POOR VECTOR EFFICIENCY OF *CULEX QUINQUEFASCIATUS* FOLLOWING INFECTION WITH *DIROFILARIA IMMITIS*

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ABSTRACT. The vector efficiency of the Haiti and Covington strains of *Culex quinquefasciatus* after feeding on dog blood infected with *Dirofilaria immitis* (~110 microfilariae (mf)/20 µl of blood) was 1.2 and 0.3%, respectively, versus nearly 20% for the Vero Beach strain of *Aedes taeniorhynchus*. At a much higher microfilaremia (~400 mf/20 µl), it was 1.6, 0.5 and 31.5%, respectively. The poor vector efficiency of the 2 *Cx. quinquefasciatus* strains probably was due to the formation of long, needle-like oxyhemoglobin crystals in the blood meal, which prevented the migration of microfilariae to the Malpighian tubules. Crystals did not form when *Cx. quinquefasciatus* ingested *D. immitis* microfilariae mixed in human blood.

INTRODUCTION

A number of reports have concluded that *Culex quinquefasciatus* Say is an important vector of dog heartworm, *Dirofilaria immitis* (Leidy), but much of this incrimination is based on circumstantial evidence. For example, Ward and Franklin (1953) assumed that this mosquito was one of the principal vectors in Mississippi simply because it was the most abundant species. Gubler (1966) arrived at a similar conclusion based on the finding that heartworm prevalence was highest in regions of Oahu, HI, where *Cx. quinquefasciatus* populations were dense, and lowest where this species was relatively scarce. Del Rosario (1936) reported that this species was an excellent vector of dog heartworm in the Philippines, and later Villavaso and Steelman (1970) had the same opinion after laboratory and field studies in Louisiana. There are, however, many reports that conflict with this view suggesting that the importance of *Cx. quinquefasciatus* as a vector of dog heartworm may be exaggerated (Summers 1943, Travis 1947, Rosen 1954, Bemrick and Moorhouse 1968, Otto and Jachowski 1981, Sauerman and Nayar 1983). This study summarizes the vector efficiency of 2 strains of *Cx. quinquefasciatus* after a *D. immitis* infective blood meal. It also reports on the identification of crystals that form in the midgut of this mosquito species shortly after the ingestion of dog blood which prevent the migration of microfilariae (mf) to the Malpighian tubules.

METHODS

Mosquito strains: Colonies of the Haiti and Covington strains of *Cx. quinquefasciatus* were established from egg rafts collected in Leogane, Haiti, and Covington, LA. The former was in the 50–54th generation at the time of this study whereas females of the latter strain were in the 5–7th generation of colonization. The Baton Rouge strain of *Cx. quinquefasciatus* was obtained from a colony that has been maintained at Louisiana State University (C. L. Meek) for nearly 20 years. The Vero Beach strain of *Aedes taeniorhynchus* (Wied.) was obtained from a colony kept at the Florida Medical Entomology Laboratory (J. K. Nayar) and maintained in our insectary for several years.

Dog blood sources: Dirofilaria immitis-infected blood was collected from the same mixed breed dog throughout the study. For each vector efficiency trial, the mf density was adjusted to the desired level using uninfective blood from another mixed breed dog. Uninfected blood for the density gradient and electrophoresis studies was obtained from a purebred Walker hound. This dog was known to be uninfected because it was born and maintained in the mosquito-free 9th floor vivarium of the Tulane Medical Center in downtown New Orleans.

Mosquito infection and dissection: With one exception, all infective dog bloods were collected in 10-ml vacutainers and kept in the refrigerator (4°C) until used the following morning. This method is routinely used in our laboratory for collecting blood infected with nocturnally periodic filariae such as *Wuchereria bancrofti* (Cobbold), *Brugia malayi* (Brug) 1958, and *Dirofilaria corynodes* (Von Linstow), and it does not impair the viability or infectivity of the mf (Lowrie 1983, Travi et al. 1986, Janousek and Lowrie 1989). The mosquitoes ingested *D. immitis* mf by feeding through a hog intestinal membrane attached to a feeder apparatus (Rutledge et al. 1964). Blood fed specimens were put into pint cardboard containers covered with bobbinet, maintained on a 10% sugar solution soaked in cotton pads, and kept at 27°C and approximately 80% RH. Freshly engorged mosquitoes were examined for mf by dissecting the blood meal in water to lyse the red blood cells (RBC). Two days postfeeding the Malpighian tubules were dissected from the gut in phosphate buffered saline (PBS) on a glass slide. It was necessary to rupture the tubules to observe and count the developing larvae. This was accomplished by placing a glass coverslip over the

tubules and gently pressing down and rotating it (Kartman 1953). On day 14, the entire mosquito was dissected in PBS so that the number of third-stage (infective) larvae (L_3s) could be counted. Forty mosquitoes were dissected at each time interval. The vector efficiency of each mosquito strain was determined by dividing the total number of L_3s recovered from 40 mosquitoes by the total number of mf initially ingested by 40 mosquitoes (Kartman 1954).

Density gradient separation of dog blood components: It is our experience that long, needle-like crystals are formed in the midgut of *Cx. quinquefasciatus* whenever dog blood is ingested, but this does not occur when blood from other sources such as humans, monkeys and guinea pigs is ingested. Therefore, we wanted to determine if the *D. immitis* mf were responsible for triggering this unusual crystal formation in this mosquito species or if it was due solely to a particular component of dog blood.

The mf and the components of dog blood were separated from each other using a self-generated continuous density gradient of Percoll. The gradient was formed in 10-ml round bottom polycarbonate tubes by centrifugation at 23,000 × g using the manufacturer's formulation (Pharmacia, Piscataway, NJ). Color-coded density marker beads were put in one of the tubes to calibrate the gradient. Several of the remaining preformed gradient tubes were overlaid with 1 ml each of uninfected dog blood, then centrifuged at 400 × g for 20 min in swing-out rotors to separate the plasma, white blood cells (WBC), and RBCs from each other. The mf from *D. immitis*-infected dog blood were separated in other preformed gradient tubes in the same manner. Each layer was removed with a bulbed transfer pipet and placed in a separate 15-ml centrifuge tube. The contents were washed once in 10 ml of PBS, centrifuged again at 400 × g, and then each pellet was resuspended in 4 ml of fresh whole human blood and fed to a separate group of *Cx. quinquefasciatus* using the membrane feeder.

Identification of the blood crystals by gel electrophoresis: A report by Nayar and Sauerman (1975) suggested that the crystals that formed in the midgut of *Cx. quinquefasciatus* after ingesting dog blood were oxyhemoglobin. We used the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method described by Laemmli (1970) to estimate the molecular weight of the proteins in the mosquito blood meals. Identification of the crystallized blood meal from this mosquito species was accomplished by comparing the banding pattern of these proteins with the banding of crystals from a commercial preparation of dog hemoglobin (Sigma, St. Louis, MO). Low molecular weight proteins (Pharmacia) were used as the calibration standard.

The crystallized dog blood was prepared for the gel by removing the blood meal from 7 *Cx. quinquefasciatus* (~25 μl) and mixing this blood with 100 μl of 2% formalin to fix the crystals. The suspension was overlaid on a gradient of 20% glycerol over 50% glycerol and centrifuged at 800 × g for 10 min. The pellet was dissolved in 100 μl of water, and 10 μl of this solution was mixed with 10 μl of SDS dissociating sample buffer in a 1.5-ml microcentrifuge tube and heated for 5 min at 100°C. Then 10 μl of this sample was loaded onto a 7.5% separating gel and electrophoresed for 5 h. The same procedure was used with 7 blood meals removed from the Liverpool strain of *Aedes aegypti* (Linn.) even though crystals do not form in the midgut of this mosquito. This served as a control for using 2% formalin although, in the absence of crystals, this solution generally lyses RBCs.

Another 7 blood meals from *Cx. quinquefasciatus* that fed on uninfected dog blood plus 7 from *Ae. aegypti* and also 1 mg of the commercially prepared dog hemoglobin crystals were treated in the same manner except they were dissolved directly in water, thus omitting the need for the glycerol gradient step.

Assessment of microfilaria damage: It is known that some mosquito species possess cibarial and/or pharyngeal armatures which physically damage and kill the mf as they pass through these pumps. We investigated whether this also occurred with *D. immitis* mf in *Cx. quinquefasciatus* using, in this one experiment, infective blood that was cryopreserved because the infected dog was no longer available to us. The blood in six 1-ml glass ampules was thawed and washed according to the method of Lowrie (1983). The mf were pooled and thoroughly mixed in 4 ml of Hanks' balanced salt solution. Then 2 ml of the solution was mixed with 5 ml of fresh human blood while the remaining 2 ml was mixed with 5 ml of fresh, uninfected dog blood, and each mixture was fed to *Cx. quinquefasciatus* using the membrane apparatus. Forty engorged mosquitoes from each group were frozen at −20°C 1 h after blood ingestion. Later, each blood meal was carefully removed and lysed in distilled water exposing the mf so that they could be microscopically scrutinized at 100× magnification for damage such as ruptures in the cuticle or a completely severed body.

RESULTS

Vector efficiency with low microfilaremia: The vector efficiency of 2 strains of *Cx. quinquefasciatus* and one strain of *Ae. taeniorhynchus* after feeding on *D. immitis*-infected dog blood with a

low density microfilaremia (108-115 mf/20 μl) is summarized in Table 1. For *Ae. taeniorhynchus*, 21% of the ingested mf migrated to the Malpighian tubule development site after 48 h (range 0–9 mf/mosquito). After the 14-day parasite development period, one or more L_3 was found in 87.5% of the mosquitoes ($\bar{x}$ = 2.0 L_3s; range 0–5), with the vector efficiency being 19.7%. The mosquito survival was 77% (266/347). Using the Haiti strain of *Cx. quinquefasciatus*, 4% of the ingested mf migrated to the Malpighian tubules after 48 h (range 0–5 mf/mosquito). On day 14, each of 5 mosquitoes had one L_3, the vector efficiency being 1.2%. The survival rate of engorged females on day 14 was 17% (40/242). For the Covington strain of *Cx. quinquefasciatus*, only 3 of the 40 mosquitoes had mf in the tubules 48 h later (range 0–2 mf/mosquito). After 14 days, one L_3 was seen in 2 of the 40 mosquitoes, the vector efficiency being 0.3%. The survival rate of engorged specimens was 63% (147/234). The overall vector efficiency of *Ae. taeniorhynchus* was more than 16 and 65 times higher than the Haiti and Covington strains of *Cx. quinquefasciatus*, respectively.

Vector efficiency with high microfilaremia: The vector efficiency of the same 2 strains of *Cx. quinquefasciatus* and one strain of *Ae. taeniorhynchus* feeding this time on high density *D. immitis*-infected dog blood (389–408 mf/20 μl) also is summarized in Table 1. For *Ae. taeniorhynchus* females, 70% had mf in the Malpighian tubules (range 0–42) 48 h after engorgement. After 14 days, 92.5% of the mosquitoes had L_3s ($\bar{x}$ = 6.1; range 0–19) with the vector efficiency being 31.5%. The survival rate of engorged females during the 14-day period was 62% (107/172). For the Haiti strain of *Cx. quinquefasciatus*, 8% of the ingested mf had migrated to the Malpighian tubules (range 0–6) after 48 h. On day 14, L_3s were seen in 27.5% of the mosquitoes ($\bar{x}$ = 1.3; range 0–3) with the vector efficiency being 1.6%. Only 29% (51/174) of the mosquitoes survived the 14-day parasite development period. For the Covington strain of *Cx. quinquefasciatus*, only 5 of the 40 (0.5%) dissected specimens had mf in the tubules after 48 h (range 0–4 mf). On day 14, 6 mosquitoes had one L_3 and one had 2 L_3s, the vector efficiency being 0.5%. The survival rate for this group was 59% (138/235). The overall vector efficiency of *Ae. taeniorhynchus* was 20 and 63 times higher than the Haiti and Covington strains of *Cx. quinquefasciatus*, respectively.

Armature damage assessment: The Haiti strain of *Cx. quinquefasciatus* fed on human blood mixed with 76 *D. immitis* mf per 20 μl. A total of 483 mf was ingested by the 40 mosquitoes with 431 mf (89.2%) showing no evidence of physical damage. All 40 blood meals contained mf ($\bar{x}$ = 12.0 mf; range 4–28) with damaged mf seen in 30 of the 40 mosquitoes ($\bar{x}$ = 1.3 damaged mf; range 0–5). When the same mosquito strain fed on dog blood mixed with 87 *D. immitis* mf per 20 μl, the 40 mosquitoes ingested 437 mf with 377 (86.3%) having no evidence of physical damage. Again, each of the 40 blood meals contained mf ($\bar{x}$ = 10.9 mf; range 4–24) with damaged mf observed in 28 of the 40 mosquitoes ($\bar{x}$ = 1.5 damaged mf; range 0–4).

Crystal formation in Cx. quinquefasciatus: We first noticed crystals in the midgut when *D. immitis*-infected dog blood was ingested by the Haiti strain of *Cx. quinquefasciatus*. Later it was discovered that the crystals began to form about 15 min after feeding, and the blood meal was approximately 75% crystallized within one h. About this time an occasional mf could be found

Table 1. Vector efficiency of 2 strains of *Culex quinquefasciatus* and one of *Aedes taeniorhynchus* following the ingestion of *Dirofilaria immitis*-infected dog blood having: a) a low density microfilaremia, and b) a high density microfilaremia.[1]

	Mosquito species and strains					
	Ae. taeniorhynchus[a]	*Cx. quinquefasciatus*[a]		*Ae. taeniorhynchus*[b]	*Cx. quinquefasciatus*[b]	
	Vero Beach	Haiti	Covington	Vero Beach	Haiti	Covington
A Microfilaremia of mosquito blood source (mf/20 μl)	110	115	108	408	405	389
B Mf uptake by mosquitoes	355	420	587	714	874	1658
C No. mf in Malpighian tubules 48 h later	75	15	4	498	70	8
D No. infective larvae (day 14)	70	5	2	225	14	8
E No. infective mosquitoes	35/40	5/40	2/40	37/40	11/40	7/40
F Vector efficiency[2]	19.7	1.2	0.3	31.5	1.6	0.5

[1] Each value in B, C and D is the total number from 40 dissected mosquitoes.
[2] Vector efficiency (%) = D ÷ B.

in the Malpighian tubule development site. The dissections initially were made in 2% formalin to lyse the erythrocytes; but, instead, this procedure fixed the crystals causing them to remain intact. If water or PBS was used, the crystals dissolved within seconds. After 2 h, the entire blood meal was engulfed with these long, needle-like crystals (Fig. 1) that restricted the movement of mf to the Malpighian tubules. These crystals never formed in the blood meal of *Ae. aegypti* or *Ae. taeniorhynchus* after ingesting dog blood.

Crystals were seen in the *Cx. quinquefasciatus* blood meal under the following circumstances: 1) when mosquitoes fed directly on a dog, 2) when the blood meal was examined in the absence of any kind of dissecting fluid, 3) when uninfected whole dog blood was ingested, and 4) when Percoll-separated dog erythrocytes were resuspended in whole human blood and fed to mosquitoes (Table 2). No crystals were formed when mosquitoes ingested whole human blood that had been mixed with either Percoll-separated *D. immitis* mf, dog WBCs or dog plasma (Table 2). The Haiti, Covington and Baton Rouge strains of *Cx. quinquefasciatus* all responded in the same manner to these various kinds of blood meals.

Identification of crystals by gel electrophoresis: The low molecular weight protein calibration standard appears in the gel of lane a (Fig. 2). The molecular weight of hemoglobin is ~65,000, which most closely corresponds with the 67-kDa band of bovine serum albumin seen on the calibration standard. The commercial preparation of dog hemoglobin (lane b) most strongly bands at this same 67 kDa molecular weight. Lane c, the crystallized blood meal from *Cx. quinquefasciatus*, shows an extremely strong band at the same 67-kDa level as the commercial prepara-

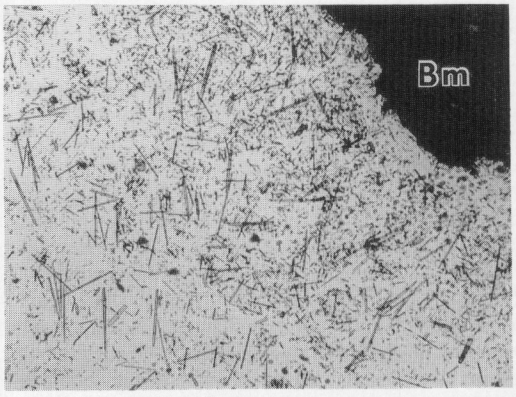

Fig. 1. Blood meal (Bm) from *Culex quinquefasciatus* 1 h after the ingestion of uninfected whole dog blood showing oxyhemoglobin crystals on the periphery.

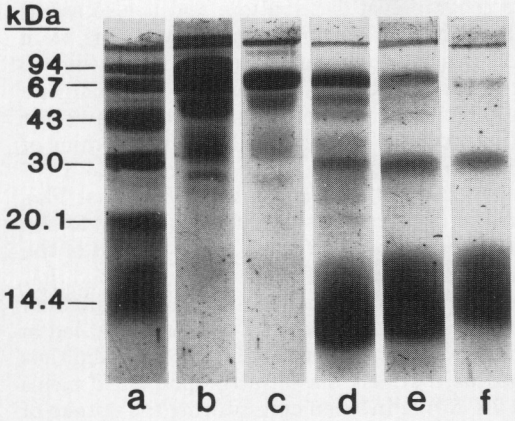

Fig. 2. SDS-PAGE analysis of mosquito blood meals. Lane a, the protein calibration standard consisting of phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa); lane b, commercially prepared dog hemoglobin; lane c, crystallized dog blood meal from *Culex quinquefasciatus* fixed in 2% formalin; lane d, dog blood meal from *Aedes aegypti* lysed in 2% formalin; lane e, dog blood meal from *Cx. quinquefasciatus* lysed in water; lane f, dog blood meal from *Ae. aegypti* lysed in water.

Table 2. Presence or absence of oxyhemoglobin crystals in the midgut of *Culex quinquefasciatus* following the ingestion of various kinds of blood meals.

Type of blood fed to mosquitoes	Strain of *Culex quinquefasciatus*	No. of mosquitoes with	
		Crystals	No crystals
D. immitis dog blood	Covington	30	0
D. immitis dog blood	Baton Rouge	30	0
D. immitis dog blood	Haiti	30	0
Uninfected whole dog blood	Haiti	30	0
Dog RBCs in whole human blood[1]	Haiti	30	0
D. immitis mf in whole human blood[1]	Haiti	0	60
Dog WBCs in whole human blood[1]	Haiti	0	30
Dog plasma in whole human blood[1]	Haiti	0	30

[1] Isolated by Percoll density gradient centrifugation.

tion of dog hemoglobin. In lane d, the blood meal from *Ae. aegypti*, a distinct band also appears at this molecular weight, suggesting that the hemoglobin is fixed when 2% formalin lyses the RBCs, but note that considerable banding occurs at the lower molecular weights as well. In lanes e and f, where the RBCs from mosquito blood meals were lysed in water, only a faint band is observed at the 67-kDa level with much heavier banding at the lower molecular weights.

DISCUSSION

The intent of this report is to put the vector importance of *Cx. quinquefasciatus* in its proper prospective. It appears from the literature that this mosquito species probably supports the complete development of *D. immitis* throughout its geographical distribution, and it also seems likely that its vector competency differs from one region to another. Whether this is due to strain differences in the mosquito or the parasite is not known. In the present study, the vector efficiency of 2 geographically distinct strains of *Cx. quinquefasciatus* ranged from 0.3 to 1.6% versus 20 to 32% for *Ae. taeniorhynchus*. This drastic impairment was due apparently to the rapid formation of crystals in the midgut of the mosquito after a meal of infected dog blood that blocked the migration of mf to the Malpighian tubules. Eventually, the mf were either killed in the blood clot or defecated. It is serendipitous that we routinely dissect arthropod blood meals in 2% formalin when quantitating the uptake of microfilariae rather than another dissecting medium such as PBS, water or Ringer's solution, otherwise the crystals would have dissolved quickly and gone unnoticed. The crystals appear identical to those induced from dog blood by the hemoglobin crystallization method (Washino and Else 1972). Nayar and Sauerman (1975) were the first to describe that this crystallization also took place within the mosquito midgut, and it occurred in the Florida strain of both *Cx. quinquefasciatus* and *Culex nigripalpus* Theobald. They speculated that secretions from symbiotic bacteria in the mosquito's midgut lysed the dog RBCs, and the released hemoglobin was oxidized to crystals of oxyhemoglobin. Our study confirms that dog RBCs are involved in the formation of these crystals and, indeed, they are oxyhemoglobin. However, because we never observe crystals when *Cx. quinquefasciatus* ingests blood from other vertebrates such as monkeys, guinea pigs, gerbils and humans, it is difficult to rationalize that bacterial secretions trigger this event; that is, these secretions specifically lyse dog RBCs but not erythrocytes from other animals. This makes us believe that other, as yet unanswered, factors are involved.

Otto and Jachowski (1981) felt that in the continental United States *Cx. quinquefasciatus* might be a prime vector of dog heartworm only in some areas of the Gulf Coast. Now, with the reports of finding oxyhemoglobin crystals in 2 strains of *Cx. quinquefasciatus* from Louisiana, one strain from Florida and one strain from Haiti following the ingestion of dog blood, one might question the importance of this mosquito even in these areas. Also, it would be interesting to learn how widespread this crystallization phenomenon is among the strains of this rather ubiquitous mosquito species. Intriguingly, Travis (1947) reported that nearly all of the *D. immitis* mf that remained in the midgut of the Guam strain of *Cx. quinquefasciatus* were dead 2 days after ingestion ($\bar{x}$ = 173 mf/mosquito), whereas a high percentage of mf were still alive in *Ae. aegypti* ($\bar{x}$ = 21 mf/mosquito). Also Kartman (1953), using a strain from Galveston, TX, that fed on a dog with a microfilaremia of 320–370 mf/20 µl of blood, obtained a vector efficiency of 1.0%, which essentially duplicates our results. He noted that a most striking feature was the nearly total absence of normal mf in the midgut 24 h after ingestion.

Obviously, an important consideration in evaluating the vectorial capacity of *Cx. quinquefasciatus* is its attraction to dogs as a blood meal source in nature. It is believed that this species has a predilection for human blood (Wharton 1951, Reeves and Rudnick 1951) and for avian blood (Colless 1959). However, Villavaso and Steelman (1970) captured over 11,000 mosquitoes during 2 summers in Baton Rouge, LA, using 10 dog-baited traps, and 91% of them were *Cx. quinquefasciatus*. But it is not stated whether the females blood fed on the animals. They went on to report that when colonized mosquitoes were put in screened cages with infected dogs, it required all-night exposure to obtain enough blood fed specimens for their laboratory studies. Travis (1947) experienced the same difficulty. Summers (1943) reported that only 3 of 500 *Cx. quinquefasciatus* blood fed when caged mosquitoes were held against a dog and, similarly, Bemrick and Moorhouse (1968) had only a 7% feeding success rate (137 of 1,913).

Among the few natural infectivity rate studies that have been conducted, Symes (1960) recovered one L_3 from 2,435 *Cx. quinquefasciatus* in Fiji; Rosen (1954) found none in 1,061 specimens in Tahiti; Villavaso and Steelman (1970) found 13 L_3s in 2,312 *Cx. quinquefasciatus* captured in Baton Rouge, LA, and Sauerman and Nayar (1983) found 3 L_3s in one of 200 *Cx. quinquefasciatus* collected in Vero Beach, FL. The CDC gravid mosquito trap (Reiter 1983) is ideal for natural infection and infectivity rate studies because nearly all of the *Cx. quinquefas-*

ciatus attracted to it are gravid females seeking to oviposit. In a recent Baton Rouge study, Niebylski et al. (1991[1]) used this trap to collect nearly 10,000 *Cx. quinquefasciatus*. They crushed the mosquitoes using the mass dissection technique (Ash and Schacher 1971), and no larvae were recovered. During the course of human lymphatic filariasis studies in Haiti, Lowrie (unpublished data) used the same gravid trap in Leogane, and no infective larvae of *D. immitis* were seen in 1,200 individually dissected *Cx. quinquefasciatus*. Also, Lowrie and Eberhard (unpublished data) dissected nearly 800 *Cx. quinquefasciatus* collected inside houses in Arcahaie, Haiti, and again none of the L_3s were *D. immitis*.

It is not uncommon for dirofilariasis in dogs to exceed 400 mf/20 μl of blood, the highest density in our vector efficiency trials. It also is known that as the mf burden in the Malpighian tubules increases, it causes excessive mortality that sometimes approaches 100% (Kershaw et al. 1953, Webber and Hawking 1955, Christensen 1978). Thus the transmission role of highly susceptible mosquito species may actually decrease as the mf density in the dog population increases. Conversely, factors that may cause a mosquito species to be a relatively poor host of *D. immitis* may actually enhance its vector significance. The first line of defense against filarial infection for some species of *Aedes*, *Culex* and *Anopheles* is the presence of pronounced cibarial and/or pharyngeal armatures that inflict lethal damage as the mf pass through these pumps enroute to the midgut (Coluzzi and Trabucchi 1968, McGreevy et al. 1978, Bryan and Southgate 1988). However, McGreevy et al. (1978) reported that *Cx. quinquefasciatus* has a weak cibarial armature and killed only 6% of the *W. bancrofti* mf that were ingested. This was comparable to our finding that only 12% of the *D. immitis* mf were damaged, which again emphasizes that the poor vector efficiency of *Cx. quinquefasciatus* is due primarily to the blockage and retention of mf in the midgut by the oxyhemoglobin crystals rather than trauma inflicted by the armatures.

It may be summarized from our study and the reports of others that the reputation of *Cx. quinquefasciatus* as a prime vector of *D. immitis* may far exceed its actual importance in nature except in areas where this mosquito has an unusually dense population.

[1] Niebylski, M. L., C. L. Meek and R. C. Lowrie, Jr. 1990. Blood-feeding of *Culex* in an urban environment. (Unpublished report).

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