3. Title : Precipitin Tests of Mosquito Blood Meals

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Objective : The host range of a mosquito vector is one of the important factors affecting the persistence and spread of mosquito - borne diseases. The vectorial capacity of a species depends, among other factors, on its preference for human blood and the frequency of its contact with man. The purpose of this study is to determine the natural hosts of the medically important mosquito species in Thailand in support of various mosquito - borne disease studies in progress.

Description : Blood engorged mosquitoes are collected during the the course of studies on malaria and arthropod - borne viruses. Saline extracts of gut contents are tested by the precipitin ring method for reaction against a battery of antisera representative of various common hosts.

Progress : The lots of lypholized antisera prepared at WRAIR for use in the precipitin tests were checked for potency. Representatives of each lot were tested by the precipitin method for reaction against the homologous serum. Results indicated that these antisera had deteriorated in storage. Consequently, the preparation of fresh antisera was initiated utilizing chickens and rabbits for their production. The preparation of antisera against man, monkey, dog, pig, horse, cow, buffalo and chicken blood has been accomplished. All were produced in chickens with the exception of the chicken antiserum which was obtained from rabbits. These antisera were checked for potency and specificity. All were found to have high titers ranging from 1/8000 to 1/32,000. Cross - reactions were observed between human and monkey and between cow and buffalo antisera when each was tested against the heterologous antigen. The absorption and elimination of the non - specific antibodies are still in progress.

Detection of blood sera in engorged mosquitoes killed and stored at ambient temperatures was compared with those stored frozen. Females of Aedes aegypti fed on humans were killed soon after engorgement and divided into two series; one was stored at 70°F while the other was kept at ambient temperatures. After variable periods of storage, saline extracts of mosquitoes of both series were tested by the precipitin method for reaction against human antiserum. The serum proteins were found to be equally detectable in both series after at least 90 days of storage. These results indicate that the collection of engorged mosquitoes from the field can be facilitated by storage of the specimens intact in a dry state at ambient temperatures eliminating the necessity for expressing mosquito gut contents on to filter paper or storage of the intact mosquitoes in a frozen state.

A study was also made on the rate of digestion of serum proteins of human blood by the mosquito Anopheles balabacensis. This test was designed to provide information on the time at which these serum proteins no longer react with specific antisera. Females of this species, fed to repletion on human blood, were held in the insectary (75°F, 65% R.H.) for intervals of 0, 6, 12, 24, 36, 48, 60, 72 hours following blood ingestion. Saline extracts of mosquitoes of all series were tested by the precipitin ring method for reaction against human antiserum. Results indicated that serum porteins were detectable up to 36 hours following blood ingestion, but thereafter the results were questionable.

Identification of blood meals of mosquitoes collected from Chiengmai and vicinity during 1964–1965 was resumed. A total of 296 engorged specimens were checked by the precipitin test for reaction with anti - human serum. These mosquitoes were collected while resting in houses with the exception of

Mansonia uniformis which was collected by light traps. Results indicated that only 37% of the 279 Culex quinquefasciatus fed on man. Since a small number of the other species (Table 5) was tested on conclusions can be drawn relative to their feeding preferences. All the saline extracts which gave negative reactions with anti - human serum are held frozen for further testing with other antisera.

Table 5. Results of precipitin reactions of mosquito blood meals with anti - human serum.

Species	Specimens tested Number	Positive reactions per cent
Aedes aegypti	12	83
Culex quinquefasciatus	279	37
Culex tritaeniorhynchus	3	33
Mansonia uniformis	2	0

Summary: Due to deterioration of lypholized antisera in storage, the preparation of eight antisera was inititated and completed during this period. These new antisera were checked for potency and specificity and the absorption of non - specific antibodies from four antisera is now in progress.

Blood sera in engorged mosquitoes killed and stored at ambient temperatures were found to be detectable after at least 90 days of storage thus eliminating the necessity for expressing mosquito gut contents onto filter paper or storage of the intact mosquito in a frozen state.

Studies on the rate of digestion of serum proteins of human blood by the mosquito Anopheles balabacensis showed that serum proteins were identifiable by the precipitin test up to 36 hours following blood ingestion.

Precipitin tests of engorged mosquitoes collected from Chiengmai showed that only 37% of the 279 Culex quinquefasciatus collected from houses had fed on man.

* Arrived on PCS 4 August 1965