LABORATORY STUDIES ON THE DEVELOPMENT AND TRANSMISSION OF *DIROFILARIA IMMITIS* BY *AEDES TRIVITTATUS*[1]

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ABSTRACT. Development of *Dirofilaria immitis* (Leidy) in *Aedes trivittatus* (Coquillett) was followed in the laboratory at 26.5 ± 1° C and 80 ± 5% RH. Juvenile *D. immitis* molted from 1st to 2nd stage and 2nd to 3rd stage at 7–8 and 10–11 days postexposure (PE), respectively. Mosquitoes showed a nearly 100% infection rate. Large numbers of microfilariae were retained within the midgut of exposed mosquitoes, and microfilariae invaded the Malpighian tubules through the first 6 days PE. A susceptible dog, exposed to the bite of 49 infective *Ae. trivittatus*, showed a microfilaremia of *D. immitis* by about 210 days PE. The role of *A. trivittatus* as a natural vector of *D. immitis* is discussed.

INTRODUCTION. The first report concerning *Aedes trivittatus* and *Dirofilaria immitis* involved laboratory studies with field-collected mosquitoes in Minnesota (Yen 1938). Yen concluded that *Ae. trivittatus* was refractive to infection with *D. immitis*. A recent study in central Iowa, however, showed that *Ae. trivittatus* supports the complete development of dog heartworm (Christensen and Andrews 1976). Possible explanations for the discrepancy between these two studies have been discussed previously (Christensen and Andrews 1976).

There are numerous reports of many species of mosquitoes supporting the development of *D. immitis* to the "infective-stage" (Ludlam et al. 1970; Bemrick and Sandholm 1966), but there is a paucity of information on the ability of these species to naturally transmit the infection to susceptible hosts. Bancroft (1904), Kume and Itagaki (1955), Newton (1957), Bemrick and Moorhouse (1968), and Bickley et al. (1977) have demonstrated transmission of *D. immitis* to susceptible dogs by the bite of infective *Culex fatigans* (=*quinquefasciatus*), *Aedes togoi*, *Anopheles quadrimaculatus*, *Ae. vigilax*, and *Ae. canadensis*, respectively. Demonstration that an arthropod can transmit a pathogen, by bite, to a susceptible host under controlled conditions is one of the criteria considered necessary for the establishment of an arthropod as a natural vector of a disease (Barnett 1960; Ludlam et al. 1970).

With the recovery of "infective-stage" *D. immitis* from 1.0% of field-collected *Ae. trivittatus*, the potential of this mosquito to function as a natural vector of dog heartworm in central Iowa was realized (Christensen and Andrews 1976). As a result, this study was designed to determine: (1) the ability of *Ae. trivittatus* to support the development of *D. immitis* under laboratory conditions, and (2) this mosquito's ability to transmit naturally the infection to a susceptible host under controlled conditions.

MATERIALS AND METHODS. Adult female *Ae. trivittatus* were field-collected, bloodfed, and allowed to oviposit on a cheesecloth substrate in the laboratory. Mosquitoes reared from these eggs were used throughout this study. Lots of 50 females were placed in 0.473-liter ice cream cartons with a fine-mesh marquisette covering. A cotton pad, moistened in 0.3 M sucrose solution, was placed on the marquisette. Sucrose pads were changed every 48 hr throughout the study, but were removed 24 hr before blood feeding. All mosquitoes were maintained at 26.5 ± 1° C and 80 ± 5% RH on a 16 hr photoperiod.

A female beagle (02), experimentally in-

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fected with *D. immitis*, was obtained from John W. McCall (School of Veterinary Medicine, University of Georgia) through a program supported by the U.S.-Japan Cooperative Medical Sciences Program-NIAID. This dog was used as the source of *D. immitis* for mosquito infections throughout the study. The dog was anesthetized with a mixture of Ketamine-HCl and Acepromazine SO_4 (9:1) administered IM at a dosage of 0.2 ml/kg body weight. Mosquitoes were allowed to feed, through the marquisette, for 15 min. Two 20-mm³ blood samples were taken immediately before feeding, and the mean microfilaremia was determined. Blood-fed mosquitoes were separated and placed in clean cartons.

Mosquitoes were dissected at 6, 9, and 12 hr, and daily until day 14 postexposure (PE) in *Aedes* saline (Hayes 1953) at 20X under a stereoscopic microscope. Malpighian tubules and juveniles recovered were examined at 100–400X with bright field or phase contrast optics. Developing stages of *D. immitis* were fixed in hot 70% glycerin-alcohol and mounted in glycerin. Blood remaining in mosquito midguts was smeared on a slide, methanol fixed, and stained with Giemsa to determine the number of microfilariae present. Measurements were obtained with the aid of an ocular micrometer and were recorded in micrometers.

At 16 days PE, 49 *Ae. trivittatus* were blood-fed on a mongrel female dog (F5). Dog F5 had been whelped during the winter and maintained in a mosquito-proof mammal room. A modified Knott's technique was used to check for a patent infection at approximately 2-week intervals beginning 5 months PE.

RESULTS AND DISCUSSION. A total of 931 of 1,217 (76.4%) *Ae. trivittatus* blood-fed when exposed to a dog (02) with a mean microfilaremia of 347/20mm³. Microfilariae were first recovered from the Malpighian tubules at 9 hr PE, and in 1 mosquito, microfilariae had already reached the distal ends of the tubules. By 12 hr PE, microfilariae were in the Malpighian tubules of all mosquitoes examined. Infection rates for various days PE are shown in Table 1. After day 1 PE, only

Table 1. Mean number of *Dirofilaria immitis* juveniles recovered from experimentally-infected *Aedes trivittatus* at various days postexposure.

Days post exposure	Mean number of J1/mosquito	Mean number of J2/mosquito	Mean number of J3/mosquito	Mean number of all juveniles/ mosquito	Mean number of mf/midgut
1	6.9 (81)*			6.9 (81)	20.9 (16)**
2	20.4 (100)			20.4 (100)	20.4 (20)
3	18.3 (100)			18.3 (100)	17.3 (17)
4	19.5 (95)			19.5 (95)	10.7 (10)
5	13.6 (100)			13.6 (100)	6.2 (6)
6	14.9 (100)			14.9 (100)	18.5 (2)
7	16.2 (100)			16.2 (100)	
8	9.8 (100)	7.4 (80)		15.8 (100)	
9	10.2 (80)	11.3 (95)		18.9 (100)	
10	5.6 (60)	11.3 (95)		14.4 (100)	
11	4.3 (28)	10.1 (93)	5.1 (67)	14.0 (100)	
12	2.5 (21)	8.9 (84)	7.2 (84)	14.1 (100)	
13	3.0 (5)	4.0 (50)	13.8 (100)	16.0 (100)	6.0 (1)
14	8.0 (5)	3.8 (20)	11.7 (100)	12.8 (100)	
20			12.8 (100)	12.8 (100)	
27		1.0 (20)	12.4 (100)	12.6 (100)	
35			13.0 (100)	13.0 (100)	

* Percent mosquitoes infected; ** Number of midguts examined.

1 uninfected mosquito was dissected. This mosquito was dissected at day 4 PE, and although no blood was evident in the midgut, ovariole development had occurred.

Juveniles molted from 1st to 2nd stage and 2nd to 3rd stage as early as 7–8 and 10–11 days PE, respectively (Table 1, Fig. 1). After day 12 PE, all mosquitoes examined contained "infective-stage" *D. immitis* outside the Malpighian tubules.

Different stages of juvenile development occurred at the same time in many of the mosquitoes examined (Table 1, Fig. 1). Similar observations have been made by other workers for several species of mosquitoes exposed to a single infective blood meal (Nayar and Sauerman 1975, Kutz and Dobson 1974, Intermill 1973, Kartman 1953). Explanations for this phenomenon, however, are lacking. With *Ae. trivittatus* exposed to *D. immitis*, it seems that this phenomenon is associated with microfilariae invading the Malpighian tubules over a period of several days. Large numbers of microfilariae were retained within the peritrophic membrane and blood clot of exposed mosquitoes (Table 1), and active microfilariae were observed in the midgut through day 6 PE. Although microfilariae were often seen passing out the hindgut with digested blood, they were also observed in the lumen of the proximal portion of the Malpighian tubules at the same time. These observations indicate that through day 6 PE it is possible for microfilariae to emerge from the digested blood and invade the Malpighian tubules as the blood is passing down the hindgut.

The ability of *D. immitis* microfilariae to survive for several days in the midgut of *Aedes* mosquitoes has previously been reported. Travis (1947) noted that *D. immitis* microfilariae remaining in the midgut of *Culex* mosquitoes were all dead 2 days after exposure, but that a large percentage were still alive in *Aedes* species. Likewise, Kartman (1953) demonstrated that some microfilariae of *D. immitis* remain alive for at least 72 hr. in the midgut of *Ae. aegypti* and *Ae. albopictus* but are rapidly destroyed in *Cx. pipiens* and *Cx. quinquefasciatus*. Studies by Coluzzi and Trabucchi (1968) and Bryan et al. (1974) on the buccopharyngeal armature of mosquitoes have helped to explain these differences between various mosquito genera.

The development of *D. immitis* in *Ae. trivittatus* relative to tissue differentiation was similar to that reported by Taylor (1960) for *Ae. aegypti*. Dimensions of developmental stages obtained from this

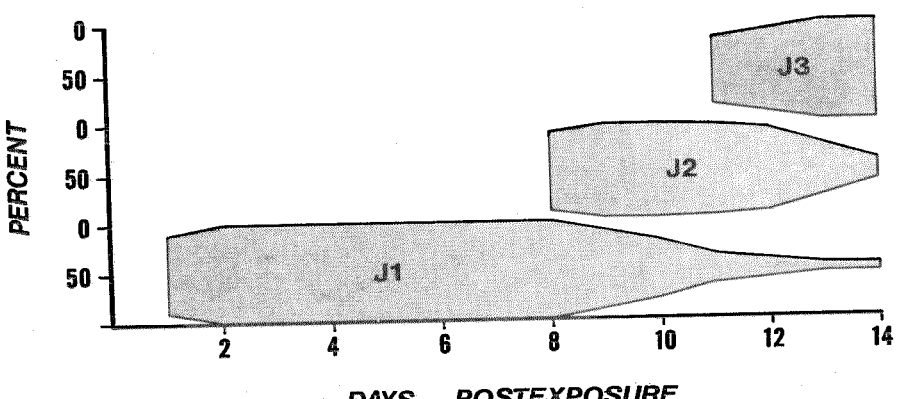

Fig. 1. Development of *Dirofilaria immitis* in *Aedes trivittatus*.

study (Table 2), however, vary considerably from those recorded by Taylor (1960). The greatest mean width of any juvenile stage from *Ae. trivittatus* was 36.0 ± 1.78 μm, and the greatest mean length 964 ± 33.3 μm. Specimens were fixed in glycerin-alcohol without coverslip pressure. Taylor (1960) reported mean widths as great as 70 ± 1 μm and mean lengths up to 1300 ± 18 μm, but the condition of worms at the time of measurement was not reported. Mean lengths of 3rd-stage *D. immitis* recovered from several mosquito species by other workers (Nelson 1959; Intermill 1973) are in agreement with the data recorded in this study for *Ae. trivittatus*.

Relatively large numbers of microfilariae were retained in the midgut of exposed *Ae. trivittatus* and often were observed passing out the hindgut. This seems to have a beneficial effect on this vector-parasite system. *D. immitis* commonly produces extremely high microfilaremias in dogs; therefore, mosquitoes may be exposed to very heavy infections. Data from this study indicate that at least the same number of microfilariae are retained in the midgut as infect the Malpighian tubules (Table 1). Kershaw et al. (1955) theorized that a reduced number of microfilariae in the mosquito is necessary for that mosquito to survive long enough to harbor infective juveniles. Data from the present study were obtained from *Ae. trivittatus* that were living when dissected; therefore, no objective statement can be made concerning worm burden as it may affect mosquito longevity. Other studies (Kutz and Dobson 1974, Lavoipierre 1958, Kershaw et al. 1953) indicate that large worm burdens, and filarial infections in general, tend to increase mosquito mortality. Data concerning the effect of *D. immitis* on the longevity of *Ae. trivittatus* will be reported later.

The dog (F5) exposed to the bite of 49 infective *Ae. trivittatus* showed a patent infection of *D. immitis* by about 210 days PE.

Ae. trivittatus, in central Iowa, is the first mosquito species in the western United States to satisfy all of the criteria considered necessary (Yen 1938, Barnett 1960, Ludlam et al. 1970) for the establishment of a principal or natural vector of *D. immitis*. It is zoophilic and readily feeds on dogs (Pinger and Rowley 1975), occurs in high populations in Iowa (Pinger and Rowley 1972; Christensen and Andrews 1976), and produces several generations per year (Rowe 1942). Preliminary studies indicate *Ae. trivittatus* is a strong flyer, flying as far as 30,000 m in a 24 hr. period on a flight mill in the laboratory (unpublished data). Field-collected *Ae. trivittatus* have been shown to harbor *D. immitis* in the infective stage (Christensen and Andrews 1976), and this study has demonstrated

Table 2. Measurements of developmental stages of *Dirofilaria immitis* from experimentally-infected *Aedes trivittatus*.

Days post exposure	Number measured	Developmental stage	Mean length ±S.D.	Mean width±S.D. (anterior-posterior)
1	7	J1	235±26.4	5.6±0.51
2	25	J1	196±17.6	6.3±0.76– 8.9±1.19
3	25	J1	154± 7.5	11.2±1.64–15.7±1.95
4	25	J1	150± 6.8	17.5±2.34–21.6±2.41
5	21	J1	205± 7.3	20.8±1.72–24.0±2.05
6	25	J1	249±17.9	23.3±2.39–27.2±2.31
7	7	J2	259± 8.8	27.7±1.60–30.6±1.13
8	21	J2	433±33.4	31.1±2.15–33.7±1.92
9	20	J2	559±41.6	30.5±2.13–36.0±1.78
11	10	J3	854±29.0	28.3±3.42
12	13	J3	964±33.3	26.0±1.65
14	25	J3	956±63.1	23.4±2.91

this mosquito's ability to naturally transmit the infection. Field studies also have provided circumstantial evidence which demonstrate an association in time and space of infective *Ae. trivittatus* and the occurrence of patent infections in the susceptible dog population (Christensen 1977).

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BITING FLIES ATTACKING HOLSTEIN CATTLE IN A BLUETONGUE ENZOOTIC AREA IN COLORADO, 1976

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ABSTRACT. The biting midge *Culicoides variipennis* (Coquillett) was the most common biting fly collected attacking cattle in Colorado in 1976; it comprised 62% of the collections and 6 species of mosquitoes totalled 33%. Concurrent New Jersey light trap collections supported the predominance of *C. variipennis*; it comprised 63% of the female biting flies collected for the species shown to attack cattle in this study.

A study area was established north of Denver, Colorado to study bluetongue disease, which appeared to be enzootic to the area. Luedke et al. (1977b) conducted laboratory studies that indicated bluetongue virus overwintered in cattle in the study area and that a vector-mediated mechanism was involved in the release of latent virus when weather conditions permitted vector activity. The data showed that the endemicity of the virus was enhanced because the virus was being transmitted both vertically through the placenta and horizontally by vector bite. Field collected data correlated with laboratory virus isolations (unpublished data of this laboratory) indicated that excessive calf mortality and related problems in the study area were associated with bluetongue virus infection: dams experimentally infected with the virus by vector bites in their 1st trimester of pregnancy commonly aborted or produced calves with congenital anomalies (Luedke et al. 1977a).

The biting midge *Culicoides variipennis* (Coquillett) was the primary suspected vector of bluetongue virus during the present studies because of pertinent previous research (Luedke et al. 1977a, 1977b). Previous research also showed that this biting fly was common to the study area, which is in the South Platte River drainage system north of Denver. Larval breeding sites had been located near Denver (Jones 1961) and northeast on the plains at Hudson, Colorado (Jones 1965). The large larval breeding site at Hudson was associated with an outbreak of bluetongue in sheep in 1963 (Jones 1965); this population of flies was subsequently shown to commonly attack sheep (Jones and Luedke 1969). Recent research (authors' unpublished data) indicated that larval breeding sites were common at 2-5 kilometers from the dairy farm that was selected as the center for our 1976 studies (closest cartographic name: Wattenberg, Weld County, Colorado). This paper presents data that show the species