

POTENTIAL MOSQUITO VECTORS OF *DIROFILARIA IMMITIS* IN BERNALILLO COUNTY, NEW MEXICO^{1,2}

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ABSTRACT. The susceptibility of *Aedes vexans*, *Culex quinquefasciatus*, and *Culex tarsalis* to *Dirofilaria immitis* infections was evaluated in Bernalillo Co., New Mexico. Mosquitoes that had taken a blood meal from a *D. immitis*-infected dog were dissected and observed for developing larvae. Infection rates for *Ae. vexans*, *Cx. quinquefasciatus*, and *Cx. tarsalis* were 68.9, 40.6, and 30.2%, respectively. Infective *D. immitis* larvae developed after a minimum of 8 days in *Ae. vexans* vs. 14 days in both *Cx. quinquefasciatus* and *Cx. tarsalis*. Vector efficiency of *Ae. vexans* was 20.4%, with a mean of 5.9 infective larvae developing from a mean of 29 microfilariae ingested per mosquito. Vector efficiency in *Cx. quinquefasciatus* and *Cx. tarsalis* was 2.7 and 0.4%, respectively. These results suggest that *Ae. vexans* has the greatest potential for *D. immitis* transmission.

INTRODUCTION

Development of canine heartworm, *Dirofilaria immitis* (Leidy), microfilariae to the infective third stage (L₃) has been reported in about 70 mosquito species (Ludlam et al. 1970, Otto and Jachowski 1981). Ecological factors such as abundance, host preference, and susceptibility reduce this number to a few species for a given geographical area (Otto and Jachowski 1981). Susceptibility of mosquitoes to *D. immitis* infection not only differs with the mosquito species but also with geographical strains of a particular species (Buxton and Mullen 1981, Christensen et al. 1984, Apperson et al. 1989).

Aedes vexans (Meigen) and *Culex quinquefasciatus* Say are considered to be efficient vectors of *D. immitis* in some regions but virtually inefficient in others, emphasizing the differences in vector competence among different geographical strains. *Aedes vexans* is considered to be an important vector of *D. immitis* in New York (Todar et al. 1977), California (Walters and Lavoipierre 1982), Maryland (Jankowski and Bickley 1976), and Canada (Ernst and Slocombe 1982, Frimeth and Aria 1983), but is considered a minor vector in Alabama (Buxton and Mullen 1980), Kentucky (Courtney and Christensen 1983), and Indiana (Pinger 1982). *Culex quinquefasciatus* is considered to be an important

vector in Louisiana by some (Villavaso and Steelman 1970), but not by others (Lowrie 1991), whereas in Florida (Weiner and Bradley 1970, Nayar and Sauerman 1975) it is not a suitable intermediate host. The vector status of *Culex tarsalis* Coq. apparently has not been reported.

Host preference data from Bernalillo County in central New Mexico show that *Ae. vexans* is the most abundant species attracted to dogs, followed by *Cx. quinquefasciatus* and *Cx. tarsalis* (K. M. Loftin, unpublished data). Data from a 1987 report on mosquito abundance (Schultz 1987⁴) and a continuing mosquito surveillance program by the Albuquerque Environmental Health Department suggest that these 3 species should be tested for their susceptibility to *D. immitis* infection.

MATERIALS AND METHODS

Mosquito rearing: *Culex tarsalis* and *Cx. quinquefasciatus* egg rafts and floodwater *Aedes* spp. larvae were collected from breeding sites located throughout Bernalillo Co., NM. Each egg raft was hatched in a paper cup (200 ml) and larvae were fed a diet of crushed Purina 5001 Rodent Chow and brewers yeast (3:1). Fourth-instar larvae were identified for species confirmation and pupae were placed in 30 × 30 × 30-cm screened cages for adult emergence. Adults were fed 15% sucrose solution (on cotton pads) and moistened raisins, with pads and raisins replaced daily. Cages were kept under controlled environmental conditions (26°C, 80% RH, and

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² The dog used in this study was given humane care in accordance with the recommendations given in the "Guide for the care and use of laboratory animals" (NIH Publ. No. 78-23) and the regulations of the USDA Animal Welfare Act.

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⁴ Schultz, J. H. 1987. Occurrence and seasonal distribution of mosquitoes in Bernalillo Co., New Mexico, 1986. Report to the Albuquerque Environmental Health Department, Albuquerque, NM.

Table 1. Competency of 3 mosquito species occurring in Bernalillo County, NM, as vectors for *Dirofilaria immitis*: infection rate, microfilaria (mf) uptake, development of infective larvae (L₃), and vector efficiency.

Species	Total		mf uptake		L ₃ Development			Vector efficiency (\bar{x} L ₃ / \bar{x} mf)
	No. dissected	% infection	No. dissected	\bar{x} mf \pm SE	No. dissected	% infective	\bar{x} L ₃ \pm SE	
<i>Aedes vexans</i>	90	68.9	10	29.0 \pm 6.1	30	73.3	5.9 \pm 0.9	20.4
<i>Culex quinquefasciatus</i>	179	40.6	10	34.2 \pm 6.3	39	41.0	0.9 \pm 0.2	2.7
<i>Culex tarsalis</i>	53	30.2	10	39.3 \pm 11.0	20	10.0	0.2 \pm 0.1	0.4

14:10 h L:D photoperiod). Daily mortality of the caged mosquitoes was recorded.

Infective dog: A 7-year-old female Labrador retriever, with a naturally occurring infection of *D. immitis*, was used for infective feedings of mosquitoes. The Knott (1939) technique was used to monitor the density of microfilariae in the dog throughout the infective feeding phase of the experiment.

Infective feeding: Before exposure to the infected dog, mosquitoes were starved for 24 h to stimulate engorgement. Two- to 5-day-old female mosquitoes were released into a screened cage (0.61 \times 0.91 \times 0.75 m) housing the dog, and allowed 4 h to complete engorgement. To facilitate handling and collection of mosquitoes, the 3 species were not fed simultaneously. Engorged mosquitoes were aspirated from the feeding cage, transferred to 30 \times 30 \times 30-cm screened cages, and maintained on 15% sucrose and moist raisins until dissection.

Dissections: To determine the mean number of microfilariae ingested, 10 freshly engorged mosquitoes of each species were dissected. To monitor development of *D. immitis*, a proportion of mosquitoes was dissected at intervals postengorgement. Seven *Ae. vexans* and 10 *Cx. quinquefasciatus* were dissected daily, whereas 3 *Cx. tarsalis* were dissected every other day from day 1 through day 9, then 3 were dissected daily for the remainder of the study. The numbers of engorged mosquitoes varied widely among species, and these procedures ensured that enough mosquitoes survived to determine infectivity rates and vector efficiency. Once infective L₃s were found within a species, all remaining live mosquitoes of that species were dissected in order to determine the infective rate and mean number of L₃s per mosquito surviving through the developmental period of *D. immitis*.

The head, thorax, and abdomen were separated and dissected in physiological saline (pH 7.2) on a glass microscope slide and examined for *D. immitis* under 100 \times magnification. The

Malpighian tubules were dissected away from the midgut and crushed to expose developing larvae. Morphology, size, and location within the host were used for assigning the appropriate developmental stage (Iyengar 1957, Nelson 1959).

RESULTS

The density of microfilariae in the heartworm-infected dog was 28 \pm 1 microfilariae per 20 μ l of blood. Ninety *Ae. vexans*, 179 *Cx. quinquefasciatus*, and 53 *Cx. tarsalis* engorged and subsequently were dissected, with results summarized in Table 1. *Aedes vexans*, *Cx. quinquefasciatus*, and *Cx. tarsalis* ingested an average of 29.0, 34.2, and 39.3 microfilariae of *D. immitis*, respectively. The minimum developmental period required for ingested microfilariae to molt into L₃s varied among species, taking 8 days in *Ae. vexans* and 14 days in both *Cx. quinquefasciatus* and *Cx. tarsalis*. Overall mortality for caged *Ae. vexans*, *Cx. quinquefasciatus*, and *Cx. tarsalis* was 18.9, 7.1, and 11.1%, respectively.

The infection rate was calculated from all mosquitoes of a species, and is the percentage of mosquitoes with any stage of *D. immitis*. The infective rate was calculated only from mosquitoes dissected after the minimum developmental period of *D. immitis* had been reached, and is the percentage of mosquitoes with infective larvae. Infection rates were 68.9, 40.6, and 30.2%, and infective rates were 73.3, 41.0, and 10.0% for *Ae. vexans*, *Cx. quinquefasciatus*, and *Cx. tarsalis*, respectively. The average number of L₃s per mosquito (including uninfected mosquitoes) included both larvae found in the head and those still in the process of migrating to the head. The highest number was observed in *Ae. vexans*, with 5.9 L₃s per mosquito, whereas *Cx. quinquefasciatus* and *Cx. tarsalis* averaged 0.9 and 0.2 L₃s per mosquito, respectively. The vector efficiency, calculated by dividing the mean number of L₃s by the mean number of microfilariae ingested,

was 20.4, 2.7, and 0.4% for *Ae. vexans*, *Cx. quinquefasciatus*, and *Cx. tarsalis*, respectively.

In addition to the 3 preceding mosquito species, an attempt was made to examine vector competence of *Aedes dorsalis* (Meigen). Due to problems in rearing this species, we decided only to determine if *Ae. dorsalis* is capable of producing infective larvae of *D. immitis*, and therefore we did not dissect any mosquitoes until we assumed the end of the developmental period had been reached. Seventeen *Ae. dorsalis* survived to be dissected and all contained infective larvae. A mean of 26.2 ± 1.8 ($n = 10$) late 2nd- or early 3rd-stage *D. immitis* larvae was found in the Malpighian tubules at 7 days postfeeding, and a mean of 10.0 ± 3.5 ($n = 7$) L₃s was found at 8 days postfeeding.

DISCUSSION

This study demonstrated that at least 4 of the mosquito species present in Bernalillo County can support development of *D. immitis*. Length of the developmental period of *D. immitis*, mosquito mortality, infection rate, and the number of infective 3rd-stage *D. immitis* varied among species, and all of these parameters have an effect on the vector competence of the species.

The minimum developmental period for *D. immitis* that we observed in *Ae. vexans* (8 days) contrasts with results from Jankowski and Bickley (1976) who found L₃s within 14 days in Maryland. Differences in geographical strains of *Ae. vexans* may account for the shorter developmental time observed in New Mexico. The minimum developmental period of *D. immitis* in *Cx. quinquefasciatus* agrees with results obtained by Villavaso and Steelman (1970). Shorter developmental periods of *D. immitis* in a vector would favor disease transmission due to an increasing risk of natural mortality for mosquitoes that sustain longer developmental periods. In the case of *Ae. vexans* versus *Cx. quinquefasciatus* and *Cx. tarsalis*, potential mortality of *D. immitis* due to vector death was decreased by 6 days (8 versus 14 days developmental period).

Mortality observed during our study was much lower than that reported by Weiner and Bradley (1970) from *D. immitis*-infected *Aedes taeniorhynchus* Wied., *Aedes aegypti* (Linn.), *Anopheles quadrimaculatus* Say, and *Cx. quinquefasciatus* in Florida. Our mortality data were collected from the groups of mosquitoes that were being dissected and thus probably underestimate actual mortality. *Aedes vexans* had higher mortality than either *Culex* species, but most (>80%) survived to be dissected. Christensen (1978) showed that ingestion of fewer microfilariae is conducive to mosquito survival and production of L₃s; there-

fore, the low density microfilaremia of the infective dog used in our study would contribute to a lower mortality rate in mosquitoes.

The infection rate we observed for *Ae. vexans* was similar to that reported by Jankowski and Bickley (1976) in Maryland and by Ernst and Slocombe (1982) in Canada. In contrast, the infection rate for *Cx. quinquefasciatus* was much lower than that observed by Villavaso and Steelman (1970) in Louisiana. Our results on infection rates, infectivity, and vector efficiency support Lowrie's (1991) conclusion that *Cx. quinquefasciatus* is generally not an important vector of *D. immitis*.

Abundance, host preference, and the data presented in this paper suggest that *Ae. vexans* has the greatest potential for *D. immitis* transmission in Bernalillo County. *Culex quinquefasciatus* could be considered a secondary potential vector, whereas *Cx. tarsalis* appears to be of minor importance. Recovery of L₃s from *Ae. dorsalis* suggests that this species is a potential *D. immitis* vector, but further studies are needed to understand its role in transmission of canine heartworm.

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