EFFECT OF DIROFILARIA IMMITIS
(NEMATODA: FILARIOIDEA)
INFECTION ON RATE OF DIURESIS IN SUSCEPTIBLE AND
REFRACTORY STRAINS OF AEDES AEGYPTI
(VERO BEACH) (DIPTERA: CULICIDAE)

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ABSTRACT

The effect of Dirofilaria immitis (Leidy) infection on the rate of diuresis was investigated for infected and uninfected blood-fed females of highly susceptible and refractory strains of Aedes aegypti L. (Vero Beach). The pattern and course of diuresis were similar for both infected and uninfected females of both strains. Infection of Malpighian tubules reduced the rate of diuresis during the peak phase for infected females as compared to that for the corresponding uninfected females. There were no differences between strains as to the rate of diuresis in infected females. Infected susceptible females showed a strong negative linear correlation between the number of developing D. immitis and the total amount of fluid excreted during the 90-min observation period, whereas in infected refractory females there was no correlation between the presence of moribund prelarvae and the total amount of fluid excretion. These results suggested that the lower rate of diuresis in both infected susceptible and refractory females was related to damage caused by the developing first stage larvae and moribund prelarvae, respectively.

RESUMEN

Se investigó el efecto de infección de Dirofilaria immitis (Leidy) en la tasa de diuresis de hembras infectadas y no infectadas de razas refractarias de Aedes aegypti L. (Vero Beach), que se alimentaron con sangre. El patrón y dirección de diuresis fue similar en hembras infectadas y no infectadas de las dos razas. La infección de los tubos de Malpígo redujo la tasa de diuresis durante la fase del auge de las hembras infectadas cuando se comparó con las correspondientes hembras no infectadas. No hubo diferencia entre las razas con respecto a la tasa de diuresis en las hembras infectadas. Hembras susceptibles infectadas demostraron una fuerte correlación lineal negativa entre el número de D. immitis en desarrollo y la cantidad total de fluido excretado durante los 90 minutos del período de observación, mientras que en hembras infectadas refractarias no hubo correlación entre la presencia de pre-larvas moribundas y la cantidad total de fluido excretado. Estos resultados sugieren que la baja tasa de diuresis en hembras susceptibles infectadas y en hembras refractarias, está relacionada al daño causado por el desarrollo de la primera etapa de larvas y de larvas pre-moribundas respectivamente.

Soon after female mosquitoes ingest a blood meal, there is rapid excretion of clear urine involving the Malpighian tubules, called diuresis. In species of mosquitoes studied thus far, the process of diuresis follows three phases: the peak phase of very rapid urine elimination during the first 10 minutes, the post-peak phase with a declining rate of elimination during the next 40 min. and the late phase of fairly constant elimination during the last 40 minutes of the 90-minute period (Boorman 1960, Nijhout & Carrow 1978, Williams et al. 1983, Nayar & Bradley 1987). Ingested microfilariae of Dirofilaria
immittis (Leidy) develop intracellularly through two molts to the infective stage in the Malpighian tubules of susceptible mosquitoes (Kartman 1953, Taylor 1960, Nayar & Sauerman 1975). Nayar & Bradley (1987) showed that, in Aedes taeniorhynchus (Wiedmann) infected with D. immittis, the rate of diuresis was significantly reduced during the peak phase of diuresis compared with that of uninfected females. Even greater reduction in secretion rates during peak and post-peak phases of diuresis were observed in infected Anopheles quadrimaculatus Say.

We have isolated highly susceptible and highly refractory strains of Aedes aegypti L. (Vero Beach) to D. immittis infection by the individual sibling mating method of McGeevy et al. (1974). In the highly susceptible strain, D. immittis larvae develop normally. The first stage of larval development occurs intracellularly in the primary cells of the Malpighian tubules and the subsequent two stages develop in the lumen (Taylor 1960). On the contrary, in the highly refractory strain the microfilariae become arrested after entering the primary cells of the Malpighian tubules and remain as moribund prelarvae (Nayar & Sauerman 1975). About 50% of the infected females of both strains die during the first 6 days after an infective blood meal (Mahmood & Nayar 1989). Among the remaining females of the susceptible strain, most of the Malpighian tubule cells are damaged due to the development of the first stage larvae, whereas in the refractory strain the proximal and distal cells of the Malpighian tubules are damaged due to the constant movement of moribund prelarvae (Nayar & Sauerman 1975). The present study was designed to investigate the effect of this damage on the process of diuresis in D. immittis infected susceptible and refractory females and to compare this process with uninfected females.

**Materials and Methods**

Highly susceptible and refractory strains of Ae. aegypti (Vero Beach) used in this study were reared and maintained as described elsewhere (Mahmood & Nayar 1989). All experiments were conducted at 26 ± 1°C under a 12:12 (L:D) photoperiod and at RH 75%.

Experimental protocol, measurements of diuresis in uninfected controls and D. immittis infected females of both susceptible and refractory strains, and the rate of infection in infected females were as described by Nayar & Bradley (1987). Diuresis was quantified after the method of Nijhout & Carrow (1978) and modified after the method of Stobart (1977) by monitoring the loss of weight of blood-fed mosquitoes. Female mosquitoes maintained on a 10% sucrose solution, were given their first blood meal to repletion on an infected dog (peripheral blood count 25 ± 5 microfilariae/μl) or (control) on a chicken 4-5 days after emergence. Blood-fed females from both groups were then maintained on a 10% sucrose solution and allowed to lay all their eggs. At 5-7 days after the first blood meal, females from both groups were individually given a second blood meal to repletion on a chicken. Diuresis in these mosquitoes (20 females per group) was measured starting immediately after the second blood meal. After measurement of diuresis, the Malpighian tubules of infected females were dissected and the number of developing larvae was assessed. Rates of weight loss at different times after the infective blood meal were compared using Student's t-test and the total amount of weight loss (diuresis) during the 90 min in relation to parasite burden were compared using linear regression analysis.

**Results**

The uninfected susceptible females of Ae. aegypti took significantly larger second blood meals and showed greater weight loss during 90 min. duration of diuresis as
compared to infected susceptible females (Table 1). There was not a significant weight loss after the second blood meal in refractory uninfected and infected females, the weight loss for the uninfected was greater (3.93 ± 1.12) than for the infected (3.58 ± 1.26) females (Table 1). The pattern and time-course of diuresis were similar in both groups of both strains (Fig. 1). In all four groups, there were significant differences (P < 0.05, n = 40) in weight loss during the peak phase (0-15 min), and post-peak phase (16-45 min) after feeding (Fig. 1), with the more rapid weight loss occurring within the peak phase (Fig. 1). Infected susceptible and refractory females showed slower rates of diuresis than uninfected susceptible and refractory females (Fig. 1). The rate of diuresis was faster in uninfected susceptible females than in uninfected refractory females, but there were no differences in the rate of diuresis in infected susceptible and refractory females (Fig. 1).

During the initial infection of susceptible and refractory females with *D. immitis* (susceptible females ingested 261.4 ± 58.4, x ± sd and refractory females ingested 376.0 ± 87.7) there was no significant difference (P > 0.05) in the number of microfilariae ingested; however, there were significant differences (P < 0.05) in the number of developing first stage larvae, 5-6 days after initial infection, in the susceptible females (30.0 ± 27.5) and the number of moribund prelarvae (11.5 ± 9.47) in the refractory females. The rate of weight loss was smaller in susceptible females with a greater number of developing first stage larvae of *D. immitis* and showed a correlation coefficient (r) of -0.38 (Fig. 2a), whereas in refractory females there was no correlation between the number of moribund prelarvae and the rate of weight loss (Fig. 2b), the correlation coefficient (r) was -0.15.

**DISCUSSION**

These studies showed that *D. immitis* infected refractory and susceptible *Ae. aegypti* females had identical patterns of diuresis and their rates of diuresis were significantly reduced as compared to those for uninfected females of both strains. These lower rates of diuresis in infected *Ae. aegypti* females were similar to those observed in *D. immitis* infected *Ae. taeniorhynchus* and *An. quadrmaculatus* (Nayar & Bradley

**TABLE 1. DIFFERENT CHARACTERISTICS OF SUSCEPTIBLE AND REFRATORY FEMALES OF Aedes aegypti (VERO BEACH) INFECTED WITH DIROFILARIA IMMITIS DURING DIURESIS. TWENTY FEMALES WERE USED FOR EACH GROUP.**

<table>
<thead>
<tr>
<th></th>
<th>Uninfected</th>
<th>Infected</th>
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<tbody>
<tr>
<td><strong>Susceptible</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mosquito wet body weight (mg)</td>
<td>2.80 ± 0.31^2</td>
<td>2.73 ± 0.27</td>
</tr>
<tr>
<td>Weight of blood meal (mg)</td>
<td>4.56 ± 1.34^1</td>
<td>3.33 ± 0.87</td>
</tr>
<tr>
<td>Weight loss during diuresis (mg)</td>
<td>2.42 ± 0.74^1</td>
<td>1.56 ± 0.62</td>
</tr>
<tr>
<td>Weight loss percent of blood meal</td>
<td>54.20 ± 14.86^1</td>
<td>44.06 ± 14.87</td>
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<tr>
<td><strong>Refractory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mosquito wet body weight (mg)</td>
<td>2.31 ± 0.48</td>
<td>2.41 ± 0.50</td>
</tr>
<tr>
<td>Weight of blood meal (mg)</td>
<td>3.39 ± 1.12</td>
<td>3.56 ± 1.26</td>
</tr>
<tr>
<td>Weight loss during diuresis (mg)</td>
<td>1.94 ± 0.42</td>
<td>1.46 ± 0.57</td>
</tr>
<tr>
<td>Weight loss percent of blood meal</td>
<td>52.57 ± 15.65^1</td>
<td>41.75 ± 2.07</td>
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^1Comparison of uninfected and infected mosquitoes after second blood meal. Mean significantly greater when tested by Student's t-test (P < 0.05).

^2Comparison of susceptible and refractory mosquitoes.
Fig. 1. Time-course of diuresis (mean total weight loss) in females after a blood meal on chicken by uninfected (—) and infected (—) *Aedes aegypti* susceptible (●) and refractory (○) to *Dirofilaria immitis*.

1987). For infected susceptible *Ae. aegypti*, lower rates of diuresis were observed as the number of developing first stage larvae increased. Similar results were recorded for infected *Ae. taeniorhynchus* and *An. quadrimaculatus* (Nayar & Bradley 1987). However, in infected refractory *Ae. aegypti* females the slower rate of diuresis observed could not be related to the number of moribund prelarvae. The impaired rate of diuresis in infected mosquitoes could be due to the damage caused to Malpighian tubules by moribund prelarvae in refractory females and developing first stage larvae in the susceptible females. In *Ae. taeniorhynchus* infected with *D. immitis* prelarvae, the ultra-structure of the Malpighian tubule cells 48 h after the infective blood meal showed significant reduction in microvillar volume, in the percent of microvillar volume occupied by mitochondria and in volume of mitochondria within the microvilli when compared to the uninfected Malpighian tubule cells (Bradley et al. 1984). Palmer et al. (1986) con-
Fig. 2a. Effect of the increasing number of developing larvae of *Dirofilaria immitis* on the total loss in weight during diuresis in infected susceptible female of *Aedes aegypti*.

Fig. 2b. Effect of the increasing number of arrested prelarvae on the total loss in weight during diuresis in refractory *Aedes aegypti*.
firmed these findings in Ae. aegypti infected with D. immitis, and further showed, that when first stage larvae were ready to molt 6 days after the infective blood meal, the cytoplasmic ground substance was highly disrupted and the cells appeared to be greatly inflated. These authors further concluded that, during the development of D. immitis larvae through the first stage, they completely destroy the Malpighian tubule cells in which they reside and after molting, the second stage larvae move to the lumen of the Malpighian tubules. They also suggested that large worm burdens could be responsible for the destruction of the excretory system and vector mortality.

In another study, Bradley & Nayar (1984) examined the rate of fluid excretion, in vitro, using both uninfected and infected D. immitis Malpighian tubules of Ae. taeniorhynchus and demonstrated that the tubules showed a decline in transport with time following infection and the reduction in transport capacity was proportional to the number of D. immitis larvae infecting the Malpighian tubules. A similar phenomenon could be occurring in the infected susceptible A. aegypti females. In infected refractory Ae. aegypti females, damage to several Malpighian tubule cells inhabited by moribund prelarvae was previously observed (Nayar & Sauerman 1975) and probably is the cause of the reduced rate of diuresis seen in this study.

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EFFECTIVENESS OF BACILLUS THURINGIENSIS SEROVAR. ISRAELENSIS (VECTOBAC 12 AS) AND BACILLUS SPHAERICUS 2362 (ABG-6232) AGAINST CULEX SPP. MOSQUITOES IN A DAIRY LAGOON IN CENTRAL FLORIDA

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ABSTRACT

A wettable powder (WP) formulation of Bacillus sphaericus 2362 (ABG-6232) and an aqueous suspension of Bacillus thuringiensis serovar. israelensis (Vectobac 12 AS) were evaluated against Culex mosquitoes in a dairy wastewater lagoon in central Florida. Culex nigripalpus and Cx. quinquefasciatus inhabited the lagoon; the former species comprised >90% of the total Culex larvae collected during the sampling periods. Vectobac 12 AS (at 1.17 L/ha) and ABG-6232 (at 1.12 kg/ha) were each applied separately to the lagoon on three different occasions during 1987-1988. Vectobac 12 AS caused a maximum 71-88% larval reduction for only one day posttreatment in the three treatments. ABG-6232 (WP) gave an average larval reduction of 84-92% in the three tests for up to 13 days posttreatment with >50% average reduction of the larvae being maintained for beyond 17 days posttreatment.

RESUMEN

Se evaluó una formulación de polvo humectante de Bacillus sphaericus 2362 (ABG-6232) y una suspensión acuosa de Bacillus thuringiensis serovar. israelensis (Vectobac 12 AS) contra mosquitos Culex en una laguna de agua de desperdicio de una lechería en el centro de la Florida. Culex nigripalpus y Cx. quinquefasciatus habitan la laguna; la primera especie constituía >90% del total de larvas de Culex colectadas durante el período de muestreo. Vectobac 12 AS (a 1.17 L/ha) y ABG-6232 (a 1.12 kg/ha) fueron separadamente aplicados a la laguna en 3 ocasiones diferentes durante 1987-1988. Vectobac 12 AS causó una reducción máxima de larvas de 71-88% solo un día después...