A NATURAL HIBERNATION SITE OF THE MOSQUITO *CULEX TARSALIS* COQUILLETT IN THE COLUMBIA RIVER BASIN, WASHINGTON

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Introduction. Encephalitis viruses in temperate zones are admirably adapted to mosquitoes of the genus *Culex* whose species, insofar as known, overwinter in the adult stage. At this writing, neither a plausible or satisfactory mechanism for maintenance of western equine encephalitis (WEE) virus through severe winters in northern climates, nor for its reintroduction each spring, has been demonstrated. Until positively ruled out, attention must be focused on the vector mosquito, *Culex tarsalis,* as a potential reservoir.

The species thrives in cold climates where it survives the winter as fertile females. Ample experimental evidence has shown that *C. tarsalis,* once infected with WEE virus, remains infected for life. Thus, if sufficient numbers of infected, hence blood-fed, females can hibernate successfully, at least one mechanism for virus maintenance will have been revealed. As briefly reviewed below, considerable effort has been expended in searching for hibernation quarters of the species and in studying overwintering populations, but not until this report has a natural site been discovered.

Review. Hammon *et al.* (1945) in the Yakima Valley, Washington, collected 18 *C. tarsalis* females among thousands of *C. pipiens* and other species in the winter. Collecting conditions are not given. Virus was not found.

Keener (1952) in western Nebraska recorded something less than 100 female *C. tarsalis* associated with *C. territans* in two outside food storage cellars from 13 January to 28 April 1951 when the last *C. tarsalis* was noted. The largest number seen in either cellar at any biweekly visit was 15 on 20 January and the lowest temperature in the cellars was 33° F. Only 18 mosquitoes were tested and no virus was found.

Blackmore and Winn (1956) in Colorado, collected and tested 1,361 *C. tarsalis* females from abandoned mines, 15 December 1953 to 9 February 1954. The temperature in one mine was about 52° F., and the relative humidity fluctuated from 30 to 80 percent. They report one isolation of WEE virus from a pool of 50 of 587 mosquitoes from three mines on 30 December. Although intensive studies appear to have ceased in February, a few mosquitoes were observed in mines after the date (not stated) in spring when *C. tarsalis* was on wing.

Dow *et al.* (1956) in northern Utah, studied small numbers of *C. tarsalis* females, along with *C. pipiens* and *Anopheles freeborni,* during the winter months of 1954–55 in two abandoned mines where Fahrenheit temperatures were in the 40's and 50's. They suggest that *C. tarsalis* in these mines did not represent a successful overwintering population, presumably because of activity induced by moderate temperatures and because the numbers diminished to zero, presumably by death, toward the end of the winter. They suggest further that the species should survive best in natural shelters where it would be inactivated by cold and conclude "If *Culex tarsalis* overwinters successfully in mine tunnels, it survives in spite of excessively high mortality. It seems more likely that the normal winter habitat is still unknown."

Reeves *et al.* (1958) in Kern County, California, following a 5-year study, report isolation of WEE virus from *C. tarsalis* in all months except December. Mosquitoes were collected from a variety of natural and artificial shelters. Sources of winter collections are not specified. Although they do not refer to the overwintering population as being in a state of "hibernation," they do note that winter temperatures and possibly other climatic factors are sufficiently rigorous to produce marked changes from normal summer behavior of the species. Male die-off in November and December is concurrent with diminished blood feeding by females which become more difficult to find through the winter. On the coldest days, at or near freezing, females in exposed places are quiescent and incapable of flight. The authors do not imply that virus found in overwintering *C. tarsalis* was due to infection acquired in late summer or fall, but on the contrary offer evidence that virus was probably acquired from infective blood meals at the onset of feeding in late January and February.

FIELD OBSERVATIONS. An excessive population of *C. tarsalis* in Grant County, Washington, during the summer of 1956 stimulated fall and winter search for hibernating individuals. Since three of the above reports (Keener; Blackmore and Winn; Dow *et al.*) record finding the species in winter only in artificial shelters, and since *C. tarsalis* presumably was here long before such winter quarters were available, an effort to discover natural hibernation sites seemed highly desirable as well as essential to a better understanding of the biology of the species.

Our activities were confined to an area of some 100 square miles in the vicinity of Moses Lake at an elevation of 1000 to 1500 feet. The land, half of which is cultivated, is semi-arid and slightly rolling. It is further characterized by abundant volcanic outcrops representing a portion of the vast lava fields of the Northwest, and by two lakes, several ponds, and two creeks. Irrigation ditches traverse the area.

The *C. tarsalis* population was under surveillance from July 1956 to April 1957. From late July until mid-September about 12,000 *C. tarsalis* females were collected and tested for virus. The WEE virus incidence was low, somewhat less than 0.3 percent.

When seasonal mosquito activity ceased, search for hibernators was begun. The *C. tarsalis* population disappeared in late September even though conditions were suitable for survival outside. During the last week of the month, temperatures ranged from 48° to 83° F. and precipitation was absent.

Searching was regular and systematic from 29 September to 7 October. Temperatures fluctuated from 36° to 78° F. and no precipitation occurred. Although emphasis was placed on natural cover, a wide variety of likely hibernation sites was investigated. Shelters inspected were animal burrows, rock crevices, standing vegetation, piles of sloughed rock at bases of outcrops, man-made rock piles, haystacks, lumber piles, chicken houses, basements, storage cellars, cisterns, outbuildings, subfloor space of houses, etc.

Mosquitoes were found only in natural and artificial rock piles, and in subfloor spaces. Volcanic outcrops with piles of loose rock at their bases (Fig. 1) varied from less than a hundred to thousands of square feet in area, and from less than 5 to more than 20 feet in height. Man-made rock piles (Fig. 2) formed by clearing fields for cultivation, ranged from 2 to 5 feet high, and the largest studied covered about 4,000 square feet. Although we were more interested in establishing presence of mosquitoes than numbers, it was immediately obvious that about 4 times as many *C. tarsalis* females were in rock piles than under floors of houses. As a rule, as soon as one mosquito was discovered another site was investigated.

During this early fall search-period, *both* sexes of *C. tarsalis*, *Culiseta inornata*, and *Anopheles freeborni* were found in rock piles at depths from 1 to 3 feet. The abdomens of all *C. tarsalis* females were full of fat bodies. In this same period, advanced-stage larvae of *C. tarsalis*, *C. ter-*

FIG. 1.—A natural hibernation site of *Culex tarsalis:* Volcanic outcrop with loose rock at base.
FIG. 2.—An artificial hibernation site of *C. tarsalis:* Man-made rock pile.

ritans, C. pipiens, and *C. inornata* were observed in various breeding places.

Observations were discontinued until 7 December when both natural and artificial rock piles were again examined. Temperatures on this day ranged from 2° to 17° F. About 3½ inches of snow had fallen 2 days before. In an artificial rock pile one female *C. tarsalis* was found at a depth of 4½ feet, and in a natural rock pile a female was discovered just beneath a surface rock. Both contained fat.

Winter inspections were made from 1 January to 5 January. Temperatures ranged from 25° to 37° F., with no precipitation. Rock piles, a basement, and storage cellars were searched. Seven of 11 artificial rock piles and each of 2 natural rock piles, yielded healthy, though quiescent, *C. tarsalis* females. These were taken at depths from 1½ to 3 feet. In one instance a mosquito was resting on the under surface of a small rock whose upper surface was covered with frost. No mosquitoes were found in a basement and 4 storage cellars.

The spring investigation for emerging hibernators was begun on 17 March. Temperatures ranged from 40° to 48° F., with a little rain. *C. tarsalis* females were found in an artificial rock pile, and a single female was taken in flight in a chicken coop. The following day (temperatures 34° to 56° F.) *C. tarsalis* females were taken from natural rock piles, but none were found in 4 chicken coops and several other outbuildings.

On 19 March an intensive search of rock piles was organized and daily collections were made through 1 April. Air temperatures ranged from 29° to 63° F., with a little rain in the last few days. During this period temperatures in a man-made rock pile, 2½ feet below the surface, ranged from 36° to 47° F. and the relative humidity fluctuated from 37 to 100 percent. A total of 555 *C. tarsalis* females were taken by aspirator. Collecting was slow and tedious and yielded only 1 to 3 mosquitoes per man hour. The largest number taken on any one day was about 100. All were examined for undigested blood and developing ova.

Early in the period mosquitoes were torpid and easily collected, but toward the end they became so active that less than half of those discovered could be captured. Until 28 March, the population in rock piles seemed to be relatively constant, as estimated by the numbers found in proportion to man-hours and effort expended. After this date, the population decreased rapidly until 1 April when only 2 mosquitoes were found in a half day.

The first blood-fed individual was found among 100 mosquitoes collected on 27 March, approximately when the rock-pile population began its rapid decline. On 29 March, 1 of 50 had blood, and 7 of 25 collected 30 and 31 March contained either blood or eggs. Also, on these last two days, 15 *C. tarsalis* collected in chicken coops had blood.

C. tarsalis and *A. freeborni* were about equally abundant in rock piles. *C. inornata,* usually present, and obviously an earlier emerger from hibernation, was not seen during the spring inspection; a lone specimen of *C. incidens* was noted in late March. In the spring, *A. freeborni* became active at the same time as *C. tarsalis.* No males of any species were found after the initial fall observation.

With almost no exception, all overwintering *C. tarsalis* females had fat bodies, no undigested blood or developing ova, and externally, had the appearance of adults recently emerged from pupation, i.e., they were unfaded, fully scaled, and had intact wings and other appendages.

During the spring study period, frequent inspections of breeding sources were made. No pre-adult stage of *C. tarsalis* was found, but early stage larvae of *Culiseta inornata, Aedes increpitus,* and *A. dorsalis* were commonly collected.

Field activities were discontinued on 2 April because of the disappearance of the hibernating population and the appearance of blood-engorged and gravid individuals.

On January 11, 3½ inches of snow fell, and from this date below-freezing temperatures persisted until 5 February. An additional 5 inches of snow fell on 19 January and another 1½ inches at the end of the month. The most severe winter weather occurred the last half of January when temperatures ranged from —20° to 21° F. for 16 days. Facilities were not always available to record internal rock-pile temperatures throughout the course of our observations, and furthermore, disturbing hibernation sites more than actually necessary seemed undesirable. However, there is little doubt that at least part of the rock-pile population was occasionally exposed to below-freezing, if not below zero, temperatures.

All of the nearly 600 *C. tarsalis* collected from 29 September to 1 April were tested for WEE virus. Blood-engorged and gravid females were tested separately. The results were negative. The first 18 days of September, just before the cessation of summer activity, we collected 2145 mosquitoes of which 7 pools of 50 each had virus. Based on these figures as well as on the incidence of virus in *C. tarsalis* throughout the entire summer, at least 2 infected mosquitoes should have been in the overwintering sample tested.

DISCUSSION. A brief dictionary definition of the relative term "hibernate" is, "to pass the winter in close quarters, in a torpid or lethargic state." The mosquitoes of the rock-pile population fulfilled these requirements, and the factors indicative of true hibernation proposed by Blackmore and Winn (1956), i.e., accumulation of fat bodies, lack of feeding, absence of males, quiescence, and temperatures prohibitive for survival outside, applied equally well.

Disappearance of the summer population when temperatures ranged from middle 40's to high 80's and emergence of the hibernating population when temperatures ranged from high 20's to low 60's suggest two physiologically different populations and that air temperature is not the fundamental motivating factor for these phenomena. Bennington, Blackmore & Sooter (1958) in Colorado have experimental evidence that air temperatures alone do not stimulate emergence of hibernating *C. tarsalis* and they have reached some interesting conclusions as to the principal factor involved. Causes of the usual abrupt disappearance of the summer population and factors stimulating adults to enter hibernation require additional study.

Although inconclusive, our findings (fresh and unworn condition of hibernators; absence of undigested blood and developing ova; presence of larvae in fall; failure to isolate virus from hibernators, probably not critical because of size of sample and low virus potential) support rapidly accumulating evidence that blood-engorged *C. tarsalis* do not hibernate successfully, hence are not a mechanism for maintenance of virus through the winter.

Parallel field and experimental observations by us in western Idaho and central North Dakota, showed that fat formation in *C. tarsalis* in late summer and fall accompanies reluctance, if not refusal, to take blood. In one experiment, only 1 of 610 fall-reared females took a minute quantity of blood after several days starvation. Studies by Bellamy *et al.* (1958) suggest that blood-feeding in autumn is prejudicial to survival through the winter, and Bennington *et al.* (1958) state, "Segments of this population in cages revealed that this species [*C. tarsalis*] developed fat bodies without blood. Other observations indicate that in this species fall broods emerge, mate, take carbohydrate and the females enter a diapause to develop fat bodies. These prepared females hibernate and emerge the next spring to take their first blood meal for ovarian development."

Our discovery of a natural hibernation site of *C. tarsalis* in the Columbia Basin unfortunately does not provide a lead to hibernation sites in the vast areas devoid of outcrops and rock piles throughout the range of this mosquito. Where it spends the winter in the plains of North Dakota and the irrigated valleys of western Montana and Idaho (regions under surveillance for some years) is still a mystery.

SUMMARY. First observations on a population of *C. tarsalis* in a state of true hibernation in a natural site (loose rock at bases of volcanic outcrops) in the Columbia Basin are reported.

Circumstantial evidence is presented that *C. tarsalis* is not *the* mechanism for maintenance of encephalitis viruses through the severe winters of northern climates.

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References

BELLAMY, R. E., REEVES, W. C. and SCRIVANI, R. P. 1958. Relationships of mosquito vectors to winter survival of encephalitis viruses. II. Under experimental conditions. Amer. Jour. Hyg. 67:90–100.

BENNINGTON, E. E., BLACKMORE, J. S., and SOOTER, C. A. 1958. Soil temperature and the emergence of *Culex tarsalis* from hibernation. Mosquito News 18(4):297–298.

BENNINGTON, E. E., SOOTER, C. A., and BAER, HAROLD. 1958. The diapause in adult female *Culex tarsalis* Coquillett (Diptera, Culicidae). Mosquito News 18(4):299–304.

BLACKMORE, J. S. and WINN, J. F. 1956. A winter isolation of western equine encephalitis virus from hibernating *Culex tarsalis* Coquillett. Proc. Soc. Exper. Biol. and Med. 91:146–148.

DOW, RICHARD P., MAIL, G. ALLEN, and RICHARDS, C. S. 1956. Observations on the overwintering of *Culex tarsalis* in northern Utah. Proceedings of the Ninth Annual Meeting of the Utah Mosqiuto Abatement Association, pages 12–15.

HAMMON, W. MCD., REEVES, W. C., BENNER, S. R., and BROOKMAN, B. 1945. Human encephalitis in the Yakima Valley, Washington, 1942. Jour. Amer. Med. Assoc. 128:1133–1139.

KEENER, GEORGE G. (JR.). 1952. Observations on overwintering of *Culex tarsalis* Coquillett (Diptera, Culicidae) in western Nebraska. Mosquito News 12:205–209.

REEVES, W. C., BELLAMY, R. E. and SCRIVANI, R. P. 1958. Relationships of mosquito vectors to winter survival of encephalitis viruses. I. Under natural conditions. Amer. Jour. Hyg. 67:78–89.

MOSQUITOES IN SEWAGE STABILIZATION PONDS IN THE DAKOTAS

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During recent years, the use of stabilization ponds as a method of sewage treatment has grown rapidly in popularity throughout the country. This growing popularity has been especially pronounced in the Dakotas and other states in the Missouri River Basin. Although the use of stabilization ponds is an accepted method of treating sewage in the Northern Plains area, some concern has been expressed relative to the possible public health hazard of these ponds from the standpoint of mosquito potential.

To evaluate the mosquito potential of sewage stabilization ponds, a special field survey was carried out in the Dakotas during the period July 30–August 8, 1956. Participating in this survey were representatives from the Robert A. Taft Engineering Center and the Communicable Disease Center of the U. S. Public Health Service. In addition, one of the district engineers of the South Dakota Department of Health assisted in the inspection of three installations in the southwestern portion of the state.