# Pathogens of Medically Important Mosquitoes of Thailand

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**OBJECTIVE :** To determine the kinds of mosquito pathogens occurring 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 :** An examination of the slide-mounted mosquito larva collection in the Department of Medical Entomology, SEATO Medical Research Laboratory, and a preliminary survey for pathogens in **Culex quinquefasciatus** in Bangkok confirmed the occurrence of pathogens in the Thai mosquito fauna (1). The present study involved a survey for mosquito pathogens in **Aedes aegypti** and **C. quinquefasciatus** at 20 locations in Thailand. These mosquito species were selected for study because of their primary medical importance, because they can be easily collected in relatively large numbers and because their breeding habits make them vulnerable to biological control by pathogens.

**DESCRIPTION :** From four to 40 man-days were spent collecting larvae of **Ae. aegypti** and **C. quinquefasciatus** at the locations in Figure 1, excluding Bangkok. Because of its size and proximity to the laboratory, more extensive collecting was done in Bangkok, here considered to include Thon Buri, Nonthaburi, and Pathum Thani. Collections from Sangkhlaburi were from numerous small villages along the Khwae Noi River between Sai Yok and Cha Deng Cheng, and up the Sang Kalia River as far as Sang Kalia. Collections from Ko Samui were from several villages on adjacent islands as well as from the villages of the main island.

**C. quinquefasciatus** larvae were collected primarily from polluted water discharged from dwellings and market places. **Ae. aegypti** larvae were collected from domestic water containers in and around homes. Other species of larvae were collected occasionally and were processed and examined, although they were not necessarily pertinent to the study. Larvae were examined successively in white enamel pans and in black pans under a bright light to detect gross signs of pathology. Watched for were any abnormality in color, size, shape or behavior. Larvae displaying such signs were separated and identified. Part were smeared on microscope slides, five per slide, in discrete smears, and the remainder were preserved in 7% neutral buffered formalin. Smears were air dried, fixed with methanol, and stained with Giemsa stain in Tris buffer at pH 7.2. Formalinized material was embedded in paraplast, sectioned and stained with hematoxylin and eosin. Specimens were examined at a magnification of 500X. A sample of larvae showing no gross signs of infection was also taken from each collection. These were identified, smeared, processed, and examined for evidence of covert infections. With the exception of eugregarines and ciliates, only those organisms observed in host tissue or hemolymph were reported. Some preliminary studies have been made to determine the infectivity. mode of transmission and host specificity of some of the pathogens found.

**RESULTS :** Twenty-five host-pathogen associations were found in 20,000 specimens prepared from eight species of mosquitoes. These are summarized in Table 1 and briefly discussed below.

## A. Protozoans

1. **Ciliates** - Ciliates were frequently seen in the midguts and occasionally in the hemolymph of **C. quinquefasciatus.** Although some species of ciliates are pathogenic for mosquitoes, they generally are regarded as having little bio-control potential.

2. **Microsporidans** - Seven apparently distinct microsporidan species were found, four in **Ae. aegypti** and one each in **C. quinquefasciatus, Anopheles vagus** and **Culex (Lutzia)** sp. Of those in **Ae. aegypti,** one was a **Stempellia** sp., two were **Thelohania** spp., and one was a **Nosema** sp.

The **Stempellia** had pyriform spores $8.0 \times 4.0$ $\mu$m. in wet mounts, and was present at 17 of the 20 locations studied. It has been transmitted in the laboratory both per os and transovarially, and about $8.0 \times 10^5$ spores were obtained per patently infected larva. The dose - infection rate curve was linear between infection rates of 15 and 85 percent, and infection rates of 100 percent have been obtained. Mortality rates approaching 100 percent occurred, but these rates varied widely in response to unknown factors in addition to infection rates.

**Thelohania** spp. of **Ae. aegypti** were found respectively at Ubon Ratchathani and at Hat Yai. One was a pathogen of the midgut epithelium, with oval spores $2.0 \times 1.5$ $\mu$m. The tissue preference of the other is unknown, because it was found only in smears. The spores were oval and $3.0 \times 2.0$ $\mu$m.

The microsporidan in **C. quinquefasciatus** was a **Stempellia** sp. It was found at 9 of the 20 locations studied but was highly prevalent only at Hat Yai. The spores were pyriform and $5.0 \times 2.75$ $\mu$m in wet mounts. Chapman (2) reported seeing a **Stempellia** sp. in **C. quinquefasciatus** in Bangkok and considered it identical to a species of **Stempellia** in the United States, subsequently described and named **S. milleri** by Hazard and Fukuda (3). Hazard aud Fukuda did not report having examined material from Thailand, so the identity of this species is still in doubt. We have transmitted it per os, and further studies are underway.

**Anopheles vagus** at Lamphun were infected with an unidentified genus of Microsporida. The spores were $5.0 \times 3.0$ $\mu$m and had structural features which will require electron microscopy for resoluton. It was distinctly different from others encountered in this study and was found primarily in the fatbody. Attempts to transmit it per os to **Anopheles maculatus** were unsuccessful.

**Culex (Lutzia)** sp. at Sangkhlaburi were infected with a microsporidan with pyriform spores $7.0 \times 4.0$ $\mu$m. The body cavity of infected larvae was filled with sporoblasts, though few spores were seen. The sporoblasts were usually disassociated, but some were associated in groups of eight. Insufficient material was examined to conclude that it was Genus **Thelohania,** so identification will require further study.

3. **Helicosporidans** – Apparently the same species of **Helicosporidium** was found widely distributed and moderately prevalent in **Ae. aegypti** and **C. quinquefasciatus.** The spores were spherical and 5.5 $\mu$m. in diameter. It has been transmitted per os to **Ae. aegypti, C. quinquefasciatus** and **Anopheles balabacensis.** There is only one previous report of Helicosporida in mosquitoes (4). The order is currently considered monotypic (5).

4. **Eugregarines** – These agents were commonly found in **Ae. aegypti, Aedes albopictus, C. quinquefasciatus,** and **Armigeres subalbatus.** They are generally regarded as having little bio-control potential.

**B. Fungi**

Apparently the same species of **Entomophthora** was found in **Ae. aegypti** and **C. quinquefasciatus** at Sangkhlaburi. It was transmitted through several passages in the laboratory and was highly virulent though not very infectious. A fungus of Genus **Coelomomyces** was found in **Culex minor** at Sangkhlaburi.

**C. Bacteria**

In several locations, **Ae. aegypti, C. quinquefasciatus,** and **Ar. subalbatus** were found infected with minute (about 1 $\mu$m long), gram negative, highly motile, vibrioform bacteria that produced an extremely virulent septicemia. Chapman (2) reported seeing possibly the same species in Bangkok and commented that the agent was found many places in the world but had never been cultured. Our attempts to culture this organism so far have been unsuccessful.

An endospore-forming bacillus was found in **Ae. aegypti, Ae. albopictus,** and **Ar. subalbatus** from Sangkhlaburi, but only in preserved material.

An apparent bacterial agent, which turned the infected host red, was found in **C. quinquefasciatus** in Bangkok.

**D. Unknown**

A pathogen of undetermined identity was found in the midgut epithelium of **Ae. chrysolineatus** from Sangkhlaburi. Epithelial cells were greatly enlarged and contained small, non-staining particles less than 0.5 $\mu$m. in diameter. The particles resemble a rickettsia of Genus **Enterella.**

The following information reflects the prevalence of mosquito pathogens in Bangkok. **Ae. aegypti** was collected 73 times at 55 collecting sites. At least one kind of pathogen was found in 37 of the 73 collections and at 45 of the 55 collection sites. **C. quinquefasciatus** was collected 151 times at 90 collecting sites. At least one kind of pathogen was found in 137 of the 151 collections and at 81 of the 90 collecting sites.

No viruses were detected during the survey.

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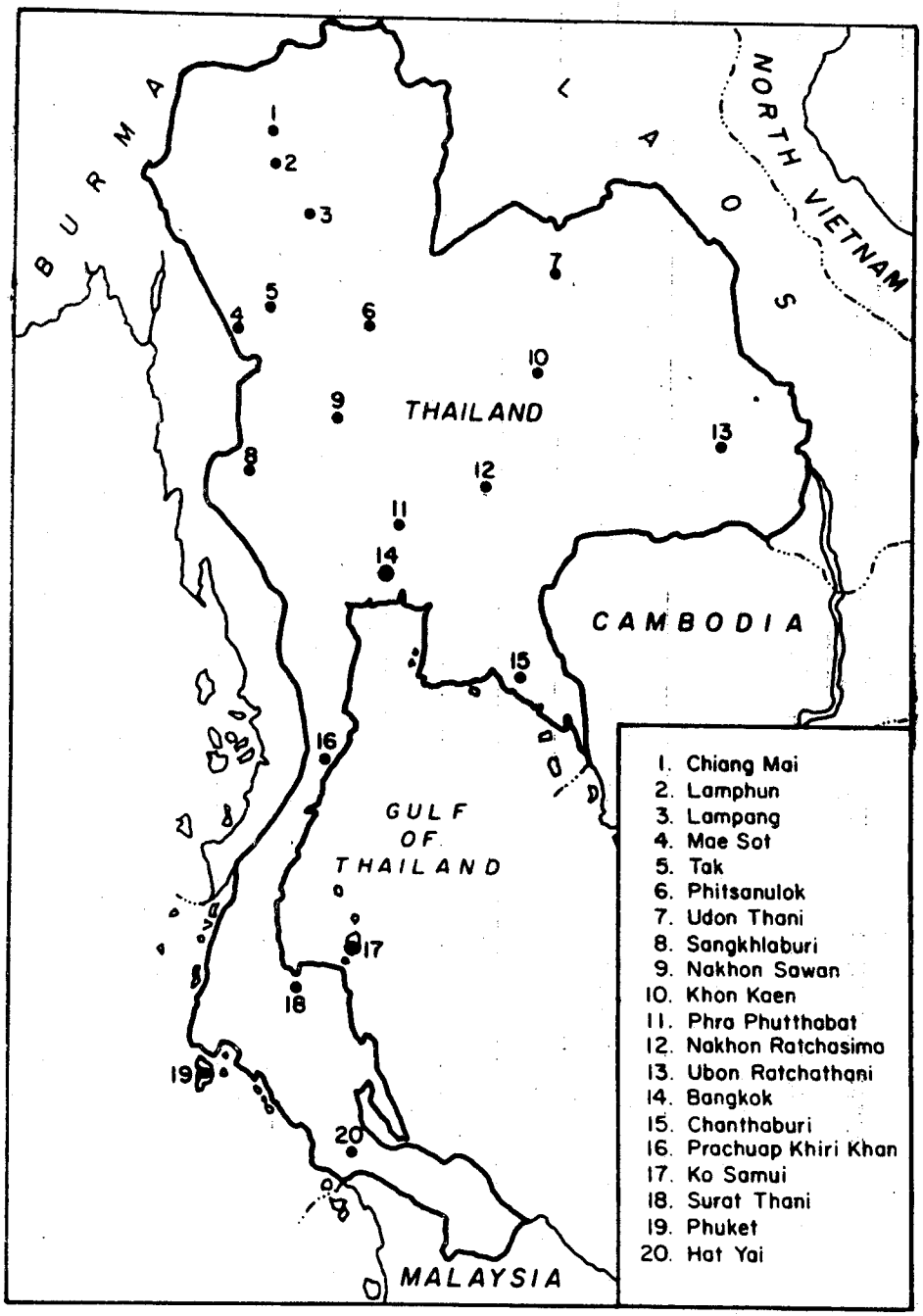


Figure 1. Locations where Aedes aegypti and Culex pipiens quinquefasciatus were surveyed for mosquito pathogens.

Table 1. Summary of pathogens found in eight species of mosquitoes in Thailand.

| Pathogens | | Host | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Aedes** | | | **Culex** | | | **Armigeres subalbatus** | **Anopheles vagus** |
| | | **aegypti** | **albopictus** | **chryso-lineatus** | **pipiens quinque-fasciatus** | **minor** | **(Lutzia) sp.** | | |
| Protozoans | | | | | | | | | |
| Ciliates | | | | | X | | | | |
| Mierosporida | 1 | X | | | | | | | |
| | 2 | X | | | | | | | |
| | 3 | X | | | | | | | |
| | 4 | X | | | | | | | |
| | 5 | | | | X | | | | |
| | 6 | | | | | | | | X |
| | 7 | | | | | | X | | |
| Helicosporida | | X | | | X | | | | |
| Eugregarines | | X | X | | X | | | X | |
| Fungi | 1 | X | | | X | | | | |
| | 2 | | | | | X | | | |
| Bacteria | 1 | X | | | X | | | X | |
| | 2 | X | X | | | | | X | |
| | 3 | | | | X | | | | |
| Unidentified | | | | X | | | | | |