Competence of *Aedes albopictus* and *Culex quinquefasciatus* as vector of *Dirofilaria immitis* after blood meal with different microfilarial density

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Abstract

*Aedes albopictus* and *Culex quinquefasciatus* were fed canine blood with different microfilarial density of *Dirofilaria immitis* ranging from 2500 to 25,000 mff/ml. Larval development in these two mosquito species did not differ significantly. Although *C. quinquefasciatus* ingested more microfilariae, the number of larvae which developed in *A. albopictus* was invariably greater than in *C. quinquefasciatus*. Mortality of the engorged *A. albopictus* was significantly greater than that of *C. quinquefasciatus*, and higher microfilarial density raised the mortality in both species. The vector efficiency index of *A. albopictus* was greater than *C. quinquefasciatus* at all microfilarial densities, but its survival time was much reduced. Thus, dogs with low microfilarial density are implicated as the main source for the transmission of *D. immitis* from dogs to mosquitoes. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Dirofilaria immitis*; Mosquitoes; *Aedes albopictus*; *Culex quinquefasciatus*; Blood microfilarial density

1. Introduction

*Dirofilaria immitis*, a nematode which is transmitted by mosquitoes, is widespread among dogs, cats and wild canines in many countries. Infected animals present a variety of symptoms, ranging from chronic cough to progressive endoarteritis and edema of the lungs and liver. Its prevalence among dogs in Taiwan increases every year, and 16.8–55% of dogs are reported to be infected (Chen et al., 1982; Wu et al., 1988; Wang, 1997). Only one human case of dirofilariosis has been reported in Taiwan (Yang et al., 1993). The number of cases...
reported is thought to understate the actual incidence because generally human dirofilariosis is asymptomatic.

As mosquitoes cannot be altogether eradicated, and the infected stray dogs are not effectively controlled in Taiwan, the prevalence of *D. immitis* in dogs and mosquitoes will continue to increase in the near future. Factors that affect the transmission of *D. immitis* include mosquito population density, mosquito species, mosquito fecundity and environmental temperature (Ludlam et al., 1970). The effect of mosquito species and environmental temperature on the spread of *D. immitis* had received considerable attention (Kutz and Dobson, 1974; Christensen and Holland, 1978; Russell, 1990). However, information on the influence of microfilarial density on the transmission of *D. immitis* remains sparse. Therefore, in this study we used *Aedes albopictus* and *Culex quinquefasciatus*, the potential vectors of *D. immitis* in central Taiwan, to investigate the competence of these mosquitoes in the transmission of *D. immitis* after engorging blood with different microfilarial density.

2. Materials and methods

2.1. Parasite

*D. immitis* microfilariae were obtained from six naturally infected dogs which were maintained in the Department of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan. These dogs were fed adult dog food pellet (Fwusow Industry Co. Ltd., Taichung) and given water ad libitum.

2.2. Mosquito rearing and maintenance

*A. albopictus* and *C. quinquefasciatus* were obtained from Dr. Chin-Chang Yeh, 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 26.5 ± 0.5°C, 80 ± 5% relative humidity (RH), with a 16 h light, 8 h darkness cycle photoperiod. Mosquito larvae were fed a slurry of tropical fish food, while adults had constant access to cotton pads seeped in a 10% sucrose solution.

2.3. Exposure to the microfilarial blood meal

Approximately 2000 5–7-day-old female mosquitoes were used in each experiment. These mosquitoes were starved for 15–20 h prior to blood feeding, and then infected with *D. immitis* microfilariae by feeding through a mosquito membrane feeder holding canine blood that contained anticoagulant. The microfilarial density ranged from 2500 to 25,000 mff/ml blood. After being exposed to the microfilarial blood meal for 30 min, individuals that had ingested a full blood meal were separated and maintained in acrylic boxes by feeding with 10% sucrose solution. Mosquitoes fed with canine blood that did not contain microfilaria were used as a negative control.
2.4. Dissection of mosquitoes

After being fed canine blood containing different microfilarial densities, 15 mosquitoes were dissected with insect pin under a dissection microscope. Their midgut contents were smeared onto a slide glass, and the number of ingested microfilariae counted. Aliquot group of the surviving mosquitoes were killed daily to observe the filarial development. Two hundred mosquitoes randomly selected from the remaining mosquitoes were immobilized in a test tube immersed in ice water and dissected 15 days after the experimental infection. Each mosquito was dissected in one drop of saline solution on a clean microscopic slide and examined under dissecting microscope for filarial larvae. A record was made of the infection rate, the average number of larvae per mosquito and the mortality of the mosquitoes following the blood meal. The vector efficiency index (VEI) (Kartman, 1954) was calculated for each species by dividing the mean number of third-stage larvae by the mean number of microfilaria ingested and converting it to a percentage. Duncan’s multiple range test was used to compare the difference between the two species.

3. Results

*C. quinquefasciatus* ingested a mean of 15.33 and 68.33 microfilariae from blood with the lowest and highest microfilarial density, respectively. *A. albopictus* ingested an average of 12.40 and 46.20 microfilariae from the blood with the lowest and highest microfilarial density, respectively (Table 1). The two species did not differ significantly in terms of the development of *D. immitis* larvae. Third-stage larvae were first found on Day 9 in *A. albopictus* and on Day 10 in *C. quinquefasciatus* after the blood meal. Although *C. quinquefasciatus* ingested more microfilariae, the number of larvae which developed in *A. albopictus* was always greater than *C. quinquefasciatus*.

Although increased microfilarial density resulted in raised mortality in both species, mortality of *A. albopictus* was significantly higher than that of *C. quinquefasciatus*. In *C. quinquefasciatus*, 51.6% of mosquitoes were still alive 15 days post feeding on the blood.

<table>
<thead>
<tr>
<th>Microfilarial density</th>
<th><em>Aedes albopictus</em></th>
<th><em>Culex quinquefasciatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean no. of L₃ b</td>
<td>Vector efficiency index (VEI)</td>
</tr>
<tr>
<td>2500</td>
<td>12.40±2.09</td>
<td>15.33±3.36</td>
</tr>
<tr>
<td>5000</td>
<td>16.33±2.70</td>
<td>22.53±4.16</td>
</tr>
<tr>
<td>10000</td>
<td>22.60±3.61</td>
<td>31.73±3.23</td>
</tr>
<tr>
<td>15000</td>
<td>29.53±4.42</td>
<td>43.20±4.02</td>
</tr>
<tr>
<td>20000</td>
<td>38.73±5.28</td>
<td>56.93±4.31</td>
</tr>
<tr>
<td>25000</td>
<td>46.20±4.56</td>
<td>68.33±5.24</td>
</tr>
</tbody>
</table>

*VEI* = mean number of third-stage larvae in mosquitoes at Day 15 after blood meal.

*VEI* = vector efficiency index (%): No. of L₃ × 100/No. of ingested MI.

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Table 1

Vector efficiency indices of *Aedes albopictus* and *Culex quinquefasciatus* at different microfilarial densities.
Fig. 1. The survival of *Culex quinquefasciatus* (top) and *Aedes albopictus* (bottom) after a blood meal at different microfilarial densities. For each group, 2000 mosquitoes were used and their survival rate were converted to percentage with respect to the initial number.

with the lowest microfilarial density, while this was reduced to 28.4% for those that fed on blood with the highest microfilarial density. However, only 23.6% of *A. albopictus* survived for the duration of the experiment after feeding on blood with the lowest microfilarial density, while in those which fed on blood with the highest density group, the percentage of live mosquito fell to just 11.3% (Fig. 1).

**Table 2**
The infection rate of *Aedes albopictus* and *Culex quinquefasciatus* at Day 15 after being fed canine blood with different microfilarial densities

<table>
<thead>
<tr>
<th>Microfilarial density</th>
<th><em>Aedes albopictus</em></th>
<th><em>Culex quinquefasciatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. dissected</td>
<td>No. infected</td>
</tr>
<tr>
<td>2500</td>
<td>200</td>
<td>38</td>
</tr>
<tr>
<td>5000</td>
<td>200</td>
<td>39</td>
</tr>
<tr>
<td>10000</td>
<td>200</td>
<td>41</td>
</tr>
<tr>
<td>15000</td>
<td>200</td>
<td>40</td>
</tr>
<tr>
<td>20000</td>
<td>200</td>
<td>41</td>
</tr>
<tr>
<td>25000</td>
<td>200</td>
<td>43</td>
</tr>
</tbody>
</table>
The infection rate of *C. quinquefasciatus* was higher than *A. albopictus*, but there was no obvious difference among different microfilarial densities. *C. quinquefasciatus* feeding on blood with the lowest microfilarial density showed 29.5% of infection with *D. immitis*, while those feeding on the highest microfilarial density showed 32.5% infection. Meanwhile the lowest and highest densities of microfilariae used for the blood meal of *A. albopictus* showed only 19.0 and 21.5% infection, respectively (Table 2).

Vector efficiency indices were calculated at each microfilarial density. The VEI of *A. albopictus* was greater than that of *C. quinquefasciatus* for all microfilarial densities, and blood with high microfilarial density reduced the VEI in both species (Table 1).

4. Discussion

The first record of dirofilariosis in Taiwanese dogs was by Hsieh and Chuang (1956). Before that, a considerable number of dogs had been imported from the US and Japan where canine heartworm infection were known to be endemic. A survey of domestic dogs in central Taiwan demonstrated that 16.8% were microfilaremic (Yang and Hsieh, 1987), while post-mortem examination of stray dogs from northern Taiwan showed 24.8% to be infected (Wu et al., 1988). Recent survey by Wang (1997) showed infection rate as high as 55%. These results indicate that *D. immitis* infection in dogs is shifting from endemic to being epizootic in Taiwan.

Female mosquitoes transmit heartworm infection, serving as the intermediate host after ingesting a blood meal from an infected dog. Approximately 60 species of mosquitoes have been shown to support complete larval development of *D. immitis* in the laboratory (Ludlam et al., 1970). Wu et al. (1997) reported that *C. quinquefasciatus* is the most important vector of *D. immitis* in northern Taiwan. We observed that *A. albopictus* and *C. quinquefasciatus* were the potential vectors of *D. immitis* in central Taiwan. Previous reports showed that although *Culex annulirostris* ingested more *D. immitis* microfilariae than *Aedes notoscriptus*, many more third-stage larvae could be recovered from the latter species (Russell and Geary, 1992). Our results also showed that although *C. quinquefasciatus* ingested more microfilariae than *A. albopictus*, more third-stage larvae could be found in *A. albopictus*.

Although the mosquitoes ingested more microfilariae when the blood microfilarial density was higher, the infection rate and the number of third-stage larvae did not increase relative to the microfilarial density. There was no significant difference in the average number of third-stage larvae in each density, indicating that only a limited number of larvae could develop within the mosquitoes. The immune responses of mosquitoes, such as melanization and encapsulation, probably killed most of the microfilariae (Christensen and Sutherland, 1984; Bradley and Nayar, 1985; Chen and Chen, 1995). After a blood meal, the mortality of *A. albopictus* was greater than that of *C. quinquefasciatus* for all microfilarial densities. However, higher microfilarial density used for feeding induced higher mortality in both mosquito species. The vector efficiency index, as defined by the percentage of development to third stage larvae of the ingested microfilariae, was higher in *A. albopictus* than *C. quinquefasciatus*. However, the higher mortality of *A. albopictus* after infection and its lower population density in Taiwan probably limit its efficiency as a vector as compared to *C. quinquefasciatus*. Therefore, besides *A. albopictus*, *C. quinquefasciatus* may also serve
as an important vector for *D. immitis* in central Taiwan. Further study on the actual parasite transmission will be needed to determine which mosquito species is more important as a vector.

In mosquitoes, the Malpighian tubules play a central role in excretion and regulation of ions and water in the haemolymph (Bradley and Nayar, 1984; Pannabecker, 1995). When mosquitoes are infected with *D. immitis*, the ingested microfilariae migrate from the midgut to the primary cells of the Malpighian tubules where they become intracellular (Bradley et al., 1984). The developing larvae destroy the primary cells and disrupt the excretion and regulation of water and ions of mosquitoes. A small number of parasites may not adversely affect the mosquitoes’ excretory process, but large worm burdens increase the possibility of destroying the excretory system (Palmer et al., 1986). Thus, the mortality of the infected mosquitoes will obviously increase after feeding on blood with a high microfilarial density. It is thought that an individual mosquito can support only a limited number of larvae and too many larvae will destroy the Malpighian tubules resulting in the death of the mosquito.

Since Taiwan is situated in a subtropical zone, its hot and humid environment is conducive for the mosquitoes’ growth and reproduction. Moreover, stray dogs are not effectively controlled, and their number increases every year. The high prevalence of *D. immitis* infection among these stray dogs will definitely increase the chances of mosquitoes being infected with *D. immitis* after a blood meal. Our results suggest that low microfilarial density among the infected dogs might be a major factor in contributing to the transmission of *D. immitis* from dogs to the mosquito vectors.

References


