13865 P

Culex tarsalis Coq. a Proven Vector of St. Louis Encephalitis.*

W. McD. HAMMON AND W. C. REEVES. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, and the Texas State Health Department, Bureau of Laboratories, Austin, Texas.

During the summer season of 1941 in the Yakima Valley, Washington, *Culex tarsalis* Coq. was thrice found infected with the virus of St. Louis encephalitis.[1] In addition the seasonal activities and feeding habits of this species were found to fit the epidemiological picture of the infection in that area.[2] Previously *Culex* mosquitoes had been suspected in the 1933 St. Louis outbreak.[3] In support of this latter observation *Culex pipiens* Linn. was found capable of transmitting the virus by Mitamura and associates,[4] and recently their results were confirmed by Reeves, Hammon and Izumi.[5]

The experimental data presented in this paper complete the evidence incriminating *C. tarsalis* as a vector of St. Louis virus, for it may now be reported that this species which was found to serve as a host in nature is also a good experimental vector.

Experiments. Two experiments were carried out in a temporary field laboratory established in San Benito, Texas. All mosquitoes used were raised from larvæ. The virus employed was strain F-103, only 3 mouse brain passages removed from that isolated by mouse inoculation from *C. tarsalis* taken in the field.[1] The chickens used as experimental hosts were raised from the incubator in a room entirely free from mosquitoes and flies.

On April 26th, 1942 two *C. tarsalis* were fed on cotton soaked with a blood-virus mixture. After 8 days incubation at laboratory temperatures varying from 22° to 38°C these same 2 mosquitoes fed on a normal 2 months-old chicken. The serum of this chicken 48 hr later contained St. Louis virus as demonstrated by intracerebral inoculation of mice. A neutralization test against the St. Louis virus with serum from the same chicken taken 20 days later indicated that antibodies had formed. The mosquitoes refused to feed again so were killed after a total of 15 days incubation. Virus was then isolated from a suspension of the two, by mouse inoculation.

On May 5th, 1942, fifty-two *C. tarsalis* were similarly given a blood-virus meal. Attempts were made to isolate virus from the serum of a series of 2-months-old chickens on which the mosquitoes had fed. Each time the mosquitoes were permitted to feed a different chicken was employed. Eight mosquitoes fed after 4 days' incubation, 15 after 6 days, 5 after 8 days, and 6 after 10 days. Virus was isolated 24 and 48 hr after the mosquitoes had fed from the serum of those chickens used on the 4th, 6th and 10th days, but not on the 8th day. Neutralization tests performed on sera taken at least 20 days later indicated that all chickens had been infected. Only 18 mosquitoes were alive on the 10th day. These were killed

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

1 Hammon, W. McD., Reeves, W. C., Brookman, B., Izumi, E. M., and Gjullin, C. M., *Science*, 1941, **94**, 328; Hammon, W. McD., Reeves, W. C., Brookman, B., and Izumi, E. M., *J. Infect. Dis.*, 1942, **70**, 263.

2 Hammon, W. McD., Reeves, W. C., Brookman, B., and Gjullin, C. M., *J. Infect. Dis.*, 1942, **70**, 278; Hammon, W. McD., Gray, J. A., Evans, F. C., Izumi, E. M., and Lundy, H. W., *Science*, 1941, **94**, 305; Hammon, W. McD., Lundy, H. W., Gray, J. A., Evans, F. C., Bang, F., and Izumi, E. M., *J. Immunol.*, 1942, **43**, 75; Bang, F., and Reeves, W. C., *J. Infect. Dis.*, 1942, **70**, 273.

3 Lumsden, L. L., unpublished official report, 1933.

4 Mitamura, T., Yamada, S., Hazato, H., Mori, K., Hosoi, T., Kitaoka, M., Watanabe, S., Okubo, K., and Tenjin, S., *Tr. Jap. Path. Soc.*, 1937, **27**, 573.

5 Reeves, W. C., Hammon, W. McD., and Izumi, E. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 125.

and from them virus was isolated.

Since the number of available *C. tarsalis* was very limited no attempt was made in these preliminary experiments to infect them by feeding on an experimental animal, for in the laboratory they feed much more satisfactorily on blood-soaked cotton. However, we have infected other mosquitoes by permitting them to feed on a chicken 48 hr after a subcutaneous inoculation of the St. Louis virus. Finding *C. tarsalis* infected in nature is evidence that it becomes infected by some natural means, probably by feeding on an infected animal host.

In addition to *C. tarsalis, Culex coronator* Beyer was shown to be capable of transmitting the St. Louis virus to chickens. This latter species is potentially important in the lower Rio Grande Valley, where the work was done. In this area during an epidemic of encephalitis in 1941 antibodies to the St. Louis virus were found in the sera of man and domestic animals.[6]

Summary and Conclusions. *Culex tarsalis* has been demonstrated to be infected in nature with St. Louis encephalitis virus and to be capable of transmitting it. Furthermore, it fits well into the epidemiological picture encountered in the Yakima Valley, Washington. We can therefore safely conclude that this species is a natural vector. This is the first instance in which a mosquito has fulfilled these 3 criteria for incrimination as a vector in respect to St. Louis encephalitis virus. *C. pipiens* previously has been shown to be capable of transmitting this virus in the laboratory and the same has just been demonstrated for *C. coronator*. In addition, these experiments demonstrate that the chicken may serve as a satisfactory reservoir of virus. This is undoubtedly shared by other birds for we have previously shown that virus may be isolated from the blood of a dove following the bite of infected *C. pipiens*[5] and unpublished work indicates that ducks may serve as reservoirs.

Collection of the larvæ for the second experiment was made by Mr. R. B. Eads, entomologist, Texas State Health Department. Valuable laboratory assistance was rendered by Mrs. Margaret Gray and Miss Margaret Smith, Hooper Foundation.

6 Hammon, W. McD., *J. A. M. A.*, in press. (Paper read at the Convention of the American Medical Association, June, 1942.)

13866

Influence of Diet on Action of the Sulfonamide Drugs.*

ESTHER M. GREISHEIMER, ROBERTA HAFKESBRING AND GRACE E. WERTENBERGER.
From the Department of Physiology, Woman's Medical College of Pennsylvania, Philadelphia.

The importance of diet in the normal physiology of the liver and in the protection of the liver from the action of such damaging substances as chloroform and carbon tetrachloride has been brought to the attention by many investigators.[1-7]

Since we found that sodium sulfapyridine exerts a definite and characteristic effect on the levels of blood sugar and liver glycogen,[8] we wished to study the effects of various diets on the action of sodium sulfapyridine and sodium sulfathiazole in rats. The diets used to date are Purina Dog Chow Checkers

* Supported by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

1 Goldschmidt, S., Vars, H. M., and Ravdin, I. S., *J. Clin. Invest.*, 1939, **18**, 277.

2 Ravdin, I. S., *Am. J. Surg.*, 1938, **40**, 171.

3 Ravdin, I. S., Rhoads, J. E., Frazier, W. D., and Ulin, A. W., *Surgery*, 1938, **3**, 805.

4 Greene, C. H., and Farrell, E., *Arch. Int. Med.*, 1940, **65**, 847.

5 Greene, C. H., and Hotz, R., *Arch. Int. Med.*, 1939, **63**, 778.

6 Channon, H. J., *Ann. Rev. Biochem.*, 1940, **9**, 231.

7 Connor, C. L., *J. A. M. A.*, 1939, **112**, 387.

8 Greisheimer, E. M., Hafkesbring, R., and Magalhaes, H., *Med. Times*, 1941, **69**, 170.