Pathogens of Medically Important Mosquitoes of Thailand

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OBJECTIVE: To determine the kinds of insect pathogens present in medically important species of mosquitoes in Thailand and to elucidate the biology of selected pathogens sufficiently to assess their potential as biological mosquito control agents.

BACKGROUND: Successful and economically feasible biological control of certain important agricultural and forest insect pests with pathogens, used alone or in combination with other control agents, has been thoroughly documented. Several pathogens for use against agricultural pests have been approved by the Food and Drug Administration and the Environmental Protection Agency and are currently being produced commercially and used in the United States. Interest in the potential of pathogens for the control of medically important arthropods is not new, but the successful use of pathogens in agriculture and forestry, combined with widespread environmental interest, has provided impetus in recent years for more vigorous investigation of their potential value to medical entomology. The SEATO Medical Research Laboratory is an ideal location for such investigations, for in Thailand there are more than four times as many species of mosquitoes (400+) as in the United States. Among these are several species of primary international importance as disease vectors.

DESCRIPTION: During the first six months of this project the slide-mounted mosquito larvae in the taxonomic collection of the Medical Entomology Department was screened for microscopically detectable pathogens. Also, a field survey for mosquito pathogens was initiated. The survey has concentrated on *Culex pipiens quinquefasciatus* in the Bangkok-Thon Buri area. Large numbers of larvae collected at various locations were transported to the laboratory. These were visually examined for gross signs suggesting presence of pathogens, such as loss of pigmentation, presence of abnormal color, aberrant swimming behavior, and conspicuously abnormal body proportions.

Larvae displaying gross signs of pathology were segregated. Part of these abnormal larvae were examined microscopically in wet-mounts and/or as Giemsa-or hematoxylin-stained squash-smears, while the balance were prepared for paraffin sectioning and hematoxylin-eosin staining. When large collections of larvae showing grossly abnormal signs were made, a portion of the collections were reared in the laboratory and the mortality rate was recorded. Smears were made of larvae and pupae that died. Surviving pupae were allowed to develop to adults and the progeny of these examined for evidence of transovarial (vertical) transmission. Attempts to transmit pathogens by *per os* exposure of uninfected laboratory-reared larvae were also made. Survivors of these tests were reared and their progeny examined for evidence of vertical transmission.

PROGRESS: Five distinct species of fungus of genus *Coelomomyces* were found in the slide-mounted larval collection. *Culex tritaeniorhynchus* and *C. fuscacephala* were infected with apparently the same species, three different species were found in *Anopheles vagus* and one in *A. nivipes*. One nematode infection was found in *C. tritaeniorhynchus*.

Seventy-eight collections of *C. pipiens quinquefasciatus* were made at 54 locations in the Bangkok-Thon Buri area. Stained smears of larvae from 67 of the 78 collections were examined. Examination of all material collected is not complete, but the following observations have been made. Of 2073 larvae displaying grossly abnormal signs, microbial agents were found in 1971 (95%). Microbial agents were often seen within tissues or hemolymph of larvae examined in wet-mounts prior to squash-smearing. The

presence of numerous microbial contaminants on the integuments and within the alimentary canals of larvae possibly obscured infections that will become apparent with the examination of sectioned material.

Two microbial agents were present in almost all specimens showing gross signs of disease. One was a dark—staining cytoplasmic inclusion, about four microns in length, that appeared to replicate by transverse fission. This agent could be seen within cells in larvae examined in wet mounts. It has been transmitted to uninfected larvae in the laboratory. The other common agent was of minute bacilliform structure at the limit of resolution of the light microscope, somewhat less than one micron in length. This agent could be detected within the hemolymph of larvae examined in wet—mounts by its intense Brownian motion and in smears stained with Giemsa's stain at pH 7.4. Larvae containing this agent died almost without exception. The agent has been transmitted to uninfected *C. quinquefasciatus* larvae in the laboratory. Microsporidia were found in 27 of 67 (40%) collections from 15 of 54 (28%) locations surveyed. Preliminary transmission attempts have not been successful, but definitive attempts are planned.

One fungal agent has been found which presents by turning mosquito larvae orange. All transmission attempts with this agent have been unsuccessful. More material is being sought in the field.

An additional agent in the size range and with the staining characteristics of a polyhedral virus was collected from 11 of 54 (20%) locations. This agent was transmitted in the laboratory, but transmission rates were lower than those expected with a polyhedral virus and the identity of the agent remains in doubt. Further studies are underway.

DISCUSSION: Distortion of *Coelomomyces* sporangia resulting from the mounting techniques used for mosquito larval taxonomic specimens made species determination impossible. Adult forms are required for identification of insect nematode parasites, so the larval nematode found could not be identified.

Studies are underway to determine the identity and to define the biological characteristics of agents found in surveys of *Culex pipiens quinquefasciatus*. Efforts in the following areas will be required: (1) culturing of bacteria and fungi, (2) electron microscopy of suspected virus, rickettsiae, and microsporidia, (3) transmission experiments to determine optimum conditions and methods for transmission and propagation, and (4) experiments to establish host ranges. The survey for mosquito pathogens will be extended both geographically and to include other species of medical importance.

Access to the scientific literature in invertebrate pathology is essential for the identification of mosquito pathogens, and the literature is a time—saving source of methods in the propagation and study of pathogens. Therefore, a high priority will be placed on accumulating a bibliography of pertinent literature and a collection of reprints of previous reports of pathogens in mosquitoes.