PRELIMINARY REPORT OF SOME MOSQUITO PATHOGENS FROM THAILAND[1]

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ABSTRACT. Surveys to find mosquito pathogens in *Aedes aegypti* and *Culex quinquefasciatus* (=*fatigans*) were conducted at 20 localities in Thailand. Specimens of a few additional mosquito species, collected incidentally, were also examined. Twenty-two mosquito host-mosquito pathogen associations were found, comprising 9 species of mosquitoes and possibly 17 pathogen species. Pathogens found included a helicosporidan in *Ae. aegypti* and *Cx. quinquefasciatus;* 3 microsporidia in *Ae. aegypti;* 1 microsporidian each in *Cx. quinquefasciatus* and *Anopheles vagus;* 1 or possibly 2 microsporidia in *Culex (Lutzia)* sp.; eugregarines in *Ae. aegypti, Ae. albopictus, Ae. chrysolineatus,* and *Armigeres subalbatus;* a flagellate in *Toxorhynchites splendens, Coelomomyces* sp. in *Cx minor;* and a highly lethal septicemia in *Ae. aegypti, Cx. quinquefasciatus, An. vagus,* and *Ar. subalbatus.* A gram positive, spore-forming bacillus was found in *Ae. aegypti, Ae. albopictus,* and *Ar. subalbatus,* but its pathogenicity was not investigated.

INTRODUCTION

The occurrence of mosquito-borne diseases in many developing tropical countries has been increasing in recent years. Insecticide resistance and higher costs of newer insecticides are major factors contributing to the problem and, along with environmental considerations, have focused attention on the need for alternatives or supplements to conventional chemical insecticides for mosquito control (Pont et al. 1977). Considering the well known precedents for successful use of pathogens in management of agricultural and forest insect pests, mosquito pathogens deserve, and are receiving, vigorous investigation and development as biological control agents. Examination of a recently accumulated bibliography of mosquito pathogens (Roberts and Strand 1977) revealed that relatively few of the known mosquito pathogens had been reported from developing nations with serious mosquito-borne disease problems and that almost none of these had been systematically evaluated as potential biological control agents. This reflects a need for concentrated research effort on the biological control of vector mosquito species in those parts of the world which have both the greatest mosquito control challenge and the most limited resources with which to meet the challenge.

With the objective of locating potential biological control agents for *Aedes aegypti* and *Culex quinquefasciatus* (=*fatigans*) in Thailand, a survey for mosquito pathogens in these species was conducted between the spring of 1975 and the summer of 1976 at 20 locations. This report provides preliminary information on the kinds of pathogens found, their hosts, and their geographical distributions.

MATERIALS AND METHODS

Collections were made at the locations shown in Figure 1. More numerous collections were made in Bangkok (14)[2], where the base lab was located. Larvae of *Ae. aegypti* and *Cx. quinquefasciatus,* and occasionally other species, were collected at several sites at each location. Detailed

[1] The opinions or assertations contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

[2] Numbers after place names refer to locations on Figure 1.

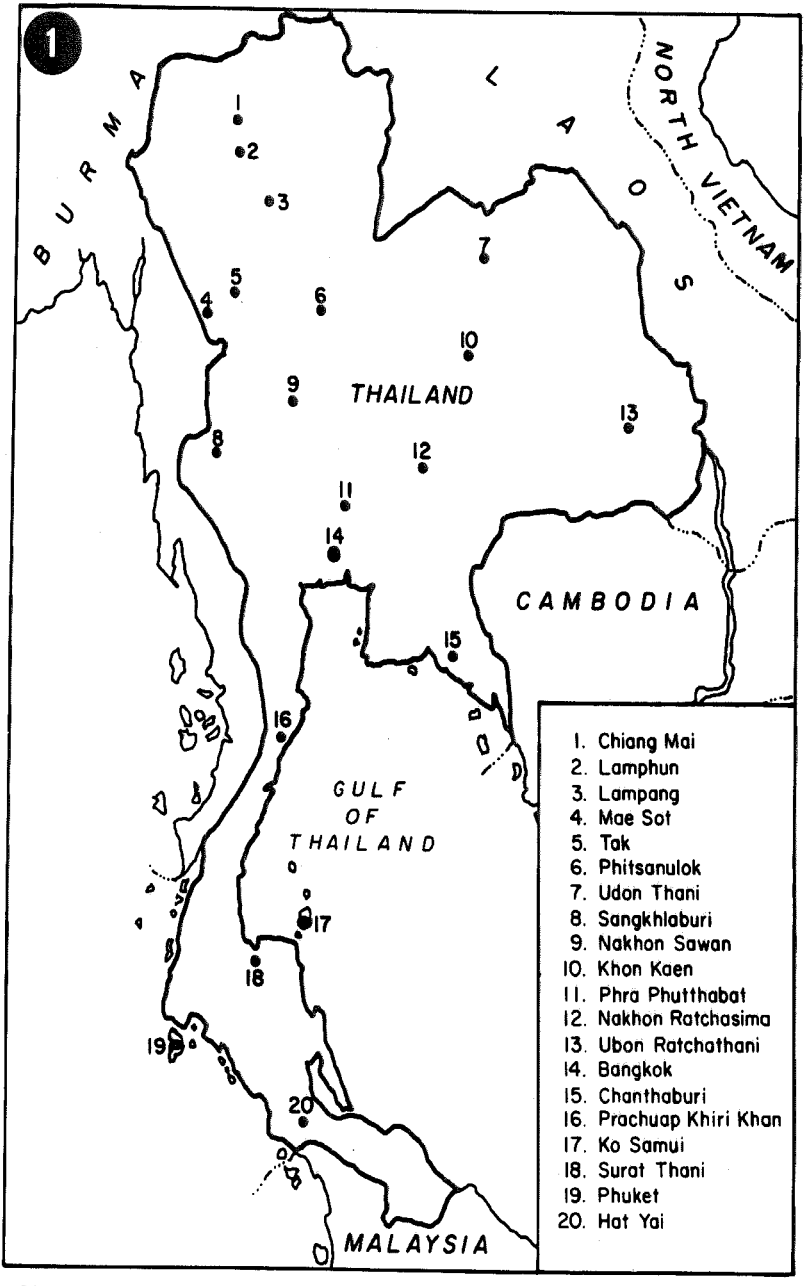

Figure 1. Locations in Thailand where *Aedes aegypti* and *Culex quinquefasciatus* (=*fatigans*) were surveyed for mosquito pathogens.

collection site data were recorded. Larvae from each site were kept separate and transported to a temporary field laboratory where they were identified and separated by species. All the larvae were placed, several at a time, successively in white and black pans and examined under bright light for gross signs of infection. Gross signs were considered to be any abnormality of color, size, shape, or locomotion. Larvae with gross signs and a sample of larvae showing no signs of infection were separated from each collection. Samples of all larvae were fixed in neutral buffered 8% formalin for subsequent sectioning. Samples of all larvae were also smeared on microscope slides with the tips of wooden applicator sticks, 5 smears per slide. The smears were air dried, fixed with absolute methanol and stained with Giemsa stain buffered at pH 7.2 to pH 7.5 with 0.01M phosphate buffer. Coverslips were affixed with a permanent synthetic mounting medium, and the smears were subsequently examined at a magnification of 500x. Formalin fixed material was sectioned at 8 μm, stained with hematoxylin and eosin, and examined at 100x and 500x magnification. Measurements were taken with a calibrated eyepiece micrometer and selected preparations were photographed.

RESULTS AND DISCUSSION

Twenty-two mosquito host-mosquito pathogen associations were found, comprising 9 mosquito species and possibly 17 pathogen species. Detailed collection site data are available to interested investigators.

A *Helicosporidium* sp. (Protozoa:Myxosporidea:Helicosporida) (Fig. 2) was found in *Ae. aegypei* from Chiang Mai (1), Lampang (3), Udon Thani (7), and Khon Kaen (10); in *Cx. quinquefasciatus* from Nakhon Sawan (9) and Hat Yai (20); and in both host species from Nakhon Ratchasima (12) and Bangkok (14). Advanced infections in *Cx. quinquefasciatus* resulted in swelling of the larval thorax and a lightening of color. The distended thorax became shiny. Decreased locomotory ability occurred shortly before death which usually occurred in the late 4th stage. No gross signs were detected in *Ae. aegypti* until shortly before death when decreased locomotory ability occurred. Primary site of infection was the fat body. The spherical spores from both hosts averaged 5.9 μm in diameter in live wet mounts and 5.5 μm in Giemsa stained material. Spores from both hosts appeared identical and were mutually cross infectious at comparable dosages.

Natural helicosporidan infections in mosquitoes have previously been reported by Chapman (1974) (*Cx. territans*, Louisiana, USA) and by Fukuda et al. (1976) (*Cx. nigripalpus*, Louisiana, USA). This appears to be the first report of a natural helicosporidan infection from either *Ae. aegypti* or *Cx. quinquefasciatus* and the first report of a helicosporidan from Asia. Fukuda et al. (1976) succeeded in transmitting the helicosporidan from *Cx. nigripalpus* to 14 of 17 other mosquito species exposed *per os* but noted that high dosages (1×10^6 to 1×10^8 spores/ml) were required to achieve modest infection rates. *Per os* exposure of 2nd stage *Ae. aegypti* larvae to the Thai helicosporidan has consistently resulted in IC_{50}'s of 500 to 1000 spores per ml. Morphological and biological studies and a preliminary assessment of the biological control potential of this pathogen are currently underway in this laboratory (The US Army Medical Bioengineering Research and Development Laboratory).

Three microsporidian species (Microspora:Microsporea) were found in *Ae. aegypti*. Species No. 1 (Fig. 3) was found at Chiang Mai (1), Lampang (3), Tak (5), Phitsanulok (6), Udon Thani (7), Nakhon Sawan (9), Khon Kaen (10), Phra Phutthabat (11), Nakhon Ratchasima (12), Ubon Ratchathani (13), Bangkok (14), Chanthaburi (15), Prachuap Khiri Khan (16), Ko Samui (17), Surat Thani (18), Phuket (19), and Hat Yai (20). This was the most wide-spread and prevalent pathogen found in *Ae. aegypti* in Thai-

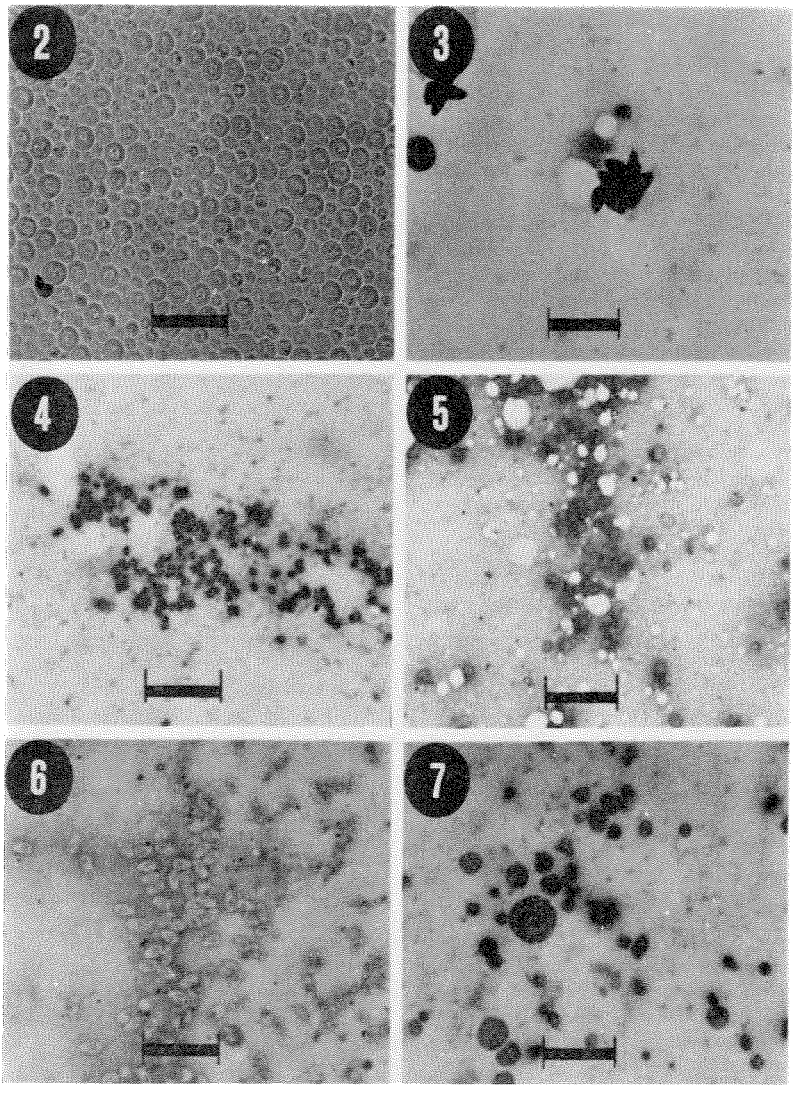

Figure 2: Water suspension of *Helicosporidium* sp. of *Aedes aegypti* and *Culex quinquefasciatus*. Bar = 19.4 μm.
Figure 3: Microsporidian species No. 1 of *Aedes aegypti* in Giemsa stained smear. Bar = 19.4 μm.
Figure 4: Microsporidian species No. 2 of *Aedes aegypti* in Giemsa stained smear. Bar = 19.4 μm.
Figure 5: Microsporidian species No. 3 of *Aedes aegypti* in Giemsa stained smear. Bar = 19.4 μm.
Figure 6: Microsporidian of *Anopheles vagus* in Giemsa stained smear. Bar = 19.4 μm.
Figure 7. Microsporidian of *Culex quinquefasciatus* in Giemsa stained smear. Bar = 19.4 μm.

land. Gross signs included stunting, prolonged development, decreased locomotory ability and, in advanced infections, conspicuous white spots on the thorax and abdomen. Primary site of infection was the fat body. Pyriform spores were most often seen loosely associated in groups of 8. However, groups of 2, 4, 6, and more than 8, but never more than 16, were common. The spores averaged 6.9 μm × 3.8 μm in Giemsa stained smears. Transmission by *per os* exposure of 2nd stage *Ae. aegypti* larvae were easily achieved with IC_{50}'s of between 500 and 1000 spores per ml. Dosages could be adjusted to result in almost total mortality in exposed groups or to result in high levels of vertical transmission through survivors. Morphological and biological studies and a preliminary assessment of biological control potential of this pathogen are currently underway in this laboratory.

Microsporidian species No. 2 of *Ae. aegypti* (Fig. 4) was found only in a few specimens from Ubon Rathathani (13) and Hat Yai (20). No gross signs of infection were detected. Primary site of infection was the midgut epithelium. The ovate spores averaged 2.9 μm × 1.8 μm in Giemsa stained smears. They were found in large numbers and were disassociated in smears. Sporoblasts in groups of 8 were seen developing in association with multinucleate sporogonic plasmodia.

Microsporidian species No. 3 of *Ae. aegypti* (Fig. 5) was found at Prachuap Khiri Khan (16). Infection with this pathogen caused no gross signs. Primary site of infection was not determined, since the pathogen was found only in smears. The ovate spores averaged 4.1 μm × 3.2 μm in Giemsa stained smears. The spores were disassociated and no associated sporogonic stages were seen.

A microsporidian was found in *An. vagus* Fig. 6) from Lamphun (2). Infected larvae had an inconspicuous mottled appearance but displayed no other gross signs. Primary site of infection was the fat body. The jug shaped spores were 5.3 μm × 3.1 μm in Giemsa stained smears. Spores in smears were not associated, but sporoblasts in groups of 8 were seen in association with multinucleate sporogonic plasmodia. Attempts to transmit this pathogen to *An. maculatus* and *An. minimus* by *per os* exposures were unsuccessful.

A microsporidian was found in *Cx. quinquefasciatus* (Fig. 7) from Mae Sot (4), Phitsanulok (6), Udon Thani (7), Nakhon Sawan (9), Phra Phutthabat (11), Nakhon Ratchasima (12), Ubon Ratchathani (13), Bangkok (14), and Hat Yai (20). Gross signs were a mottled appearance resulting from melanization of clumps of the pathogen visible through the larval integument. Primary site of infection was the fat body. The pyriform spores were 4.3 μm × 2.5 μm in Giemsa stained smears. Spores in smears were often loosely associated in groups of 8. However, groups of 2 and 4 and more than 8, but never more than 16, were also seen. Attempts to transmit this pathogen by *per os* exposure of 2nd stage *Cx. quinquefasciatus* larvae gave irregular results. Most attempts were negative or gave very low transmission rates, but 1 attempt at a dose of 1×10^4 spores per ml gave a 77% transmission rate. This pathogen possibly is the same species found in Bangkok by Chapman (1974), who thought it was *Hazardia milleri.*

One or possibly 2 species of microsporidia were found in *Cx. (Lutzia)* sp. (Fig. 8) from Sangkhlaburi (8). Only 2 infected larvae were collected. Both were conspicuously light in color. One was smeared and 1 was sectioned. Two shapes of spores were seen, both in the smear and in the fat body of the sectioned specimen. A barrel shaped spore 5.7 μm × 4.5 μm vastly predominated. It occurred in associations of 8 spores and was accompanied by a large number of pansporoblasts containing 8 sporoblasts. Also present were occasional pyriform spores, 8.0 μm × 3.7 μm, that were not associated. No developmental stages were seen that appeared to be related to the pyriform spore. However, in both the smears and the sections, the barrel-shaped spores and their pansporoblasts

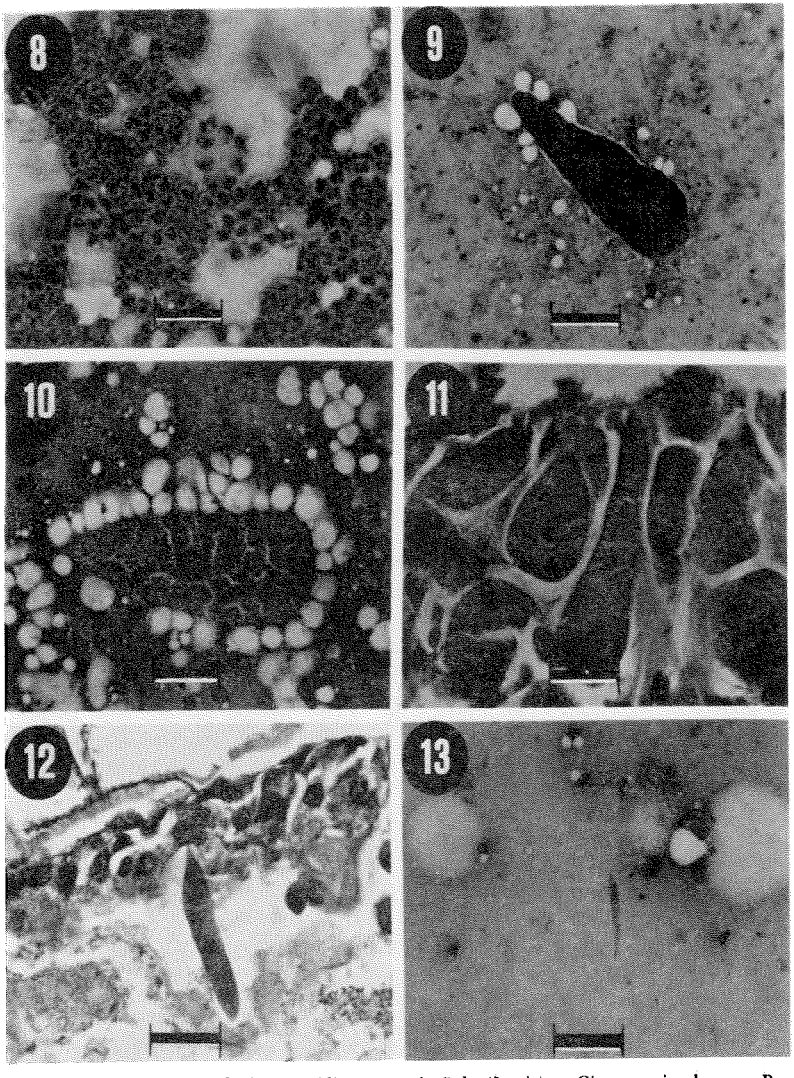

Figure 8: Two shapes of microsporidian spores in *Culex (Lutzia)* sp. Giemsa stained smear. Bar = 19.4 μm.

Figure 9: Trophozoite of acephaline eugregarine from *Aedes aegypti* Giemsa stained smear. Bar = 29.4 μm.

Figure 10: Trophozoite of acephaline eugregarine from *Aedes albopictus* Giemsa stained smear. Bar = 29.4 μm.

Figure 11: Trophozoite of acephaline eugregarine from *Aedes chrysolineatus.* Hematoxylin and eosin stained section. Bar = 29.4 μm.

Figure 12: Trophozoite of acephaline eugregarine from *Armigeres subalbatus.* Hematoxylin and eosin stained section. Bar = 29.4 μm.

Figure 13: Promastigote flagellate from *Toxorhynchites splendens.* Giemsa stained smear. Bar = 19.4 μm.

were so numerous, developmental stages for the pyriform spore might have been concealed. It could not be determined whether or not 2 pathogen species were present or 1 species which produced 2 kinds of spores. *Culex (Lutzia)* spp. are commonly predatory, but the presence of the pyriform spores in tissue precluded the possibility that the pyriform spores were consumed within a prey organism.

Trophozoites of acephaline eugregarines (Protozoa:Sporozoa:Gregarinida) were found in *Ae. aegypti* (Fig. 9) from Tak (5), Phitsanulok (6), Udon Thani (7), Sangkhlaburi (8), Phra Phutthabat (11), Nakhon Ratchasima (12), Chanthaburi (15), and Hat Yai (20); in *Ae. albopictus* (Fig. 10) and *Ae. chrysolineatus* (Fig. 11) from Sangkhlaburi (8) and in *Ar. subalbatus* (Fig. 12) from Bangkok (14). None of the gregarine infections found presented gross signs. Since only trophozoite stages were observed, the number of gregarine species represented could not be determined. However, the gregarines in *Ae. albopictus* appeared distinctive in that its cytoplasm was highly granular in Giemsa stained smears, and the gregarine in *Ar. subalbatus* appeared to be consistently smaller than those seen in other hosts.

A promastigote form of trypanosome (Fig. 13) was common in *Toxorhynchites splendens* from Bangkok (14) but infections presented no gross signs. The body of the flagellate was 17.6 μm × 1.5 μm in Giemsa stained smears. It was often present in great numbers in infected larvae, but no mortality could be related to its presence.

A *Coelomomyces* sp. was common in *Cx. minor* from Sangkhlaburi (8). Lightened color and decreased locomotory ability were gross signs. The body cavities of sectioned larvae were filled with sporangia, and fat body tissues were virtually absent. Highly rugose sporangia were 53.8 μm × 30.0 μm in Giemsa stained smears.

Septicemic infections were seen in *Ae. aegypti* from Chiang Mai (1), Lampang (3), Udon Thani (7), Khon Kaen (10), Phra Phuttabat (11), Ubon Ratchathani (13), Prachuap Khiri Khan (16), Ko Samui (17) and Hat Yai (20). An apparently identical organism caused septicemic infections in *Cx. quinquefasciatus* at all locations surveyed and in *An. vagus* and *Ar. subalbatus* in Bangkok (14). This was the most common pathogen found in *Cx. quinquefasciatus*. Infected larvae were conspicuously light colored, and larvae showing gross signs were never seen to survive past the 4th larval stage. The causative agent was a minute, gram negative, motile organism that appeared vibrioform in darkfield illumination. Attempts to culture it in various media were unsuccessful. Transmission attempts by *per os* exposures resulted in consistently less than 5% transmission. Chapman (1974) reported finding what might have been the same organism in Bangkok, Thailand, in 1971. He commented that the organism appeared to be distributed worldwide and that it had never been cultured. An epizootic involving this organism, the microsporidian and the helicosporidan in *Cx. quinquefasciatus* was observed in Hat Yai. The bottoms of shallow pools of filthy water flooding a slum area were covered with dead and dying white larvae infected with this organism and either the microsporidian, the helicosporidan or both. No quantitative observations of the eipzootic were made.

Spore-forming, gram positive bacilli were observed in sections of midgut of *Ae. aegypti* from Sangkhlaburi (8) and Ubon Ratchathani (12) and in *Ae. albopictus* and *Ar. subalbatus* from Sangkhlaburi (8). No gross signs of infection were observed, and whether or not the agent was pathogenic was not determined. The finding is included here because a new strain of *Bacillus thuringiensis* pathogenic for mosquitoes has recently been reported (de Barjac 1978).

The following reflect the prevalence of pathogens in *Ae. aegypti* and *Cx. quinquefasciatus* in Bangkok (14). *Ae. aegypti* were collected 73 times from 55 collection sites. At least 1 kind of pathogen was

found in 37 of the 73 collections and at 45 of the 55 collection sites. *Cx. quinquefasciatus* were collected 151 times at 90 collection sites. At least 1 kind of pathogen was found in 137 of the 151 collections and at 81 of the 90 collection sites.

These data indicate that mosquito populations in the tropics are a lucrative source of diverse mosquito pathogens for evaluation and possible development as biological control agents. The frequency and ease with which they can be found suggests they are an important factor in natural population control.

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