ISOLATION OF EASTERN ENCEPHALITIS VIRUS FROM THE MOSQUITO *Culex restuans* COLLECTED IN NEW JERSEY DURING 1959

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Eastern encephalitis (EE) virus previously has been isolated from several species of naturally infected mosquitoes: *Mansonia perturbans* (1), *Culiseta melanura* (2–5), *Anopheles crucians* (6, 7), *Aedes mitchellae* (7), *Culex salinarius* (5), *Culex taeniopus* (8), and *Culex nigropalpus* (8). The virus isolation from *Culex salinarius* was presumed to be due to recently ingested blood containing virus rather than from the mosquito tissue (5). Laboratory investigations have shown additional species to have potential as vectors of this virus (9). The isolation of EE virus from the mosquito *Culex restuans* Theobald collected in New Jersey during the encephalitis outbreak which occurred in the autumn of 1959 is herewith reported.

A pool consisting of 6 *C. restuans* collected in a New Jersey-type mosquito light trap at Woodbine, New Jersey was made by combining specimens obtained on the nights of October 2, 3, and 4, 1959. Following each night of collecting, the mosquitoes were hermetically sealed in glass vials and frozen on dry ice. The specimens, along with other mosquito pools, were shipped in the frozen state to the laboratory for testing. There was no evidence that any of the 6 *C. restuans* had recently taken a blood meal.

In the laboratory, the mosquitoes were ground in 3 ml. of a Hanks balanced salt solution containing 10 percent normal calf serum, 1000 units of penicillin per ml. and 500 micrograms of streptomycin per ml. The large particulate matter was allowed to settle out while the emulsion was held in an ice bath for about 1 hour. The supernatant fluid was inoculated intracerebrally into 5 mice, which were observed daily for signs of illness or death. No work with EE virus was in progress in the laboratory during the time when efforts were being made to isolate virus from these pools, nor was another isolation of EE accomplished from any other pool ground on the same day.

In the primary isolation attempt, 1 out of 5 mice died on the third day, and the other mice remained well. The brain of the dead mouse was harvested under aseptic conditions, ground in sterile Hanks BSS with antibiotics, and the supernatant fluid inoculated intracerebrally into 5 mice, and later into 6 additional mice. All 11 mice died on the second or third day. Reisolation was achieved from the original mosquito emulsion, and 2 of 6 mice died on the third and eighth days. An emulsion of the brains of these mice was passed intracerebrally into 6 mice, resulting in the death of all 6 on the second day. The virus concentration in a brain suspension was $10^{8.3}$ mouse IC LD_{50}. In a neutralization test in mice this virus was neutralized by EE hyperimmune rabbit serum, but not by WE hyperimmune or normal rabbit serum.

The epidemiologic implications of this virus isolation from *C. restuans* are considerable because of several facts. The residence of a confirmed human (2 years of age) case of EE was within 100 yards of the place of collection. *C. restuans* has been found to have good vector potential in laboratory studies (9). The geographic distribution (10) of the mosquito species coincides with that presently known for EE in the United States. The females of this species probably feed by preference on avian hosts, and they will also attack man (11). *C. restuans* could readily obtain virus infections from wild birds, considered to be the natural reservoirs of EE virus (12), and subsequently transmit them to other birds or mammals.

References and Notes

1. HOWITT, B. F., DODGE, H. R., BISHOP, L. K. and CORRIE, R. H. Science, 110:141 (1949).

2. CHAMBERLAIN, R. W., RUBIN, H., KISSLING, R. E. and EDISON, M. E. Proc. Soc. Exp. Biol. and Med. 77:396 (1951).

3. HOLDEN, P., MILLER, B. J. and JOBBINS, D. M. Proc. Soc. Exp. Biol. and Med. 87:457 (1954).

4. FEEMSTER, R. F., WHEELER, R. E., DANIELS, J. B., ROSE, H. D., KISSLING, R. E., HAYES, R. O., ALEXANDER, E. R. and MURRAY, W. A. New England J. Med. 259:107 (1958).

5. CHAMBERLAIN, R. W., SUDIA, W. D., BURBUTIS, P. P. and BOGUE, M. D. Mosquito News, 18:305 (1958).

6. KISSLING, R. E., CHAMBERLAIN, R. W., NELSON, D. B. and STAMM, D. D. Am. J. Hyg. 62: 233 (1955).

7. KARSTAD, L. H., FLETCHER, O. K., SPALATIN J., ROBERTS, R. and HANSON, R. P. Science 125 395 (1957).

8. DOWNS, W. G., AITKEN, T. H. G. and SPENCE, L. Science 130:1471 (1959).

9. CHAMBERLAIN, R. W., SIKES, R. K., NELSON D. B. and SUDIA, W. D. Am. J. Hyg. 60:278 (1954).

10. CARPENTER, S. J. and LACASSE, W. J. Mosquitoes of North America. Univ. of California Press, Berkeley, 1955.

11. BARR, A. R. Univ. Minn. Tech. Bull. 228 133 (1958).

12. BEADLE, L. D. Public Health Rpts. 74:8 (1959).

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