

Coelomomyces infection in mosquito larvae in Peninsular Malaysia

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Abstract

During a survey for biological control agents of mosquito larva, a variety of larval habitats, including rubber cups (2,506), artificial containers (200), coconut shells (94), bamboos (25), tins (12) and tree holes (11) were examined from all states, except Penang, in Peninsular Malaysia. The following species of mosquito larva were examined, namely *Aedes albopictus* (56,217), *Aedes aegypti* (104), *Aedes* spp. (5,378), *Armigeres* (Ar) spp. (9,924), *Armigeres* (*Leicesteria*) spp (31), *Anopheles vagus* (39), *An. subpictus* (23), *Anopheles kochi* (1), *Anopheles* spp. (21), *Culex* spp (1,367), *Tripteroides* spp (133) and *Toxorhynchites* spp (138). The fungal pathogen, *Coelomomyces*, the sporangial stage of which is capable of causing high mortality in mosquito larvae, was isolated from 228 *Ae. albopictus* and 3 *Armigeres* (Ar.) spp. All the isolates were identified as *Coelomomyces stegomyiae stegomyiae*. This species is recorded from *Ae. albopictus* from all the states surveyed and from *Armigeres* (Ar.) sp. from Selangor and Kelantan.

Key words: *Coelomomyces*, mosquito larvae, distribution

Introduction

Coelomomyces species are obligate organisms with a life cycle that includes a sporophytic phase in aquatic immatures, especially mosquito larva, and a gametophytic phase in copepod (Couch & Bland, 1985). The genus was established by Keilin (1921) for a fungal organism which he named *Coelomomyces stegomyiae*. This was recovered from a larva of *Aedes* (*stegomyia*) *albopictus* (= *Stegomyia scutellaris*) collected by Lamborn (1921) in an earthen pot from Kajang, in Selangor, Peninsular Malaysia. Subsequently, Thompson (1924) reported the occurrence of an unidentified *Coelomomyces* in dead larvae of *Anopheles*. Later, Cheong (1965) and Sulaiman & Jeffery (1987) found the ovaries of *Anopheles philippinensis* from the northern state of Perlis and *Ae. albopictus* from Selangor, with sporangia of unidentified *Coelomomyces*, respectively. In addition, the following known species have been reported from Peninsular Malaysia, namely *Coleomomyces quadrangulatus var lamborni* (Couch & Dodge, 1947) in *Ae. albopictus* from Kajang, Selangor; *Coelomomyces macleayae* (Ramalingam, 1984); *Coelomomyces reticulatus* in larvae of *Anopheles kochi* from Tampin, Negri Sembilan (Couch & Walker, 1985) and *Coelomomyces africanus* in the larvae of *Anopheles nigerrimus* and *Anopheles sinensis* from Penang (Chapman *et al.*, 1987).

This fungus is now reported from all continents except South America (Couch & Umphlett 1963). Infected larvae are brown orange to red and are usually easy to see in the field. Most often *Coelomomyces* alternates between mosquitoes and copepods or ostracods (Whisler *et al.*, 1974).

Recently, during a survey in Peninsular Malaysia to

determine the natural distribution of the ciliate, *Lambornella* (Vythilingam *et al.*, 1996), this fungal pathogen - *Coelomomyces* was found and we report here on its speciation and distribution.

Materials and Methods

Larval surveys

Larval surveys were carried out in all states of peninsula Malaysia with the exception of Penang. Surveys were carried out over a period of two years from 1992 to 1993. Each state was surveyed at different times of the year. All types of artificial containers, bamboos stumps and tree holes were examined for larvae both in rural and urban areas. Larvae from each container were collected into plastic collection bottles and brought back to the laboratory for study.

Larval collections were examined in the laboratory under a compound microscope (40X) for infections. Samples of positive larvae were processed for electron microscopic study.

Electron Microscopic Study

Samples were fixed in Karnovsky fixative (2.5% Glutaraldehyde + 2% Paraformaldehyde in 0.1M Phosphate Buffer pH 7.3) at 4° C for 5 hours. Fixed specimens were washed several times in 0.1M Phosphate Buffer and postfixed in 0.1% Osmium Tetroxide in the same buffer for another 2 hrs. It was then dehydrated in ascending concentrations of Acetone and dried in Polaron E 3000(SII) critical point drying apparatus. Specimens were mounted on aluminium stubs and coated with gold palladium in polaron E-500 sputter coater. Specimens were then examined by Cambridge 150 scanning electron microscope.

Identification of *Coelomomyces*

Identification was based on the following criteria in Couch & Bland (1985): resting sporangia ellipsoid (15-14 X 25-100 (m); surface ornamented with scattered punctate (0.5 µm in diameter and 1-5 µm apart); margins of punctae depressed and papillae interspersed among the punctae.

Results

A total of 2,848 containers from various states in Malaysia were examined and of these 60 were found to be positive for *Coelomomyces* (Table 1). *Coelomomyces* was found in all the states and Kelantan had the highest percentage positive (5.6%), followed by Pahang.

The different types of containers examined are shown in Table 2. Although a large number of rubber cups were examined, only 39 were positive (1.6%). However 36% of the tree holes examined were positive followed by 25% of the tins.

Although various species of mosquito larvae were examined, *Coelomomyces* were found only in *Aedes albopictus* and *Armigeres* spp. None of the *Anopheles* spp. were positive (Table 3).

All the isolates of *Coelomomyces* were identified as *Coelomomyces stegomyiae* var *stegomyiae* Keilin, 1921.

Table 1. Total number of *Coelomomyces* infections in mosquito larvae from field collections in different localities in peninsular Malaysia (1992-1993)

Locality	Total collections examined	Number of infections
Perak	445	4
Kedah	225	6
Selangor	718	12
Negeri Sembilan	307	5
Pahang	215	8
Trengganu	179	3
Kelantan	282	16
Malacca	261	4
Johore	264	2
Total	2,896	60

Table 2. Different containers examined and found positive for *Coelomomyces*

Type of container	Number examined	Number positive
Rubber Cup	2,506	39
Other artificial containers	200	8
Coconut shell	94	6
Tins	12	3
Bamboos	25	0
Tree holes	11	4

Table 3. Mosquito larvae identified and found positive for *Coelomomyces*

Mosquito species	Number identified	Number positive
<i>Aedes albopictus</i>	56,217	228
<i>Aedes aegypti</i>	104	0
<i>Aedes</i> spp.	5,378	0
<i>Armigeres</i> spp.	9,924	3
<i>Armigeres (Leicesteria)</i> spp.	31	0
<i>Toxorhynchites</i> spp.	138	0
<i>Tripteroides</i> spp.	133	0
<i>Culex</i> spp.	1,367	0
<i>Anopheles kochi</i>	1	0
<i>Anopheles subpictus</i>	23	0
<i>Anopheles vagus</i>	39	0
<i>Anopheles</i> spp.	21	0

Discussion

This incidental study shows that *Coelomomyces* is more adapted to various breeding habitats compared to *Lambornella stegomyiae*. Although a large number of rubber cups were examined for pathogens only 1.56% were positive for *Coelomomyces* compared to 5% positive for *L. stegomyiae* (Vythilingam *et al.*, 1996). A larger percentage of other artificial containers like tins, earthen jars and coconut shells seem more conducive for the breeding of *Coelomomyces*. Larvae from tree holes were also found to be positive for *Coelomomyces*. All the infected larvae were found with the sporangia stage of *Coelomomyces*.

In some containers there was mixed breeding of *Aedes albopictus* and *Armigeres* spp. and both species from the same container were positive for *Coelomomyces*. However, in the case of *L. stegomyiae*, it was found only in one of the species in containers that had mixed breeding (Vythilingam *et al.*, 1996).

The east coast states of Kelantan, Pahang and Terengganu had the most number of infected larvae compared to the rest of the country, while *L. stegomyiae* was found in larger numbers in the central zone of Selangor and Negeri Sembilan. We did not carry out a survey in Penang Island, but studies carried out by Chapman *et al.* (1987) showed that *Coelomomyces africanus* was found in *Anopheles nigerrimus* and *Anopheles sinensis*. However, they did not find infection in *Ae. albopictus*. *Coelomomyces stegomyiae* var. *stegomyiae* have been shown to occur in a variety of habitats in most of the states surveyed for it. This widespread distribution of *Coelomomyces* should be exploited for its potential as a natural biological control agent.

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