Demonstration of vector competence of *Culex quinquefasciatus* (Diptera: Culicidae) for *Setaria digitata*

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Abstract

In Taiwan, *Setaria digitata* infection causes a lumber paralysis in increasing number of cattle. *Culex quinquefasciatus* is one of the predominant mosquitoes, and it has been suspected that *C. quinquefasciatus* acts as a vector to *Setaria* nematodes prevalence but this was not confirmed. *C. quinquefasciatus*, *Aedes albopictus* and *A. aegypti* of various strains were investigated using an artificial infection system to evaluate their vector competence. After blood feeding at day 14, the number of larvae (stage III) per infected mosquito in *A. aegypti* (Liverpool strain), *A. aegypti* (Kaohsiung strain), *A. aegypti* (Tungan strain), *C. quinquefasciatus* (Taichung strain) and *A. albopictus* (Taichung strain) was 1.3 ± 0.1, 1.3 ± 0.1, 1.4 ± 0.1, 1.0 ± 0.0 and 0 ± 0.0 (mean ± S.E.M), respectively. The vector efficiency index of *A. aegypti* (Liverpool) was the highest among mosquitoes whereas *A. albopictus* showed a complete refractoriness to the infection. In conclusion, *C. quinquefasciatus* demonstrates its potential competence for serving as a transmission vector of *S. digitata*. This mosquito might therefore be responsible, at least in part, for the prevalence

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of cattle lumbar paralysis in Taiwan. This is the first report of *C. quinquefasciatus* demonstrating its vector competence for *S. digitata*. © 2004 Elsevier B.V. All rights reserved.

**Keywords:** Aedes aegypti; Culex quinquefasciatus; Setaria digitata; Vector efficiency index (VEI); Lumbar paralysis

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### 1. Introduction

Recently, we reported *Setaria digitata* is an important parasitic factor, which induces lumbar paralysis (LP) in cattle (Tung et al., 2003). Infected animals generally manifest various degree of malnutrition and are eventually sacrificed. Clinical cases of LP have been significantly identified during the past few years (Fei et al., 1989; Wang et al., 1991; Tung et al., 2003). In some of them, adult filarial worms of *S. digitata* and *S. marshalli* (cattle: Wang et al., 1990) and *S. cervi* (deer: Wang et al., 1988; Wang et al., 1991) were found to colonize the peritoneal surface and cavity. Recently, cerebrospinal setariosis caused by *S. digitata* (goat: Ooi et al., 1998; cattle: Tung et al., 2003) and *S. cervi* (deer: Wang et al., 1991) have also been described as increasing clinical manifestations.

Although *S. digitata* is proven to be the causative factor of LP in Taiwan (Tung et al., 2003), actual species involved in the transmission were never fully discovered. Various mosquito specimens harboring *Setaria* larvae have been recorded (Soulsby, 1982; Cancrini et al., 1997). Infective larvae are produced in the thoracic muscle within 12–16 days. For instance, *S. digitata* develops in *Aedes pembaensis*, *A. aegypti*, *Anopheles lyrcanus*, *A. togoi*, *Armigeres obturbans* and *Culex pipens*. In contrast to previous observations, experimental infections indicate that the larval development of *S. digitata* in *Armigeres subalbatus* and *A. togoi* may not be accomplished (Lee and Wang, 1991). *C. quinquefasciatus* is predominantly distributed throughout Taiwan. Epidemiologically, it has been suspected of being a vector contributed to *S. digitata* prevalence but this was not confirmed. This is the first study of *Culex quinquefasciatus* to demonstrate its vector competence for *S. digitata*.

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### 2. Materials and methods

#### 2.1. Parasite

Adult filarial worms identified as *S. digitata* were collected from the peritoneal cavity of slaughtered cattle. Microfilaria (Mff) obtained from the uterus were mixed with microfilaria-free cattle blood for fresh use.

#### 2.2. Mosquito rearing and maintenance

*A. albopictus* (Taichung strain), *A. aegypti* (Liverpool strain; Tungan and Kaohsiung strain) and *C. quinquefasciatus* (Taichung strain) were obtained from Department of
Entomology, National Chung Hsing University. All mosquitoes were kept in 15 cm 20 cm 25 cm acrylic cases, and maintained in a walk-in environmental chamber set at 27.0 ± 0.5°C, 80 ± 5% relative humidity (RH), with a 16 h light and 8 h darkness cycle photoperiod. Mosquito larvae were fed with the slurry of tropical fish food supplemented with dried pig liver powder and yeast powder. The adults gained constant access to cotton pads seeped in a 10% sucrose solution.

2.3. Exposure to the microfilarial blood meal

Six to 7-day-old female mosquitoes were starved for 15–20 h prior to blood feeding. They were infected with Mff by feeding through a mosquito membrane feeder. The feeder held cattle blood supplemented with Na2EDTA anti-coagulant. After blood meal for 30 min, individuals that had fully ingested were separated and maintained in acrylic boxes by feeding with 10% sucrose solution.

2.4. Dissection of mosquitoes

After the blood meal, 10 mosquitoes were immediately dissected with insect pin under a dissection microscope. Midgut contents were smeared onto a slide glass and the numbers of ingested Mff were recorded. The rest of infected mosquitoes were dissected after 14 days to observe the development of third-stage larvae (L3). The L3 infection rate was calculated by dividing the number of infected mosquitoes by the number of dissected mosquitoes. A vector efficiency index (VEI) (Kartman, 1954) was calculated for each species by dividing the mean number of L3 by the mean number of ingested Mff and then converting it into a percentage.

3. Results

Immediately after blood feeding, the number of Mff were 49.9 ± 6.9 (mean ± S.E.M, n = 10) in A. aegypti (Liverpool strain), 40.8 ± 7.9 in A. aegypti (Kaohsiung strain), 39.0 ± 7.2 in A. aegypti (Tungan strain), 36.0 ± 2.9 in A. albopictus (Taihng strain) and 29.0 ± 3.4 in C. quinquefasciatus (Taihng strain), respectively.

The L3 infection rate was 48.0% (61/127) in A. aegypti (Liverpool strain), 30.4% (38/125) in A. aegypti (Kaohsiung strain), 25.5% (40/157) in A. aegypti (Tungan strain) and 24.2% (23/95) in C. quinquefasciatus (Taihng strain), respectively. There was no trace of L3 found in A. albopictus (Taihng strain). The mean ± S.E.M of L3 per infected mosquito in A. albopictus (Taihng strain), A. aegypti (Kaohsiung strain), A. aegypti (Tungan strain), C. quinquefasciatus (Taihng strain) and A. albopictus (Taihng strain) was 1.3 ± 0.1, 1.3 ± 0.1, 1.4 ± 0.1, 1.0 ± 0.0 and 0 ± 0.0, respectively.

The VEI in A. aegypti (Liverpool strain) was 1.4 and 1.0 in A. aegypti (Kaohsiung strain), 0.8 in A. aegypti (Tungan strain), 0.7 in C. quinquefasciatus (Taihng strain) and 0 in A. albopictus (Taihng strain), respectively (Table 1).
The relatively high prevalence of *S. digitata* infection has been reported in slaughtered Holstein–Friesian cattle (46.2%, Taichung), buffalo (22.6%) and local yellow cattle (56.4%, Wang et al., 1990). In addition, Fei et al. (1989) documented that 8.5% (44/518) of cattle examined in 13 counties of Taiwan were positive for *S. digitata* microfilaria. Recently, we discovered the first case of bovine cerebrospinal setariosis that caused LP (Tung et al., 2003). These incidences generate an important issue regarding vector control efficiency. As far as the host range is concerned, these mosquito species investigated commonly feed on cattle, buffalo, sheep, goats and horses (Soulsby, 1982). The present study has clearly demonstrated for the first time that larvae III (L3) develops in *C. quinquefasciatus*, indicating its vector capacity for *S. digitata* transmission, whereas *A. albopictus* showed a complete refractoriness to the infection. The hypothesis of a possible contribution of *C. quinquefasciatus* to *S. digitata* infection is thus supported.

The developing stage of Mff to L3 on day 14 after blood meal is different among mosquitoes. Similar to the observation of Lee and Wang (1991), *S. digitata* did not develop to infective stage (L3) in *A. albopictus*. This development, however, succeeds in other *A. aegypti* local strains. Thus, *A. albopictus* might act as a refractory vector in which many of the microfilariae may become extracellularly encapsulated and melanized soon after they reach the hemocoel following ingestion (Nayar et al., 1995). Humoral encapsulation is the most commonly seen vector defence reaction that involves initially the deposition of pigmented material on the surface of microfilarial sheaths. Subsequently, hemocytes adhere to the pigmented layer, resulting in loss of microfilarial integrity (Chikilian et al., 1994). Actually, such microfilarial melanization was observed in the stomach of some (3/122) *A. albopictus* on day 14. Between days 5 and 9, there was no trace of larvae stage L1 or L2 in thoracic muscle, meaning that insect defence probably eliminate microfilariae prior to their further development. In addition to the extracellular response, it has been reported that intracellular melanization could interfere with developing process of L1 of *Brugia*sp. in *Anopheles quadrimaculatus* (Nayar et al., 1989).

### Table 1

The development of microfilariae (Mff) of *Setaria digitata*, the infection rate and the vector efficiency indices (VEI) in various mosquito species during experimental infections of *S. digitata* filaria

<table>
<thead>
<tr>
<th>Mosquitoes</th>
<th>Strains</th>
<th>Mff(^a)</th>
<th>L3(^b) %(^d)</th>
<th>No(^e)</th>
<th>VEI(^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Liverpool</td>
<td>43.6 ± 6.6</td>
<td>48.0 (61/127)</td>
<td>1.3 ± 0.1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Kaohsiung</td>
<td>40.8 ± 7.9</td>
<td>30.4 (38/125)</td>
<td>1.3 ± 0.1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Tungan</td>
<td>39.0 ± 7.2</td>
<td>25.5 (40/157)</td>
<td>1.4 ± 0.1</td>
<td>0.8</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>Taichung</td>
<td>36.0 ± 2.9</td>
<td>0 (0/122)</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>Taichung</td>
<td>29.0 ± 3.4</td>
<td>24.2 (23/95)</td>
<td>1.0 ± 0.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>

\(^a\) Mean (±S.E.M) of microfilariae ingested in the midgut of 10 mosquitoes soon after blood meal.

\(^b\) L3 = third-stage larvae developed at day 14 after blood meal.

\(^c\) Vector efficiency index (VEI, %) = number of L3 × 100/number of ingested microfilariae.

\(^d\) % = infection rate (number of infected mosquitoes/number of total dissected mosquitoes).

\(^e\) No = mean (±S.E.M) of L3 per infected mosquito at day 14 after blood meal.

\(^f\) Vector efficiency index (VEI, %)

### 4. Discussion

The relatively high prevalence of *S. digitata* infection has been reported in slaughtered Holstein–Friesian cattle (46.2%, Taichung), buffalo (22.6%) and local yellow cattle (56.4%, Wang et al., 1990). In addition, Fei et al. (1989) documented that 8.5% (44/518) of cattle examined in 13 counties of Taiwan were positive for *S. digitata* microfilaria. Recently, we discovered the first case of bovine cerebrospinal setariosis that caused LP (Tung et al., 2003). These incidences generate an important issue regarding vector control efficiency. As far as the host range is concerned, these mosquito species investigated commonly feed on cattle, buffalo, sheep, goats and horses (Soulsby, 1982). The present study has clearly demonstrated for the first time that larvae III (L3) develops in *C. quinquefasciatus*, indicating its vector capacity for *S. digitata* transmission, whereas *A. albopictus* showed a complete refractoriness to the infection. The hypothesis of a possible contribution of *C. quinquefasciatus* to *S. digitata* infection is thus supported.

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The observed VEI in *A. aegypti* indicates this typical mosquito species found here is the most efficient vector. Liverpool strain serves the greatest competence as compared to Tungan and Kaohsiung local strains. Although such a high index is not seen in *C. quinquefasciatus*, its VEI still reveals that this species could function as a potential transmission vector. Comparisons of these two mosquito genera, Cancrini et al. (1997) also showed lower susceptibility and vector efficiency of *Setaria labiatopapillosa* in *C. pipiens* as compared to *A. caspius* and *A. vexans*. Interestingly, Taichung strain of *A. albopictus* lacks vector competence. This might be explained by penetrating capacity of Mff through muscular barriers (Rodriguez, 1973). Relevant mechanisms remain to be clarified. Thus, *A. albopictus* should not account for the spreading of *S. digitata*.

Knowing and identifying the vector is an important process in the parasite and vector control programs. The epidemic survey of setariosis ought to include the factor of mosquito activity patterns throughout the day. Learning from this study, *C. quinquefasciatus* and *A. aegypti* are the potential suspected vectors of *S. digitata*. It is known that *A. aegypti* is diurnally active and *C. quinquefasciatus* is active nocturnally. Geographically, *A. aegypti* are distributed in the southern regions of Taiwan whereas *C. quinquefasciatus* highly populates around the whole island. Therefore, the increasing incidence of cattle with cerebrospinal setariosis is likely associated with the high distribution level of *C. quinquefasciatus* in the area. In conclusion, *C. quinquefasciatus* demonstrates its potential competence for serving as transmission vector for *S. digitata* and this species might therefore account for, at least in part, the prevalence of cattle lumbar paralysis in Taiwan.

References


