yuill. 1975. *Aedes hendersoni*: Failure of laboratory-infected mosquitoes to transmit LaCrosse virus (California encephalitis group) J. Med. Entomol. 12:451–53.

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

Zavortink T. J. 1972. Mosquito studies (Diptera:Culicidae) XXVIII. The new world species formerly placed in *Aedes (Finlaya)*. Contrib. Amer. Entomol. Inst. 8:1–206.