

ANNUAL PROGRESS REPORT

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| SEATO Medic Study No. 43    | Colonization of Vector Mosquito Species  |
| Project No. 3A 025601 A 811 | Military Medical Research Program<br>S. E. Asia  |
| Task 01:                    | Military Medical Research Program<br>S. E. Asia  |
| Subtask 01:                 | Military Medical Research Program<br>SEASIA (Thailand)   |
| Reporting Installation:     | US Army-SEATO Medical Research Laboratory<br>APO 146, San Francisco, California<br><br>Division of Medical Research Laboratories<br><br>Department of Medical Entomology |
| Period Covered by Report:   | 1 April 1963 to 31 March 1964  |
| Principal Investigator:     | Major John E. Scanlon, MSC   |
| Assistant Investigator:     | Mr. Sahem Esah   |
| Reports Control Symbol:     | MEDDH-288  |
| Security Classification:    | UNCLASSIFIED   |

ABSTRACT

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Species

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The objective of this study was to develop methods of maintaining colonies of mosquitoes. Colonies of several important species of mosquitoes have been developed and are being maintained to supply mosquitoes of known background for insecticide testing and disease transmission experiments. Colonies of the readily adapted species Aedes aegypti and Culex quinquefasciatus were reestablished in a newly built insectary, and both species were subjected to insecticide tests to provide baseline data. Aedes albopictus was also colonized from material collected in the Bangkok area, and the colony is being expanded. The colonization of the malaria vector, Anopheles balabacensis proved to be much more difficult. Lack of natural mating in cages required the use of the artificial mating or forced mating technique. The larvae also required conditions simulating their natural environment. This was provided by the use of mechanical aeration and by placing boiled sand in the larval rearing trays.

BODY OF REPORT

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Objective: To establish, and develop methods of maintaining, colonies of mosquito species which are vectors of human disease, or which are important for other reasons. Mosquitoes from the colonies are used for experimental transmission of disease organisms in the laboratory, and for insecticide tolerance tests (Study 50).

Description: Wild caught mosquitoes are brought into the insectary and permitted to feed on normal animals and lay eggs. Each species is reared according to methods which produce the best results for it, and these are initially determined by trial and error when dealing with previously uncolonized species. The ultimate aim is to develop the simplest possible methods for the production of large numbers of

mosquitoes on demand.

Progress: During the year the work with living mosquitoes was transferred from the insectary on the grounds of the School of Tropical Medicine to new facilities in the main laboratory. An integrated system of temperature and humidity control was developed by a local contractor, and appeared to be working adequately six months after installation. Supplementary humidifier units were installed to cover any possible malfunction of the equipment. Level of 80 degrees farenheit and over 80 percent relative humidity have been maintained without difficulty with this equipment which consists essentially of a large compressor and heat exchange unit (without cooling tower) and a water injection unit which humidifies the air just before it is expelled into the insectary rooms. Three rooms are being controlled by the system.

New colonies of Aedes aegypti, Culex quinquefasciatus and Aedes albopictus were established in the new insectary. Stocks of A. aegypti and C. quinquefasciatus were used for full series of insecticide tests with DDT and dieldrin. Late in the report period approximately six thousand A. aegypti females were used for a test of effeciency of recovery of chikungunya virus from pools containing known numbers of infected mosquitoes and subjected to varying isolation techniques. No other demands were placed on the A. aegypti colony, but large numbers of eggs were accumulated for future demands. No A. albopictus have been withdrawn from the colony as yet, and the build-up of this species has been much slower than A. aegypti.

Most of the effort in the past year has been devoted to development of a colony of Anopheles balabacensis, the most important malaria vector in many parts of Thailand. An earlier attempts to establish a colony from larvae collected in Chantaburi Province ended unsuccessfully at the beginning of the report period. No viable eggs were obtained from the adults reared from these larvae. Adults were then obtained from females collected at the malaria study site at Khao Mai Kaeo, Cholburi Province. At that time the facilities of the old insectary at the School of Public Health were still being used. Considerable difficulty was experienced with loss of eggs, presumably to ants, and no normal mating occured. From July to September approximately one thousand females were brought from the malaria study area. Almost all of them survived the trip well and many thousand eggs were produced. The eggs from three hundred females were also supplied to WRAIR for a satellite colony. Survival of the field collected females was excellent, some surviving up to three weeks. Egg production and fertility were also excellent. Almost all of the females had been taken while coming to feed on man, and it would appear that females in that physiological state are almost

universally inseminated. None of the resulting F1 adults mated in the insectary, despite being kept in cages of various sizes up to one cubic yard, and despite exposure to various light schedules. On dissection, none of the females were found to be inseminated. A check on wild caught females indicated that insemination continued at a very high rate in the field. In addition, the F1 laboratory generation adults did not appear to be as vigorous as the wild stock. In September the colony was moved entirely into the new insectary and a number of procedures were attempted in order to insure insemination and to improve the vigor of the resulting laboratory generations.

#### Forced Insemination

From November onward attempts at natural mating were abandoned, and the forced insemination technique has been employed. The methods used are essentially the same as those used to maintain a colony of A. maculatus at the Institute for Medical Research, Malaysia. Adult males 3 to 5 days of age are pinned through the pleural region of the thorax while being held with a vacuum pipette. The legs and head are removed. When 10 to 20 males have been so prepared blood fed females are placed in individual vials and anaesthetized with ether. Each female is then placed in a supine position on the table top and pinned. A male is brought into contact with her. If copulation does not begin almost immediately another male is presented, and the first male is saved for another attempt. When copulation is properly affected the pinned male may be lifted from the table and the female will remain attached. Inseminated females are placed in paper drinking cups for recovery and fed on maintenance fluid for three days, at which time they are transferred to individual paper cups with moist filter paper on the bottom for egg laying. Insemination rates and fertility have been excellent, and additional technicians are being trained in the technique. It appears, however, that not all persons are equally adept at the fine detail needed to perform the task.

#### Oviposition

Eggs were deposited in a minimum of 4 days, a maximum of 14 days, and an average of 6 days after forced mating. Egg production compares favorably with the production from wild-caught females, as does fertility, as can be seen in the comparative figures given below:

|             | <u>Number of females</u> | <u>No. of eggs</u> | <u>Larvae hatched</u> |
|-------------|--------------------------|--------------------|-----------------------|
| Wild caught | 63                       | 3869               | 2360                  |
| Colony      | 69                       | 5724               | 3279                  |

### Feeding and Maintenance of Adults

Adults brought in from the field were originally kept in a room at 76-78 degrees Far. on a twelve hour daylight schedule. However, it was found that these did not produce eggs or feed, although longevity was good. They were moved to a room at 80-82° F. and a thirteen hour light schedule and fed and laid eggs normally. Adults are offered a maintenance feed on a multi-vitamin syrup diluted with tap water shortly after emergence and at all other times when not being prepared for a blood meal. This syrup is also used to feed adults being transported from the field for various purposes, and it has been found to be an excellent maintenance food. Guinea pigs are used as a source of blood meals, and are offered to the females in cubic foot cages. The female A. balabacensis also feed well on man.

### Rearing of Immature Stages

In December, it was noted that larvae produced by the artificially inseminated females, or from wild-caught females were not nearly as vigorous as larvae found in the field. An examination of the rearing records also indicated that mortality of the immature larvae was excessive. Up to that time rather routine larval rearing methods had been used. A number of approaches were tried, and the method presently used seems to produce excellent larvae. Since A. balabacensis are generally found in small streams and seepages in the study area an aeration system was employed in the insectary to duplicate the water movement. Standard aquarium aerators are used, without air stones, to produce movement of the water surface in the rearing trays. Ordinary drinking straws are bent in circles to produce egg harbors, and after hatching straight straws are laid on the water surface to allow the larvae to take refuge from the direct currents produced by the aeration. Boiled sand from the known breeding sites of A. balabacensis has also been added to the trays, and this appears to have a definitely favorable effect. Larval food consists of commercial animal biscuits finely ground. This is suspended in water. A few drops of the suspension are added to the larval trays twice a day, avoiding overfeeding.

The techniques listed above have been routinely followed since February. It appears that the colony is now secure, and that it can be expanded. As additional generations appear in the laboratory aliquots of the adults will be placed in large cages to see if natural mating will occur. In addition, simplified larval rearing techniques will be attempted with succeeding generations. Even if the present techniques continue to be required it is estimated that a production of adults per week can be sustained with the present facilities and personnel. Adults

will be used for feeding experiments with malarious humans and animals in the coming year.

Some effort was made to colonize the even more difficult malaria vector, Anopheles minimus during the year. However, the time and space devoted to the A. balabacensis work precluded development of a colony. Since the A. balabacensis colony is now in the routine stage, additional efforts will be made to colonize A. minimus when large numbers again become available. In addition, a small colony of A. maculatus was developed late in the period, using the forced insemination technique.

Summary: Normal colonies of Aedes aegypti, A. albopictus and Culex quinquefasciatus were maintained and expanded during the report period. A colony of Anopheles balabacensis was developed, with the use of the forced insemination technique. The larvae of this malaria vector proved to be quite fastidious in their requirements. The use of an air stream and boiled sand in the larval rearing trays produced vigorous larvae.

Conclusions: Satisfactory techniques have been developed for the maintenance of Anopheles balabacensis, a difficult species to rear. The same techniques should give good results when applied to other refractory Anopheles species, such as A. minimus; but attempts will be made to simplify the procedures even further.