FIELD AND LABORATORY OBSERVATIONS OF TWO VIRAL DISEASES IN *AEDES SOLLICITANS* (WALKER) IN SOUTHWESTERN LOUISIANA

T. B. CLARK AND T. FUKUDA [1, 2]

Entomology Research Division, Agr. Res. Serv., U.S. Department of Agriculture
Lake Charles, Louisiana 70601

ABSTRACT. A viral epizootic involving both a cytoplasmic polyhedrosis and a nuclear polyhedrosis occurred in populations of *Aedes sollicitans* near Hackberry, Cameron Parish, Louisiana. The frequency of viral infections in the affected populations was followed through three periods of flooding and compared with that of a control population.

Laboratory studies of larva-to-larva transmission, transovum transmission, and cross transmission, were made to aid our understanding of the events that occurred in the field.

Our findings suggest that a series of overlapping broods of *Aedes sollicitans* may lead to a buildup of infective viral material in the habitat, but a period of drying between broods appears to reduce it very significantly. Oil pollution in the area may act as a stressing agent. Transovum transmission and lateral transmission could explain the levels of infection found after the dry period. The introduction of infective material into an area previously almost free of the disease resulted in a significant rise in the rate of infection.

Of the many mosquito species inhabiting southwestern Louisiana, probably none is more important nor presents greater problems of control than *Aedes sollicitans* (Walker). Thus, biological control with pathogenic microorganisms, which may be a solution, has occupied the attention of the Gulf Coast Marsh and Rice Field Mosquito Research Laboratory, Lake Charles, Louisiana for several years. In the search for pathogens that we might test for potential control, we have found a number of parasites, but the rate of infection was usually less than 1 percent and often less than 0.1 percent. However, in May 1970, our attention was called to an unusually large number of intestinal viral infections in larvae collected from a salt marsh pasture near the community of Hackberry, Cameron Parish, Louisiana. This pasture had been the site of frequent collections over several years and had never, to our knowledge, produced the two viruses found in 1970. Subsequently, the nuclear polyhedrosis virus (NPV) found in the *Aedes sollicitans* from Hackberry was identified as the virus described earlier, along with a cytoplasmic polyhedrosis virus from *Culex salinarius* Coquillett (Clark *et al.*, 1969). The cytoplasmic polyhedrosis virus (CPV) found in conjunction with the NPV at Hackberry, though similar to that found in *C. salinarius*, appeared to differ in its host specificity.

After the discovery of the viral infections, their frequency in the infected populations was followed through three periods of flooding and compared with that in a control area. Also, studies to help explain these findings were made in the laboratory.

MATERIALS AND METHODS. The infected larvae were found near Hackberry on the west side of Lake Calcasieu in an area that consisted of about 5 acres of salt marsh pastureland which was subjected to frequent floodings by rains and high tides. When the area was flooded, many shallow pools formed and then slowly grew smaller as a result of drainage, percolation, and evaporation. The pools from which the collections were made varied in size from 1 foot square to 10 yards square; the maximum depth was about 8 inches. An oil pumping station and oil storage tanks located about 200 yards from the study site caused a heavy film of oil to spread over much of the water.

Most infected larvae were identified by examination with a dissecting microscope;

[1] In cooperation with McNeese State University, Lake Charles, Louisiana 70601.

[2] The senior author's current address is: Department of Biology, Fresno State College, Fresno, California 93726.

however, when diagnosis was particularly difficult because of the stage of infection or because of multiple infections, the digestive tracts of the larvae were removed and examined under the higher magnifications of a phase contrast microscope.

The supply of *Aedes sollicitans* larvae, a species that has not been colonized, was assured by collecting adult females in the field with mechanical aspiration as they landed to feed. They were then transported to the laboratory in screened cages where they were blooded on guinea pigs. Plastic boxes 8 x 11 x 14 cm half filled with wet sphagnum moss were placed in the cages as oviposition sites.

When egg batches from single females were needed to determine the extent of transovum transmission of the viral diseases, blooded females were placed in 10-dram shell vials containing moist cotton (oviposition site). Squares of organdy held in place by rubber bands served as tops for the vials.

The infection of larvae with both CPV and NPV was usually accomplished by placing 48-hour-old larvae into a brei composed of 50 infected fourth instar larvae that were ground and suspended in 200 ml of water. After a 4-hour exposure to the virus, the larvae were transferred to 18 x 18 x 5 cm enameled pans, maintained on a diet of pelletized rabbit food and high-protein cattle food, and aerated.

Viral material to be preserved was freeze-dried and stored at 8° C. When it was to be used in experiments, it was placed in distilled water and finely ground.

Larvae from our laboratory colonies of *Aedes taeniorhynchus* (Wiedemann), *Aedes triseriatus* (Say), *Aedes tormentor* Dyar and Knab, *Aedes aegypti* (L.), *Aedes sierrensis* (Ludlow), *Psorophora varipes* (Coquillett), *P. ferox* (Humboldt), *Culex salinarius, Culex pipiens quinquefasciatus* Say, *Culex territans* (Walker), and *Anopheles quadrimaculatus* Say were challenged in cross-transmission tests.

RESULTS AND DISCUSSION. The unusual rate of infection in the larvae at Hackberry was first noticed in a collection made May 8, and a few pupae from that same brood were still present in the field when the present study began May 12. Table 1 shows the numbers of immature infected *Aedes sollicitans* in our collections from May 12 through June 5. The total level of viral infections did not exceed 8.6 percent

TABLE 1.—Frequency of viral infections in *Aedes sollicitans* at the Hackberry collecting site.

Date	Larval instars present	Number insects examined	Percent insects examined	
			With CPV	With NPV or with NPV and CPV
May 12	1, 2 & pupae	643	0	0
13	2 & 3	388	0.2	0.2
14	3 & 4	966	1.7	0.5
15	4 & pupae	918	2.8	5.8
16	No collection			
17	Rain; no collection			
18	2	396	1.7	3.2
19	2 & 3	1234	5.2	21.2
20	3 & 4	296	14.1	56.7
21	4 & pupae	332	15.9	48.7
22–28	Dry; no collections			
29–31	Rain; no collections			
June 1	2	508	0	0
2	2 & 3	658	0	0
3	3	328	0.6	0.3
4	3 & 4	693	2.5	0.8
5	4	320	2.8	1.2

in the May 12 to 17 brood though the study area did not dry up completely. Also, complete drying did not occur between May 12 to 17 and May 18 to 22. In the May 18 to 22 brood, the level of infection reached a maximum of 70.8 percent. Then between May 21 and 29, the soil of the study area became parched and cracked because of the intense heat and sunlight. The drying that occurred may have either destroyed or made unavailable to the following brood much if not all the residual viral material because the level of infection in the June 1 to 5 brood never exceeded 4 percent.

The Grand Lake area of Louisiana, which has been sampled regularly since the NPV of *Aedes sollicitans* was originally described from this site, is very similar to the Hackberry area and is located on the eastern shore of Lake Calcasieu. Since no epizootic of which we were aware had occurred in the Grand Lake area (though both CPV and NPV were found there in frequencies of less than 0.1 percent) we selected a pond (10 ft. x 20 ft. x 8 in.) there and seeded it with 5 grams of freeze-dried infected larvae that had been collected May 20 from the Hackberry site.

Table 2 shows the results. The highest levels of infection achieved (16.3 percent) were large compared with the normal levels of less than 0.1 percent. Also, in subsequent samplings (not included in table 2), the virus persisted in the area through at least two more broods at levels of about 5 percent; and then it dropped to less than 0.1 percent.

We feel that the percentages of larvae infected shown in Tables 1 and 2 are probably rather poor indices of the actual mortality caused by the infections. In laboratory tests, the NPV has a prepatent period of between 24 and 48 hours at 22° C. and infected larvae never survived more than 32 hours after patency. We do not know the length of patency at the field temperatures, but if these larvae survived no longer than 32 hours, then the patently infected portion of the population in the field on any day may have included only a part of the population sampled the following day. Therefore, since we have no estimates of the size of the total population at either the beginning or the end of each generation, we can only state that though some adults did emerge from each brood, the total number was greatly diminished by the disease.

An evaluation of the separate rôles of the two diseases involved was precluded by the presence of an unknown number of double infections. Laboratory tests showed that CPV was usually not fatal; however, the presence of CPV in the larvae may have predisposed them to NPV, which we found to be invariably fatal in the patent condition. The possibility of a synergistic effect was supported by the finding that even when very high doses of virus (one patently infected fourth instar larva/test larva) were used in the laboratory, the rate of infection was rarely more than 30 percent though as many as 56.7 percent of the larvae sampled each day in the field showed NPV infections.

Some double infections of CPV and NPV were easily recognized, even at low

TABLE 2.—Frequency of viral infections in *Aedes sollicitans* at the Grand Lake collecting site.

Date	Larval instars present	Number insects examined	Percent insects examined	
			With CPV	With NPV or with NPV and CPV
June 3	2 & 3	280	0.7	0.7
4	3 & 4	614	3.9	6.0
5	4 & pupae	590	3.8	12.5

magnification (40 X), but most were not. Nevertheless, it was nearly always possible to recognize infections of NPV because in this disease, the cells in the anterior portion of the midgut are infected; inclusion bodies of both CPV and NPV occur in the cells lining the gastric caeca and the posterior portion of the midgut. Electron micrographs (Fig. 1) made by D. W. Anthony of our Gainesville, Florida laboratory have confirmed the existence of double infections.

The drastic reduction in the levels of infection in the brood that hatched after the dry period seemed to indicate that much of the infectivity of the residual viral ma-

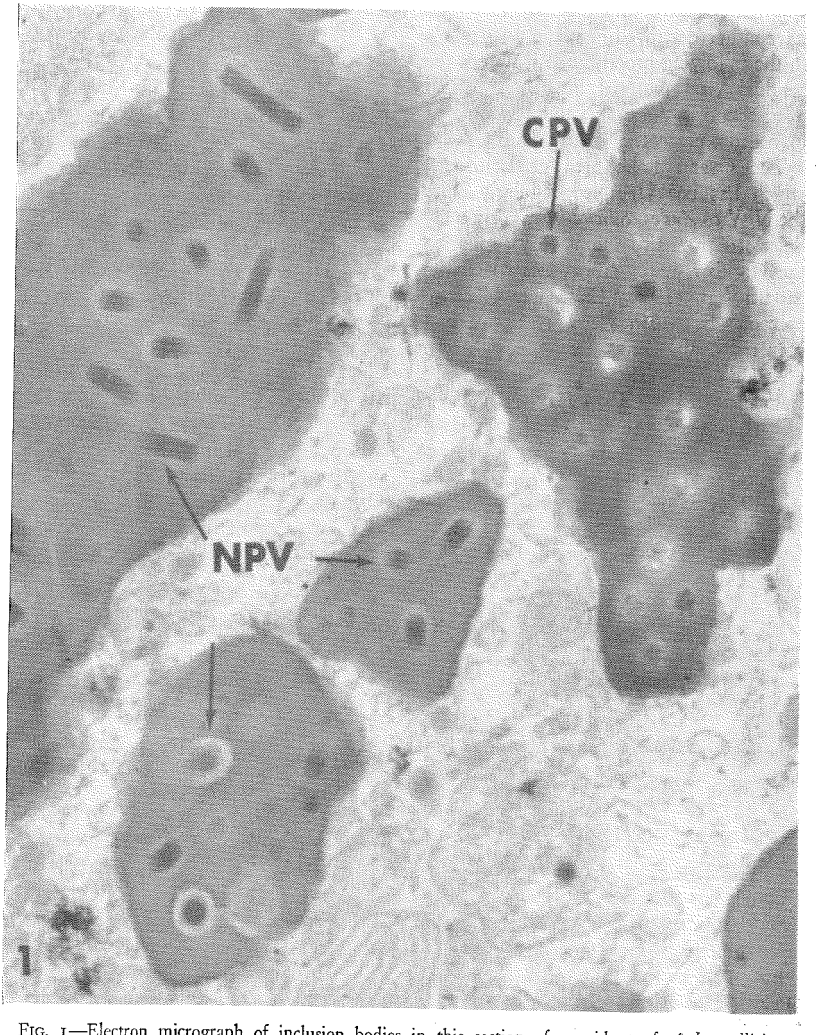

FIG. 1—Electron micrograph of inclusion bodies in this section of a midgut of *Aedes sollicitans* infected with both nuclear polyhedrosis virus (NPV) and cytoplasmic polyhedrosis virus (CPV). 77,500 X.

terial had been destroyed. Then the infections that occurred in the June 1 to 5 brood may have resulted largely from transovum infections which spread the diseases among brood mates. Therefore, during the May 22 to 28 dry period, adult mosquitoes were collected and egged so we could estimate the amount of transovum transmission that was occurring in nature. Thirty-three of the adults collected during this period laid viable eggs, and 28 produced egg batches free of both CPV and NPV. Four adults produced larvae infected with CPV (14 of a total of 495 were infected), and one adult produced one larva in 56 with NPV.

In the laboratory, routine transmissions were accomplished by placing uninfected larvae in water containing inclusion bodies from larvae that had been killed by the disease. However, we also investigated the possibility that patently infected larvae could transmit the disease while they were still alive since such a method of transmission was more likely to contribute to the levels of infection that were present in the June 1 to 5 brood. Thus, two hundred 24-hour-old larvae were placed in each of six plastic freezer boxes with 20 ml of water, and 10 live infected fourth instar larvae were added to five containers; the sixth was held as a control. The larvae were maintained at 22° C. and were examined daily for infections. The percentages of infection were calculated by dividing the total number of patent infections by the number of larvae that survived until the first patently infected larva was found. When the infected fourth instar larvae died, they were removed from the boxes as were the newly infected larvae. The results of this test are shown in Table 3 and together with the results of the test of transovum transmission, suggest that the viral residue left by the previous brood may not have contributed to the level of infection of the June 1 to 5 brood.

Since the sequence of events that led to the epizootic at Hackberry were unknown, we could only speculate about the factors involved. At least two overlapping broods preceded the May 18 to 22 period of peak infection, and such a sequence may have allowed a significant buildup of infective material in the habitat. However, a similar sequence of broods at Grand Lake did not have that effect. The proportion of CPV to NPV in the Hackberry population may have also been a critical factor in the buildup, but this rate was variable. Also it is possible that the heavy oil pollution in the Hackberry area was important in weakening the larvae, and this possibility is supported by the fact that when larvae at Hackberry and at Grand Lake hatched at the same time, the Hackberry brood was often from one to two days slower in developing to pupae. (The oil pollution at the Grand Lake area was much less pronounced.)

In the evaluation of any potential biological control agent, it is important to learn what species other than the suspected normal host might be affected. Other than *Aedes sollicitans,* only single specimens of *Culex salinarius* and *Psorophora confinnis* (Lynch-Arribálzaga) have been found with natural infections of NPV. Neither infection was confirmed by either electron microscopy or by tests of transmission, but the appearance of the disease in each case was very similar to that seen in *Aedes sollicitans.* The results of attempts to cross-transmit NPV to other mosquitoes are presented in Table 4: two species of *Psorophora* were susceptible to NPV from

TABLE 3.—Transmission of NPV from live infected 4th-instar larvae to uninfected larvae of *Aedes sollicitans* (200 larvae per container).

Container number	Number of survivors	Percent infected
1	9	22.2
2	110	13.6
3	30	33.3
4	102	34.3
5	126	39.7
Control	119	0
	Average infection = 29.0%	

TABLE 4.—Susceptibility of some colonized mosquitoes to NPV and CPV from *Aedes sollicitans*.

Experimental host	Susceptibility to NPV		Susceptibility to CPV	
	Number of larvae exposed	Percent patently infected	Number of larvae exposed	Percent patently infected
Aedes				
sollicitans[1]	1494	14.25	2437	7.02
triseriatus	590	6.62		
tormentor	220	14.09		
aegypti	1127	0.26		
taeniorhynchus	2329	0.0	2769	1.81
sierrensis	100	0.0		
Psorophora				
varipes	591	9.81	501	0.0
ferox	2129	0.42	461	1.07
Culex				
salinarius	542	0.0	349	0.0
p. quinquefasciatus	357	0.0		
territans	125	0.0		
Anopheles				
quadrimaculatus	1978	0.0	243	0.0

[1] Not colonized

Aedes sollicitans but *Culex salinarius* was not.

In contrast, CPV has been seen in the larvae of many species of mosquitoes in southwestern Louisiana, though the disease agent has been confirmed by both electron microscopy and studies of transmission only in *Culex salinarius* and *Aedes sollicitans*. We have had insufficient material to test the CPV viruses from *Culex* and *Culiseta* extensively, but we have found *Culex salinarius* and *Culex territans* susceptible to CPV from *Culiseta melanura* (Coquillett), *Culex salinarius,* and *Culex territans*. In contrast, *Culex salinarius* is apparently not susceptible to CPV from *Aedes sollicitans* (Table 4).

CONCLUSIONS. The present study seems to have raised more questions than it has answered. Certainly, the two viral diseases do not appear to be the immediate answer to the control of *Aedes sollicitans* in southwestern Louisiana. However, some of our results were encouraging. The viral diseases were clearly effective at Hackberry, but it was discouraging to discover that the rate of infection was so greatly reduced by the dry period. It was also discouraging to find that the rate of transovum transmission was low and that CPV by itself was apparently not very pathogenic. The successful introduction of the disease at the Grand Lake site produced levels of infection which were not as spectacular as those of the Hackberry epizootic, but the results do suggest that an inundative approach might be effective in controlling *Aedes sollicitans* in this area.

Is there a synergistic relationship between NPV and CPV? What effect does tide water compared with rain water have on the survival of the viral agents outside hosts and on the course of the disease in the host? Was the oil pollution at Hackberry important in bringing about the epizootic? What was the fate of the viral residue during the dry period? Was it actually destroyed, temporarily inactivated, or did it become merely inaccessible to the larvae? These are some of the more obvious questions left unanswered.

It is conceivable that efficient mass production techniques and a method of protecting the virus during dry periods might lead to the eventual use of these viral diseases in mosquito control.

ACKNOWLEDGEMENTS. We wish to thank Osborne R. Willis for bringing to our at-

tention the epizootic at Hackberry. We also thank Frank Glenn, Jr., who found the first natural NPV infection in *Psorophora confinnis* and Donald B. Woodard for his assistance in field collections.

References Cited

Clark, T. B., Chapman, H. C. and Fukuda T. 1969. Nuclear-polyhedrosis and cytoplasmic-polyhedrosis virus infections in Louisiana mosquitoes. J. Invert. Pathol. 14:284–286.

CULICOIDES FROM SOUTHERN PART OF LUT DESERT, IRAN WITH TWO NEW SPECIES (DIPTERA: CERATOPOGONIDAE)[1]

SHAHIN NAVAI[2]

In November, 1968, in order to study the insect fauna of the Lut desert, particularly its southern part (Lut-e-Zangi Ahmad), the author accompanied the team of the Geographical Institute of the University of Teheran.

The study area lies between Sistan mountains in the east, Kerman mountains in the west, the range of Jabel-e-Barez, Shahsavaran and Bazman mountains in the south and the desert itself in the north. See Figure 15.

The area is 500 km. long and 200 km. wide. It is criss-crossed by the deep (3–4 m.) usually dry ditches made by the surface waters.

The features of the study localities are as follows:

Baluch-Ab (alt. 418 m.): A spring opening in one of the ditches with water not overflowing. The water is slightly brackish, but more or less potable. The study group raised their tents in this region and stayed there for two weeks.

Shahrokh-Abad (alt. 444 m.): A little (seven household) village about 40 km. south of Baluch-Ab.

Keshit (alt. 480 m.): Located on the western edge of the Lut desert where Keshit river enters the desert. The river is perennial with palm groves on both banks.

MATERIALS AND METHODS. The insects were collected both day and night by hand catch, sweeping with insect nets, in light traps, and with castor oil coated paper. In one instance, a good number were collected around the camping lamp in the tent.

RESULTS. The present paper deals with 583 *Culicoides* collected during this survey. They included two new species as well as five other species. The types of the new species were deposited in the collection of the Institute of Public Health Research, Teheran, Iran.

Culicoides faghihi n.sp.

Brownish species of moderate size. With clear wings. Thorax without any distinct spot.

Female:

Eyes separate and bare. The diameter of fronto vertex (fig. 1 a, b) usually less than one facet. Antennae (fig. 2): Last five segments of the antennal flagellum well elongated, sensilla present on segments III-X and absent on XI-XV.

[1] This study was supported in part by project No. 6395, the Ministry of Health and Plan Organization, Government of Iran.

[2] Research associate, Department of Environmental Health, School of Public Health and Institute of Public Health Research, University of Teheran, P.O. Box 1310, Teheran, Iran.