Semi-Annual Progress Report

REPORT PREPARED BY: Dr. Lloyd E. Rozeboom

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PRINCIPAL INVESTIGATOR: Dr. Gilbert F. Otto

Collaborators: Lt. Leo A. Jachowski, Jr., MSC, U.S.N.

Dr. Lloyd E. Rozeboom

Dr. Otto has resigned from his position in the School of Hygiene and Public Health. As of 22 September, 1953, Dr. Lloyd E. Rozeboom was appointed Principal Investigator. This report, however, covers the first half of the year when Dr. Otto still held this position.

ASSISTANTS: Dr. Robert Ingram

Mrs. Barbara N. Gilford

TITLE OF PROJECT: Studies on the epidemiology and control of filariasis, with particular reference to the biology of the vector, Aedes pseudoscutellaris in the South Pacific.

Objectives: To determine (a) effects of climatic factors on the biology of Aedes polynesiensis; (b) the genetic relationship of Aedes polynesiensis with A. pseudoscutellaris; (c) the importance of A. polynesiensis and A. pseudoscutellaris as actual or potential vectors of other disease organisms; (d) to complete the statistical analysis of the mass of data accummulated from Samoa bearing on the epidemiology and control of filariasis in the Pacific.

SUMMARY OF RESULTS:

A. The effect of climatic factors on the biology of Aedes polynesiensis. Dr. Robert Ingram completed this phase of the work during the past year, and additional information is available from the investigation completed by Dr. Robert

Wallis on oviposition responses of mosquitoes. The purpose of a study of this kind is to establish the basic developmental cycle of a species, and the effect of certain environmental factors on this cycle. With this information, one can arrive at a more definite understanding of the biology of the species in its natural habitat. As a result of the work done in our laboratory, we can make the following summary of the biology of Aedes polynesiensis.

The eggs are laid just above the water line, on a moist substratum. The females appear to prefer a rough surface, and force their eggs into crevices. These oviposition habits may be one reason why the breeding habitat of this species in nature is comprised of coconut husks and tree holes. The rough surface of these plant water containers would furnish the ovipositing female with many small fissures and irregularities within and between which the eggs could be deposited.

Eggs which were kept in water began to hatch four days after they were deposited; most of these hatched after five or six days; but some did not do so until after 72 days. A more rapid and complete hatching took place when eggs were placed in media with low dissolved oxygen content; Ingram suggests that in nature this might be advantageous to the species in that breeding waters low in dissolved oxygen content might indicate the presence of suitable food, inasmuch as such media would be rich in organic matter.

Eggs, if kept moist for four days after having been deposited, can withstand complete desiccation at room temperature for at least 30 days. At the end of this period, only about 10 percent of the eggs hatched. In nature, eggs deposited in coconut husks would thus be capable of surviving dewatering for periods which, in a tropical climate, would more than carry them from one period of rainfall to another. Furthermore, as submerged eggs were shown to hatch for as long as 72 days after deposition, eggs in coconut husks might survive for extremely long periods of time under conditions of incomplete desiccation and in a container with very high humidity. Later experiments showed that in a climatizer, in which temperature and humidity could be controlled, mature eggs kept in a saturated atmosphere survived for a significantly longer time at lower than at higher temperatures. From data obtained by Dr. Ingram, it would appear that at 80° F. and 95 per cent RH, eggs could survive for at least two months, and much longer at 70° F.

A mosquito species, adapted to life in small water containers, might be expected to be faced with the problem of survival during frequent periods of desiccation, of long or short duration. Long periods of drought, as during a dry season, could readily be passed by A. polynesiensis in the egg stage, but certainly short periods of dewatering (but not of complete desiccation) must occur frequently in coconut husks even during the rainy season. If such short periods could be passed only in the egg stage, drastic set-backs in the population of the species through wholesale destruction of the larvae might occur. It is not too surprising, therefore, that Dr. Ingram found that the larvae and pupae of this species were remarkably resistant to dewatering. Some larvae could survive dewatering for as long as four days; but younger stages were more susceptible than were the older stages. Almost all of the pupae were capable of surviving dewatering for two days; these gave rise to apparently normal adults.

Another hazard which might be encountered by a mosquito with an island distribution is contamination of the breeding habitats with sea water. That A. polynesiensis is capable of withstanding such contamination was shown in an experiment in which fourth stage larvae and pupae were able to develop normally in 1.5 per cent saline solutions. The younger larvae were more susceptible, but 60 per cent of even the first stage larvae survived 1.0 per cent saline, while 0.5 per cent had no apparent harmful effect.

Under normal conditions, at temperatures between 70-90° F., the larval period was passed in 4.6 to 9.6 days, and the pupal period in 1.8 to 3.7 days.

In the laboratory, the adult females fed upon any animal, even frogs, which were offered as a source of blood. Man was the preferred host; however, it would seem that this species would not be dependent upon the presence of man for its survival in nature. The females fed most actively from about noon to late afternoon.

Adults survived for periods of 32 to 100 days at temperatures of 70 to 90° F. and a relative humidity of 75 to 95 per cent. The females, therefore, are fully capable of surviving long enough to permit the development to the infective stage not only of Wuchereria bancrofti, but of other parasites as well of which this species may be a potential vector. A. polynesiensis is widely distributed in the Pacific; this distribution in part at least must be due to the ability of the adults to survive for three months or more. It seems evident that this species is capable of extending its range even beyond its present limits to areas where temperatures range from 70 to 90° F. and where a fairly high humidity prevails.

Adults were active within the colony cages through most of the day, but most mating took place early in the morning. Following the first blood meal, there was a preoviposition period of four days; oviposition was followed by a period of two to three days before a second blood meal was taken. Individual females took as many as six blood meals, each of which was followed by deposition of eggs. These females laid from 60 to 89 eggs per blood meal, and most of them deposited a total of 100 to 300 eggs; a few deposited over 400 eggs.

In summary, these studies show that Aedes polynesiensis is well adapted to its natural habitat. Its reproductive potential is high, and it can survive adverse conditions which would be fatal to many other mosquitoes. It is probably capable of extending its range beyond the limits of its present distribution. It can take advantage of the alterations in its environment created by man to build up high population densities, but appears also to be entirely capable of surviving in areas where man is absent. With respect to control, Dr. Ingram points out that one can be certain of complete destruction of the eggs only by burning or burial of coconut husks and other small water containers used by this species as breeding habitats. As the larvae are able to survive dewatering for four days, and the pupae are not affected at all by drought, these stages may not be adequately destroyed by haphazard overturning of coconut husks. The observations made by Lt. Jachowski and Dr. Otto in Samoa indicate that control measures directed against the immature stages would be the most effective inasmuch as the adults are active and rest primarily in the bush where they are not affected by residual household sprays.

B. Genetic relationship between A. polynesiensis and A. pseudoscutellaris. Up to last year we were referring to the species on Samoa as A. pseudoscutellaris. Elizabeth Marks observed that the mosquito originally named A. pseudoscutellaris was taken on Fiji. Type material in the British Museum, and also specimens from a colony, maintained in the London School of Tropical Medicine which was established from material collected in Fiji, showed small but distinct morphological differences from the form which has a wide-spread distribution in the South Pacific. Marks named the latter A. polynesiensis. This situation creates many questions of interest to workers in the field of population genetics. Are these two kinds of mosquitoes true species, or are they sub-specific populations of one species? If experimention confirms the morphological evidence that they are distinct populations, either at the specific or subspecific level, what are the mechanisms which led to this division? Are there differences in the climatic and other environmental requirements between the two forms? Are they equally capable of disease transmission? Do they require different measures for their control?

During the past year a colony of A. pseudoscutellaris was established in this laboratory from eggs sent to us from the London School of Tropical Medicine. Cross breeding experiments now under way have given us an indication that these populations are indeed different from one another, but as yet we have insufficient evidence to determine whether they are species or subspecies. A. pseudoscutellaris appears to be more susceptible to adverse climatic conditions than is A. polynesiensis; this may be a reason why the latter now has such a wide distribution, whereas the former, so far as we know, is found only on Fiji. The results of these studies will be presented in more detail in our next report.

C. A. polynesiensis as a vector of other diseases. A. polynesiensis is a member of the scutellaris complex; and certain other species in this group have been suspected of being responsible for outbreaks of dengue. Dr. Leon Rosen, of the

U.S. Public Health Service, has observed such an outbreak on Tahiti which seemed to be associated with A. polynesiensis; and during the past year he and the principal investigator set up an experiment to prove that this species is capable of transmitting dengue. Dengue virus was obtained from Dr. Albert Sabin; this was inoculated into a monkey (Maccacus cynomolgus). After an appropriate incubation period several lots of Aedes polynesiensis were allowed to feed on this monkey. These mosquitoes were stored in the climatizer at 80° F. and approximately 95 per cent R.H.; after suitable intervals they were fed again on three recipient monkeys (one M. cynomolgus and two M. rhesus). Paired sera of each of these monkeys were sent to Dr. Sabin for testing of antibodies. These tests showed that whereas none of the four monkeys was immune to dengue prior to their inoculation with the virus, after they had been inoculated, the donor monkey and two of the three recipient monkeys showed high antibodies titres. We interpret this as strong evidence that A. polynesiensis transmitted dengue by their bites in this experiment.

D. Analysis of data bearing on the epidemology and control of filariasis in the Pacific. In March and April 1953, Lt. L. A. Jachowski, Jr., and a technician returned to Samoa for the final evaluation of the experimental control program (NM005-048.08). Blood smears were made from nearly 1200 individuals in the experimental villages. These slides, received at Johns Hopkins in September 1953, will be examined and the results incorporated in a paper being written on the experimental control studies.

Additional atypical larvae of Aedes polynesiensis were collected. These have been studied along with material from other sources by Drs. Rozeboom and Rosen.

Sera from 25 Samoans were collected and preliminary studies undertaken to determine whether the reaction of "immune" serum with infective filaria larvae might serve as a diagnostic tool. Due to time limitations this investigation had to be curtailed and only inconclusive results were obtained.

The environmental sanitation program initiated by the research unit in 1949 has continued and the appearance of the villages on the island of Tutiula has markedly improved.

The new civilian public health officer was briefed on the results of the field studies. In "A report of health services in American Samoa 1952", Dr. J. C. Haldeman, U S P H S has limited discussion of filariasis to a reprinting in toto of the article by G. F. Otto and L. A. Jachowski entitled "New Facts on an old Disease" which appeared in Research Reviews in August.

PLANS FOR THE FUTURE

With Lt. Jachowski's trip to Samoa, the field investigations have come to an end. The analysis of the data collected in this field will be completed, and Lt. Jachowski is preparing a paper on experimental control of filariasis. He also plans to prepare a report on the mosquitoes of Samoa, which will include data on distribution and biology. He has found larvae of Culex sitiens on Samoa with unusual preclypeal spines, and he hopes to determine the taxonomic significance of this anomaly.

We hope to complete the cross breeding experiments with A. polynesiensis and A. pseudoscutellaris during the next few months. With Dr. Ingram's study as a base line, it would be interesting to carry out experiments on the comparative physiology of A. polynesiensis and A. pseudoscutellaris. We are making certain comparisons, such as the relative resistance of the eggs to desiccation, but a thorough study of the various aspects of the biology of A. pseudoscutellaris will require more time than is available up to the end of the present project.

It would be most desirable to take advantage of the presence of colonies of these two exogenous species in our laboratory to determine their respective roles as actual or potential vectors of diseases other than filariasis. We have shown that A. polynesiensis can transmit dengue. Many details as to its efficiency as a vector remain to be determined: the titres of the infecting and infective doses; the length of time the mosquito remains infective, and whether the bite of a single mosquito may cause an infection. Parallel studies should be made with

A. pseudoscutellaris, and for that matter, with other species of this important complex of which colonies could be established in our laboratory. The role of monkeys as reservoir hosts for dengue is of considerable epidemologic importance. Are these mosquito species potential vectors of the various encephalitic virus? Expansion of the present filariasis project to include other diseases would be dependent on further support.

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