

Encephalitis in Midwest V. Western Equine Encephalomyelitis Virus Recovered from Mites *Dermanyssus americanus* Ewing. (18789)

VIRGIL I. MILES,* BEATRICE F. HOWITT,† RACHAEL GORRIE,† AND T. AIDAN COCKBURN.*

From the Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Ga.

During routine testing for natural infection with neurotropic viruses, the virus of Western equine encephalomyelitis (WEE) was recovered from a collection of approximately 500 mites obtained from a nest of an English sparrow, *Passer domesticus*, in Weld County, Colorado, July 18, 1950, and identified as *Dermanyssus americanus* Ewing.

The mites were collected through a Berlese funnel, sealed in glass tubing, and while still alive, were frozen in dry ice and sent frozen to the Virus and Rickettsial Laboratory at Montgomery, Ala. for test. In the laboratory the mites were triturated in a sterile mortar with buffered saline containing 30% by volume of inactivated normal rabbit serum, diluted to a volume of 1.5 ml and the suspension centrifuged in a cold room at 13,000 rpm in an angle centrifuge. The supernatant fluid was then inoculated both intracerebrally (0.02 ml) and intraperitoneally (0.04) into 12- to 14-day-old white mice. The brains of mice which died or showed symptoms of encephalitis were removed, tested for bacteria, and suspensions of them inoculated intracerebrally into fresh mice.

An agent was thus isolated which had the following characteristics: it passed through a Seitz filter pad, was free from bacteria, was lethal to mice in a dilution of $10^{-6.5}$ to $10^{-8.5}$ and was neutralized by an antiserum of WEE virus, but not by that of the Eastern equine encephalomyelitis or the St. Louis encephalitis viruses. The identification of the virus as that of the Western equine type was completed by the challenge inoculation of guinea pigs previously immunized against this virus.

The sparrow nest was taken from an open cattle shed on a farm where a human case with

symptoms of encephalitis had recently occurred. The patient was a 14-year-old boy who became ill on July 7, 1950. Antibodies neutralizing the Western equine encephalomyelitis virus were demonstrated in the serum withdrawn from the patient July 11th, the neutralization index being 50. A later serum sample drawn August 24th had an index of only 36, however, and did not indicate infection with Western equine encephalomyelitis virus since a rising titre of antibodies was not demonstrated.

Heart blood from a 9-day-old nestling occupant of the nest from which the virus-positive mites were taken was tested for the presence of virus, but none was isolated. Furthermore, no virus was recovered from the combined heart bloods of five 12- to 14-day-old nestlings from an additional English sparrow nest in the same building, nor from a collection of about 125 *D. americanus* infesting the nest.

The virus recovery here reported was the only isolation of a neurotropic virus in tests of

TABLE I. Mite Lots from Birds' Nests, Weld County, Colo. Tested for Presence of Neurotropic Viruses, May-August, 1950.

From nest of:	<i>Dermanyssus americanus</i>	<i>D. gallinae</i>	<i>Liponyssus sylviarum</i>	<i>D. americanus</i> and <i>L. sylviarum</i>	<i>D. americanus</i> and <i>D. gallinae</i>	<i>D. gallinae</i> and <i>L. sylviarum</i>	Total
English sparrow	28*	3	2	8	1		42
Barn swallow	5	10	2		1	3	21
Pigeon		9		1		1	11
Magpie	1		1				2
Northern cliff swallow		1					1
Double-crested cormorant	1						1
Total	35	23	5	9	2	4	78

* Encephalitis Investigations Unit, Office of Midwestern CDC Services, Kansas City, Kan.

† Virus and Rickettsia Section, Montgomery, Ala.

* WEE virus isolated from one lot, July 18, 1950.

a total of 78 lots of *Dermanyssus* and *Liponyssus* mites from wild birds' nests collected in Weld County, May through August, 1950. The bird nest sources of the mites and the species tested are presented in Table I.

The tests were made on approximately 500 mites sealed for each nest, except where this number was not available, as at the first part of the nesting season. Mite species determinations were based on samples of usually 10 to 30 specimens preserved in alcohol from each nest. The methods of collecting and of testing as described above in the recovery of WEE virus were used in all tests.

Identification of the mites from the virus-infected lot was made on 34 specimens, all *D. americanus*, 6 males, 26 females and 2 nymphs. This is the first definite recovery of a neurotropic virus from *D. americanus*, although Reeves and his associates(1) obtained evi-

dence of a possible infection of this mite with WEE virus in Kern County, California, where, in recoveries of the virus from *Liponyssus sylviarum*, they found *D. americanus* included with *L. sylviarum* in one pool of mites found infected with WEE virus. Natural infections with this virus have been reported from the chicken mite *Dermanyssus gallinae* by Sulkin(2) and from the tropical fowl mite *Liponyssus bursa* by Sulkin and Izumi(3).

Summary. Virus of Western equine encephalomyelitis was isolated from mites (*Dermanyssus americanus*) collected in Weld County, Colo. in July 1950.

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2. Sulkin, S. E., *Science*, 1945, v101, 381.

3. Sulkin, S. E., and Izumi, E. M., *Proc. Soc. Exp. Biol. and Med.*, 1947, v66, 249.

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1. Reeves, W. C., Hammon, W. McD., Furman, D. P., McClure, H. E., and Brookman, B., *Science*, 1947, v105, 411.

Recovery of Virus of Eastern Equine Encephalomyelitis from a Mosquito, *Culiseta melanura* (Coquillett)* (18790)

ROY W. CHAMBERLAIN, HARRY RUBIN, R. E. KISSLING, AND M. E. EIDSON.
(Introduced by Morris Schaeffer.)

From the Communicable Disease Center, Public Health Service, Federal Security Agency,
Atlanta, Ga.

Although the virus of eastern equine encephalomyelitis has been transmitted by several species of *Aedes* mosquitoes in the laboratory(1,2) and is generally considered to be arthropod-borne, isolations from arthropods in nature have been few. Howitt *et al.*(3) made the first isolation of EEE virus from wild-caught arthropods when they recovered

it from common chicken mites (*Dermanyssus gallinae* (De Geer)) and chicken lice (*Eomenacanthus stramineus* (Nitzsch)), collected in Tennessee in 1947. The only other isolation of EEE virus from arthropods in nature has been from a mosquito, *Mansonia perturbans* Walker in Georgia, also reported by Howitt *et al.*(4).

Western equine encephalomyelitis, on the other hand, has been recovered from wild-caught *Culex tarsalis* Coquillett(5), *Culiseta inornata* (Williston), *Culex pipiens* Linnaeus and *Anopheles maculipennis freeborni* Aitken

* This note constitutes part of an investigation on encephalitis in Louisiana conducted by the Virus and Rickettsia Section, Montgomery, Ala.

1. Merrill, M. H., Lacaillade, D. W., Jr., and TenBroeck, C., *Science*, 1934, v80, 251.

2. Davis, W. A., *Am. J. Hyg.*, 1940, v32, 45.

3. Howitt, B. F., Dodge, H. R., Bishop, L. K., and Gorrie, R. H., *Proc. Soc. Exp. Biol. and Med.*, 1948, v68, 622.

4. Howitt, B. F., Dodge, H. R., Bishop, L. K., and Gorrie, R. H., *Science*, 1949, v110, 141.

5. Hammon, W. McD., Reeves, W. C., Brookman, B., and Izumi, E. M., *J. Infect. Dis.*, 1942, v70, 263.