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# Biology of *Dirofilaria immitis* (Leidy, 1850) (Nematoda: Onchocercidae) and *Aedes trivittatus* (Coquillett, 1902) (Diptera: Culicidae) in Central Iowa

by

Bruce Martin Christensen

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# INTRODUCTION

Since Grassi and Noe (1900) first suggested that mosquitoes might serve as vectors for dog heartworm, Dirofilaria immitis (Leidy, 1850), and Newton and Wright (1956) discredited fleas as intermediate hosts for this species, many published accounts have appeared on the vector-parasite relationships of D. immitis and numerous species of mosquitoes. Although the literature contains reports of more than 60 species of mosquitoes supporting the complete development of D. immitis, no investigators have established a given species of mosquito as anything more than a potential vector (Ludlam et al. 1970). The recovery of "infective-stage" juveniles from laboratory-infected mosquitoes does little to prove the ability of a mosquito species to actually function as a vector of D. immitis.

Barnett (1960) outlined four criteria, or "rules of proof", necessary for the incrimination of arthropods as vectors of human diseases. A modification of three of these, applicable to D. immitis and mosquitoes, follows: (1) demonstration that the mosquito will feed on dogs under natural conditions; (2) demonstration that the mosquito, collected under natural conditions, harbors the parasite in the infective stage; and (3) demonstration that the mosquito transmits D. immitis by bite to a susceptible host under controlled conditions. Ludlam et al. (1970) and Yen (1938) suggested additional criteria to be considered in the evaluation of a particular species as a vector of D. immitis. These include size of the vector population, habitat require-

ments, longevity, flight capability and range, and number of generations produced each year. No one has yet satisfied all these criteria for any of the potential vectors of dog heartworm.

This study was designed to elucidate the role Aedes trivittatus (Coquillett, 1902) plays as a vector of D. immitis in central Iowa. The objectives were: (1) to critically follow the development of D. immitis in Ae. trivittatus in the laboratory, (2) to determine the incidence of D. immitis infection in field-collected mosquitoes, (3) to relate this infection rate in mosquitoes with the acquisition of D. immitis infection in the susceptible dog population, (4) to determine the effect of D. immitis on the longevity and fecundity of Ae. trivittatus, and (5) to determine the ability of Ae. trivittatus to transmit D. immitis by bite.

# REVIEW OF LITERATURE

## Mosquito Vectors of *Dirofilaria immitis*

*Dirofilaria immitis* was originally described by Leidy in 1850 as *Canis cordis*, but association of adult *D. immitis* with microfilariae in the blood was not made until six years later (Leidy 1856). The ability of mosquitoes to function as vectors of filarial worms was first determined by Manson (1878) who established *Culex fatigans* as a vector of *Wuchereria bancrofti*. This monumental addition to the knowledge of parasitology eventually led to the experimental demonstration by Grassi and Noe (1900) of *D. immitis* development in mosquitoes. Bancroft (1904) verified the role mosquitoes play as vectors of *D. immitis* when he demonstrated transmission of the parasite to susceptible dogs by the bite of infected *Cx. fatigans*.

Conflict of opinion as to the actual vector of *D. immitis* arose, however, when Breinl (1921) reported the development of *D. immitis* within Malpighian tubules of two species of flea, *Ctenocephalides felis* and *C. canis*. Brown and Sheldon (1940) and Summers (1940, 1943) also reported development of *D. immitis* in fleas, although Brown and Sheldon discovered developing forms in the Malpighian tubules while Summers found them in the hemocoele. The most extensive study of fleas as vectors of *D. immitis* was conducted by Stueben (1954). He found *D. immitis* juveniles in advanced stages of development in 446 of 1203 *C. felis* collected from 71 dogs, and also reported that *C. canis*, *C. felis*, *Xenopsylla cheopis*, *Pulex irritans*, *Echidnophaga gallinacea*, and

Orchopeas wickhami supported development of D. immitis to the "infective-stage". None of these studies (Breinl 1921; Brown and Sheldon 1940; Summers 1940, 1943; Stueben 1954), however, verified the presence of D. immitis infections by identification of adult worms in dogs used to infect fleas. Dogs were only reported as having a microfilaremia, presumed to be D. immitis.

In other studies, fleas have been found resistant to infection with D. immitis. In many of these studies, adult D. immitis were identified following host necropsy. Grassi (1888) was the first to discredit fleas as vectors of dog heartworm. He examined several hundred fleas recovered from dogs shown at necropsy to have adult D. immitis in the heart, and found no fleas supporting juvenile development. Kosuge (1924), Joyeux and Sautet (1938), and Phillips (1939) also noted the inability of fleas, C. canis, to function as vectors of D. immitis.

Work on D. immitis in French Oceania by Rosen (1954) also showed fleas, Ctenocephalides sp., to be refractive to infection. He found only undeveloped microfilariae in the gut in any of 82 fleas examined. In Rosen's study, however, adult worms obtained from dogs used in experimentation were identified as D. immitis.

Work by Grassi and Calandruccio (1890) described the development of another dog filarial species, Dipetalonema reconditum, in the hemocoele of C. canis, C. felis, and P. irritans. Workers reporting fleas as vectors of D. immitis were evidently unaware of this study, for it was never cited in their publications. This fact was emphasized

by Rosen (1954) when he stated:

> . . . since the workers who have reported the development of D. immitis in fleas, apparently unaware of the data on D. reconditum, have described neither the adult filariae nor the microfilariae with which the dogs were infected, there would seem to be considerable doubt as to the ability of fleas to serve as intermediate hosts of D. immitis.

This led to the work by Newton and Wright (1956), and a clarification of the questions surrounding flea-D. immitis relationships. They reported the occurrence of a dog filarioid nematode (D. reconditum) other than D. immitis in the United States. Dipetalonema reconditum developed in the hemocoele of C. canis and C. felis, but failed to develop in Anopheles quadrimaculatus, and the reverse was found for these arthropods exposed to D. immitis (Newton and Wright 1956). It is now accepted that only mosquitoes function as vectors of D. immitis.

Since mosquitoes were first found to serve as intermediate hosts for D. immitis, numerous species of culicids have been reported as potential vectors of dog heartworm. Mosquitoes known to support complete development of D. immitis were summarized by Bemrick and Sandholm (1966) and Ludlam et al. (1970). These are included in a summarization of information through 1976 (Table 1).

Only 18 of 72 potential vector species have been reported naturally infected with third-stage juveniles ($J_3$) of D. immitis (Table 1). Aedes trivittatus and Cx. pipiens quinquefasciatus have been the only reported species found naturally infected in North America (Christensen and Andrews 1976; Villavaso and Steelman 1970). Bemrick and Sandholm (1966) recovered microfilariae and first-stage juveniles ($J_1$) of Dirofilaria from 5 Ae.

Table 1. Summary of the mosquito species known to support development of *Dirofilaria immitis* to third-stage juveniles

| Mosquito species | Experimental infection | Natural infection | Reference |
|---|---|---|---|
| *Aedes aegypti* | X | | Bancroft (1901) |
| *Aedes albopictus* | X | | Inoue, as cited in Ludlam et al. (1970) |
| *Aedes atropalpus* | X | | Keegan et al. (1968) |
| *Aedes canadensis* | X | | Hu (1931) |
| | | X[a] | Crans and Feldlaufer (1974) |
| *Aedes cantator* | | X[a] | Crans and Feldlaufer (1974) |
| *Aedes caspius* | X | | Grassi and Noe (1900) |
| *Aedes cinereus* | X | | Yen (1938) |
| *Aedes dorsalis* | X | | Weinmann and Garcia (1974) |
| *Aedes edgari* | X | | Rosen (1954) |
| *Aedes excrucians* | X | | Phillips (1939) |
| *Aedes fijiensis* | X | X | Symes (1960) |
| *Aedes fitchii* | X | | Bemrick and Sandholm (1966) |
| *Aedes geniculatus* | X | | Roubaud and Colas-Belcour (1937) |
| *Aedes guamensis* | X | | Travis (1947) |
| *Aedes infirmatus* | X | | Summers (1943) |
| *Aedes koreicus* | X | | Feng (1930) |
| *Aedes notoscriptus* | X | | Bemrick and Moorhouse (1968) |

[a]Juvenile identity questioned by the author reporting the natural infection of *D. immitis* from that mosquito species.

Table 1 (Continued)

| Mosquito species | Experimental infection | Natural infection | Reference |
|---|---|---|---|
| Aedes pandani | X | | Travis (1947) |
| Aedes pampaensis | X | | Heisch et al. (1959) |
| Aedes poecilus | X | X | Estrada (1965) |
| Aedes polynesiensis | X | X | Rosen (1954) |
| | | X | Ramalingam (1968) |
| | | X | Symes (1960) |
| Aedes pseudoscutellaris | X | X | Symes (1960) |
| Aedes punctor | X | X | Roubaud and Colas-Belcour (1937) |
| Aedes samoanus | | X | Ramalingam (1968) |
| Aedes sierrensis | X | | Weinmann and Garcia (1974) |
| Aedes sollicitans | X | | Hu (1931) |
| Aedes sticticus | X | | Bemrick and Sandholm (1966) |
| Aedes stimulans | X | | Yen (1938) |
| Aedes taeniorhynchus | X | | Hu (1931) |
| Aedes togoi | X | | Inoue, as cited in Ludlam et al. (1970) |
| | | X | Keegan et al. (1967) |
| Aedes triseriatus | X | | Phillips (1939) |
| Aedes trivittatus | X | X | Christensen and Andrews (1976) |
| Aedes vexans | X | | Hu (1931) |
| Aedes vigilax | X | | Bemrick and Moorhouse (1968) |
| Aedes zoosophus | X | | Keegan et al. (1968) |
| Anopheles crucians | X | | Summers (1943) |
| Anopheles earlei | X | | Yen (1938) |

Table 1 (Continued)

| Mosquito species | Experimental infection | Natural infection | Reference |
|---|---|---|---|
| *Anopheles franciscoi* | | X | Cabrera (1968) |
| *Anopheles freeborni* | X | | Kartman (1953a) |
| *Anopheles hyrcanus pseudopictus* | X | | Grassi and Noe (1900) |
| *Anopheles maculipennis* | X | | Grassi and Noe (1900) |
| *Anopheles minimus flavirostris* | | X | Cabrera (1968) |
| *Anopheles plumbeus* | X | | Roubaud and Colas-Belcour (1937) |
| *Anopheles punctipennis* | X | | Hu (1931) |
| *Anopheles quadrimaculatus* | X | | Phillips (1939) |
| *Anopheles sinensis* | X | | Feng (1930) |
| *Anopheles superpictus* | X | | Rosario (1936) |
| *Anopheles tessellatus* | | X | Estrada (1965) |
| *Anopheles walkeri* | X | | Bemrick and Sandholm (1966) |
| *Coquillettidia perturbans* | X | | Bemrick and Sandholm (1966) |
| *Culex annulirostris* | X | | Travis (1947) |
| | | X | Symes (1960) |
| *Culex bitaeniorhynchus* | X | X | Estrada (1965) |
| *Culex gelidus* | X | | Estrada (1965) |
| *Culex nigripalpus* | X | | Nayar and Sauerman (1975) |
| *Culex pipiens pipiens* | X | | Hu (1931) |
| *Culex pipiens molestus* | X | | Webber and Hawking (1955) |
| *Culex pipiens pallens* | X | | Inoue, as cited in Ludlam et al. (1970) |

Table 1 (Continued)

| Mosquito species | Experimental infection | Natural infection | Reference |
|---|---|---|---|
| Culex pipiens quinquefasciatus | X | | Bancroft (1901) |
| | | X | Villavaso and Steelman (1970) |
| Culex restuans | X | | Bemrick and Sandholm (1966) |
| Culex salinarius | X | | Seeley and Bickley (1974) |
| Culex sitiens | X | | Travis (1947) |
| Culex tarsalis | X | | Yen (1938) |
| Culex territans | X | | Hu (1931) |
| Culex tritaeniorhynchus | X | | Inoue,as cited in Ludlam et al. (1970) |
| | | X | Poynton and Hodgkin (1938) |
| Culex tritaeniorhychus summorosus | X | X | Estrada (1965) |
| Mansonia annulata | X | | Wharton (1962) |
| Mansonia bonneae | X | | Wharton (1962) |
| Mansonia dives | X | | Wharton (1962) |
| | | X | Poynton and Hodgkin (1938) |
| Mansonia indiana | | X | Poynton and Hodgkin (1938) |
| Mansonia titillans | X | | Bacigalupo, as cited in Ludlam et al. (1970) |
| Mansonia uniformis | | X | Estrada (1965) |
| Psorophora ferox | X | | Stueben (1954) |

vexans among 4,746 mosquitoes caught by light traps in an urban area of Minnesota. Bickley et al. (1976) recovered $J_3$, thought to be D. immitis, from field-collected mosquitoes in Maryland, but the species of mosquitoes harboring the $J_3$ was not indicated. Likewise, Crans and Feldlaufer (1974) found "infective-stage" filarial worms in field-collected Ae. canadensis, Ae. cantator, and Cx. salinarius in an area of New Jersey where D. immitis was endemic, but an identification of the filarial species was not made.

A paucity of information also exists on the ability of most potential vectors to transmit D. immitis to susceptible hosts by bite. In addition to the first report of successful transmission by Cx. fatigans (Bancroft 1904), only three other mosquito species have apparently been reported to transmit D. immitis by bite. In Japan, Kume and Itagaki (1955) recovered heartworms from two dogs exposed to the bite of infective Ae. togoi, and in Australia, Bemrick and Moorhouse (1968) reported successful transmission of D. immitis to two dogs by the bite of infective Ae. vigilax. In the United States, Newton (1957) exposed a dog to 55 An. quadrimaculatus heavily infected with D. immitis. Only eight mosquitoes fed, but D. immitis microfilariae were obtained from the dog after nine months postexposure.

Hinman (1935) failed in his attempts to transmit D. immitis by bite of infective Ae. aegypti, and suggested that $J_3$ were unable to escape the labium of infected mosquitoes. Similarly, Bickley (1976) reported Cx. salinarius incapable of transmitting D. immitis.

## Effects of *Dirofilaria immitis* on Mosquito Hosts

Filarial nematodes may be deleterious to their arthropod hosts, but evidence for this is based almost entirely on histological observations and mortality studies of parasitized arthropod populations (Lavoipierre 1958). Considerations of the effects *D. immitis* may have on such mosquito functions as fecundity and flight ability are lacking, despite the fact that such effects could greatly increase our understanding of the epidemiology of dirofilariasis.

Noe, as cited by Lavoipierre (1958), first described the histological damage occurring in mosquitoes infected with *D. immitis*, and his observations have been confirmed by later workers (Yen 1938; Taylor 1960). *Dirofilaria immitis* microfilariae penetrate cells of the Malpighian tubules and during development destroy the cytoplasm, cell membranes, and eventually the nuclei, until the parasites are enclosed in what appears as a clear tubule.

Verification that this damage is detrimental to the mosquito has most often been shown in mortality studies. Increased mortality due to *D. immitis* infection has been reported in controlled studies of several mosquito species; *Ae. aegypti* by Kershaw et al. (1953), *Ae. polynesiensis* by Rosen (1954), *Ae. sollicitans* by Beam (1966), and *An. quadrimaculatus* by Kartman (1953a) and Kutz and Dobson (1974).

It has been noted (Kartman 1953b) that mosquitoes feeding on a dog with a high microfilaremia will have a greater parasite burden and a

higher mortality than those feeding on a host with a low microfilaremia of D. immitis.

Kartman reported 47.6% survival at 15 days postexposure of An. quadrimaculatus exposed to a dog (S) with from 16,000 to 18,000 D. immitis microfilariae per $cm^3$ blood, but only a 5% survival at 15 days postexposure for those exposed to a dog (D) with 30,000 to 34,000 microfilariae per $cm^3$ blood. Anopheles quadrimaculatus fed on dogs S and D contained a mean number of 17.3 and 93.3 D. immitis juveniles per mosquito, respectively.

Kutz and Dobson (1974) showed an increased mortality rate in D. immitis infected An. quadrimaculatus at temperatures supporting juvenile development. They concluded that the greatest mortality occurred when "infective-stage" juveniles migrated from the Malpighian tubules to the head and labium.

No data are evident on what effects, if any, D. immitis has on the fecundity of mosquito vectors. Wülker (1964) cited several examples of nematodes causing a decrease in fecundity of their insect hosts, although in most cases cited, the nematode species parasitized the hemocoele and caused a physical suppression or destruction of gonadal tissue. Hacker (1971), however, showed a reduced fecundity of Ae. aegypti infected with the hemosporidan, Plasmodium gallinaceum, a parasite that does not invade gonadal tissue.

The only report evident in the literature on filarial worms as they affect vector fecundity is that of Javadian and Macdonald (1974). They

studied Ae. aegypti infected with either Brugia pahangi or Dirofilaria repens. Egg production was not significantly different between control mosquitoes and those infected with B. pahangi. Following a second blood meal (uninfective), however, there was a significant decrease in the number of eggs produced by infected Ae. aegypti. Mosquitoes infected with D. repens had a significantly reduced egg production at both the first and second egg laying. In both mosquitoes infected with B. pahangi and in those infected with D. repens, there was a negative linear correlation between parasite numbers and the number of eggs produced following infective and uninfective blood meals.

Although no literature deals with D. immitis infections and mosquito egg production, data provided by Javadian and Macdonald (1974) on D. repens, a filarial worm developing in the Malpighian tubules of mosquitoes, suggest that D. immitis may also be detrimental to the fecundity of its mosquito vectors.

The effect of a filarial infection on a mosquito's ability to fly determines to a great extent the ability of that mosquito to function as a successful vector under natural conditions. Despite this fact, few studies have evaluated the effect of filarial infections on mosquito flight ability. Townson (1970) reported a significantly greater number of nonflying mosquitoes among Ae. aegypti infected with B. pahangi than among uninfected controls. He also reported a significantly higher parasite burden in infected nonflying than infected flying mosquitoes. In wind tunnel experiments flight duration decreased in infected compared with uninfected Ae. aegypti, although significance was measured at the

93.5% probability level.

A recent study involved the same parasite-vector system, but used a flight mill system which produced more quantitative data (Hockmeyer et al. 1975). Infected mosquitoes flew significantly shorter distances, had a reduced total flight time during 24 hr flight tests, and there were more nonflyers in the population than in uninfected controls. Hockmeyer et al. (1975) concluded that the reduced flight ability of Ae. aegypti infected with B. pahangi decreased this mosquito's ability to survive and transmit its infection.

No experimental work has been done with filarial worms developing in tissues other than the flight muscles of mosquitoes. The apparent disruption of excretory function due to Malpighian tubule destruction in D. immitis-infected mosquitoes warrants quantitative investigations on the effects this disruption might have on a mosquito vector's flight ability.

## Effects of Mosquito Hosts on *Dirofilaria immitis*

The susceptibility of mosquitoes to filarial infections varies among species and even among strains of the same species. Roubaud (1937) reported a greater susceptibility of certain strains of Ae. aegypti to D. immitis infections, and suggested that susceptibility was an inherited character. Likewise, Kartman (1953a) compared susceptibilities of various strains of Ae. aegypti, Ae. albopictus, Cx. pipiens pipiens, and Cx. p. fatigans to D. immitis, and was able to obtain by mass

selection, strains of Ae. aegypti more refractive and more susceptible to D. immitis.

In a series of studies, Macdonald (1962a, 1962b, 1963) clarified the mode of inheritance of susceptibility of Ae. aegypti (Liverpool strain) to semiperiodic B. malayi. From an initial population of Ae. aegypti, showing a 17 to 31% susceptibility to B. malayi, Macdonald (1962a), by familial selection through 15 generations, obtained a strain with a mean susceptibility of 84.4%. Through a series of crosses and backcrosses between this highly susceptible strain of Ae. aegypti and uniformly refractory strains, and by testing the offspring (F1, F2, and backcross) for susceptibility to B. malayi, Macdonald (1962b) showed that this susceptibility was controlled by a sex-linked recessive gene. This was designated $f^m$, for filarial susceptibility (B. malayi).

Macdonald (1963) then tested refractory individuals from his highly susceptible strain and found that they also were homozygous for $f^m$, therefore showing that $f^m$ does not entirely control susceptibility. It was therefore concluded that there must also be modifying genes present, and that $f^m$ was the major factor, but did not entirely control susceptibility.

Macdonald and Ramachandran (1965) demonstrated that the $f^m$ gene in Ae. aegypti also controlled susceptibility to periodic B. malayi, B. pahangi, periodic W. bancrofti and sub-periodic W. bancrofti, but had no influence on susceptibility to D. immitis or D. repens. The fact that

$f^m$ homozygotes are susceptible to all muscle inhabiting filarial species tested was discussed by Barr (1975). He suggested that selection for the $f^m$ homozygote disrupted a basic defense mechanism of the mosquito, for natural vectors of one muscle-parasitizing filarial species are usually resistant to other, similar species.

Raghavan et al. (1967) showed that susceptibility of Ae. aegypti to D. immitis infections is inheritable, thus verifying the earlier suggestion made by Roubaud (1937). Zielke (1973) reported that susceptibility of Ae. aegypti to D. immitis was also controlled by a sex-linked recessive gene, but not $f^m$. He reported the gene responsible for D. immitis susceptibility was at a different locus. McGreevy et al. (1974) reported similar findings and designated the sex-linked recessive gene responsible for D. immitis susceptibility as ft.

Although the mode of action of genes $f^m$ and ft is not understood, a report from India, cited by Macdonald (1976), suggests that gene ft may influence alkaline phosphatase activity in Ae. aegypti. High levels of alkaline phosphatase activity were recorded in ft homozygotes, and no marked differences were noted in acid phosphatase levels in susceptible and refractory Ae. aegypti. Possible effects this increased or decreased enzyme level might have on D. immitis or D. repens juveniles were not discussed.

The destruction of microfilariae in the midgut of mosquitoes has been frequently reported. Additional reports indicate that this destruction is more common in certain genera of mosquitoes. Travis (1947) noted that D. immitis microfilariae remaining in the midgut of Culex

mosquitoes were all dead two days after exposure, but that a large percentage were still alive in Aedes species. Kartman (1953a) demonstrated that some microfilariae of D. immitis remain alive for at least 72 hours in the midgut of Ae. aegypti and Ae. albopictus, but are rapidly destroyed in Cx. quinquefasciatus and Cx. pipiens. Wuchereria bancrofti microfilariae were also reported to be destroyed after one day in the midgut of Culex species (O'Connor and Beatty 1938).

Kartman (1953a) suggested that microfilariae are digested by the mosquito, and that only dead worms are digested. He hypothesized that some factor in the salivary glands or midgut of Cx. pipiens and Cx. quinquefasciatus, but not Ae. aegypti or Ae. albopictus, was capable of killing microfilariae before they were digested.

Coluzzi and Trabucchi (1968) investigated the mechanism by which D. repens microfilariae were rapidly destroyed in the midgut of Anopheles and Culex mosquitoes. They tested 18 species of mosquitoes and found physically damaged microfilariae in 7 of these (An. farauti, An. stephensi, An. superpictus, An. gambiae, An. pharoensis, An. albimanus, and Cx. pipiens). Because these species all have a well developed bucco-pharyngeal armature, it was proposed that many microfilariae are physically damaged by the armature, and therefore susceptible to digestion by the mosquito. These 7 species were found either not infected with D. repens or infected with very few juveniles. Microfilariae introduced into the gut through the anus, however, were undamaged, and even completely resistant species became infected. When

these mosquitoes ingested smaller microfilariae (Eufilaria sergenti), only a very few were found damaged.

Bryan et al. (1974) described the bucco-pharyngeal armature as consisting of the cibarial armature, a row of well developed teeth projecting into the anterior lumen of the cibarial pump, and the pharyngeal armature, which is a ring of slender teeth at the posterior end of the pharynx. Mosquitoes with both armatures (An. gambiae and An. farauti) had a much greater proportion of damaged B. pahangi microfilariae than did mosquitoes with only a pharyngeal armature (Ae. aegypti and Ae. togoi) (Bryan et al. 1974).

Shortly after mosquitoes engorge with blood, the midgut epithelial cells begin to lay down an amorphous layer of material eventually solidifying to form a hard, inelastic membrane separating the blood bolus from the epithelium (Orihel 1975). This layer, the peritrophic membrane, has often been considered a barrier which might hinder parasitic infections. The peritrophic membrane, however, has not been shown to limit the migration of microfilariae from the midgut. Esslinger (1962) and Ewert (1965) reported that B. pahangi migrated through the midgut before the peritrophic membrane had hardened. Similar findings have been reported for B. malayi in Ae. aegypti by Ramachandran (1966) and in M. longipalpis by Wharton (1957a).

Filarial worms developing in the Malpighian tubules do not penetrate the midgut, but only migrate to openings of the tubules. Kartman (1953a) reported that clot formation could prevent or hinder this

migration in the Culex and Aedes species he exposed to D. immitis. Anopheles species he studied had a very slow clot formation and microfilariae migrated rapidly to the tubules. By adding anticoagulant to the infective blood meal, he obtained rates of microfilarial migrations in Ae. aegypti similar to those in An. quadrimaculatus.

Lavoipierre (1958) stated that the peritrophic membrane of Ae. aegypti was completely destroyed by heavy infections of D. immitis microfilariae, therefore making digestion of blood impossible, and killing the mosquito host.

The most commonly recorded defense mechanism of mosquitoes against filarial infections is that of encapsulation (Lavoipierre 1958). Capsule formation occurs principally by aggregation and adhesion of hemocytes over foreign particles too large for phagocytosis by individual hemocytes (Salt 1970; Nappi 1975). This reaction is generally accompanied by oxidation and polymerization of phenols (tyrosine and dopa) by phenoloxidases to form melanin (Lipke 1975; Nappi 1975).

Encapsulation was first thought to occur only around dead parasites (Brug 1932; Hu 1939a). Later work by Hu (1939b), Highby (1943), and Kartman (1953a), however, demonstrated encapsulation of living juveniles in several mosquito species. Encapsulation and melanization have since been considered mosquito defense mechanisms. Kartman (1953a), however, from his work with Ae. aegypti, An. quadrimaculatus, Cx. quinquefasciatus, and D. immitis, stated:

> The phenomena of filarial encapsulation apparently was of little consequence to the developmental ability of D. immitis in either of the three species under consideration, although it appeared to be a consistent reaction to the parasite in the Malpighian tubules of A. aegypti. Likewise, the presence of degenerate larval forms of D. immitis in A. quadrimaculatus appeared to be of no quantitative significance.

Although numerous reports of encapsulation or "chitinisation" of filarial juveniles by mosquito hosts have been published (Sambon 1902; Brug 1932, Yen 1938; Hu 1939a,b; Highby 1943; Newton et al. 1945; Kartman 1953a,b; Lavoipierre 1958; Esslinger 1962; Intermill 1973), such studies have not determined what effect, if any, this reaction has on the capability of a mosquito to function as a vector.

# MATERIALS AND METHODS

## Maintenance of Aedes trivittatus

Aedes trivittatus used in these experiments were reared from eggs obtained from adult females field-collected at the Iowa State Nursery, Ames, Iowa. Most adult females were field-collected with a mouth aspirator after they had blood fed on a human host, but some Ae. trivittatus were blood fed in the laboratory on a rabbit or dog following field-collection. Mosquitoes were maintained in lots of 50 in 0.473 ℓ ice cream cartons with fine-mesh marquisette coverings. Cotton pads, moistened in 0.3 M sucrose solution, were placed on the marquisette.

One to six mosquitoes were transferred to individual oviposition cages three days after blood feeding (Horsfall et al. 1973). Cages were placed on a wet cheesecloth substrate in white enamel pans (25 x 42 x 7 cm) to allow mosquitoes to oviposit. Eggs, collected with a Pasteur pipette, were counted, and transferred to moist filter paper in petri dishes. Eggs were maintained at 26.5 ± 1° C and 80 ± 5% RH for 14 days to allow for embryonation, and were then stored at 4° C for future use. Two weeks before hatching, eggs were removed from refrigeration and conditioned at 26.5 ± 1° C and 80 ± 5% RH.

Two steps were involved in hatching Ae. trivittatus eggs: (1) Eggs were immersed in deoxygenated water for 2 hr, and larvae were then removed and counted, and (2) remaining eggs were transferred to a mild nutrient broth (approximately 1 part nutrient broth: 5000 parts water) for 12 to 14 hr (overnight). Larvae were removed and counted,

and remaining unhatched eggs were discarded.

Larvae were reared in lots of 200 in deionized water (1.0 to 1.5-cm deep) in white enamel trays. Eight pinches of sterile, washed sand were dispersed evenly in each tray. Larvae were fed a slurry of finely-ground fish food (Tetramin® ) pipetted on top of the sand in amounts that mosquitoes would consume in 24 to 36 hr.

Pupae were harvested on developmental days six and seven, and lots of 50 were placed in deionized water in ice cream cartons with marquisette coverings. After emergence, water was drained, and mosquitoes were lightly anesthetized with $CO_2$ and separated according to sex on a refrigerated cold table. Adult mosquitoes were maintained on sucrose pads that were changed every 48 hr. Sucrose was removed from mosquitoes 24 hr before blood feeding.

All Ae. trivittatus developmental stages were maintained at 26.5 ± 1° C and 80 ± 5% RH during all studies. Mosquitoes were reared in a 16 hr photoperiod with a 90 min crepuscular period at the beginning and end of each light period.

## Development and Transmission Studies

A female dog (02), experimentally-infected 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. 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. Approximately 30 min before anesthesia, 1.0 ml Atropine-$SO_4$ was administered SQ to reduce salivation.

Five day old mosquitoes were exposed to D. immitis by allowing them to feed on dog 02 through the marquisette for 15 min. Two 20-$mm^3$ blood samples were taken immediately before feeding, and the mean microfilaremia was determined. Blood-fed mosquitoes were separated and placed in lots of 50 in clean cartons.

Mosquitoes were dissected at 6, 9, and 12 hr, and daily until day 14 postexposure (PE). All dissections were done in Aedes saline (Hayes 1953) at 20X under a dissecting microscope. Malpighian tubules were examined at 100 to 400X with bright field or phase contrast optics. Developing stages of D. immitis were fixed without pressure in hot 70% glycerin alcohol, and mounted in glycerin or glycerin jelly. Blood remaining in mosquito midguts was smeared on a slide, methanol fixed, and stained with Giemsa to determine the number of microfilariae present. Sectioned material was fixed in Bouin's or 10% buffered formalin, dehydrated in tertiary butyl alcohol, and embedded in paraffin. Sections were stained in hematoxylin and eosin. Measurements and illustrations were accomplished with the aid of an ocular micrometer. All measurements were recorded in micrometers.

The viability of $J_3$ and the ability of Ae. trivittatus to transmit D. immitis was ascertained as follows. At 16 days PE to dog 02, mosquitoes were dissected in Earle's solution, and 100 $J_3$ were in-

oculated SQ with a tuberculin syringe on the medial surface of the hind leg of a mongrel male dog (F4). Also, at 16 days PE, 49 Ae. trivittatus were blood fed on a mongrel female dog (F5). Both dogs (F4 and F5) were whelped during the winter and had been maintained in a mosquito-proof, indoor mammal room. A modified Knott's technique was used to check dogs for patent infections at approximately two week intervals beginning five months PE. All blood samples were taken at approximately 1600 hr.

## Vector Mortality Studies

Five day old Ae. trivittatus were exposed to either dog 02, dog F5, or dog F6 (uninfected) by the method previously described. Two 20-$mm^3$ blood samples were taken from each dog immediately before feeding and mean microfilaremias were determined. Additional mosquitoes were not blood fed, but were maintained on sucrose to determine the effect blood feeding had on mosquito mortality. Experimental groups were maintained in separate lots of 50 in ice cream cartons with marquisette coverings.

Mosquitoes were examined daily and dead individuals were counted and removed. Dead Ae. trivittatus previously exposed to D. immitis were dissected as described above and the number, location, and developmental stage of D. immitis juveniles recovered were recorded for each mosquito. These data could not be determined for all mosquitoes, because a few mosquitoes had been dead too long and the resulting desiccation made accurate dissections impossible. Mosquitoes were

checked in the above manner until all individuals were dead. Daily percentage mortalities and cumulative percentage mortalities were calculated.

## Vector Fecundity Studies

Aedes trivittatus do not mate naturally in captivity; therefore, mosquitoes used in this study were manually copulated. The method used was a modification of several previously reported techniques (McDaniel and Horsfall 1957, Hayes 1953; Ow Yang et al. 1963; Baker 1964; Hayes 1968; Horsfall et al. 1973).

Adult Ae. trivittatus were separated according to sex at one day postemergence, and maintained on sucrose until they were four to five days old. At this time, males were collected with an aspirator and gently blown between layers of cotton. A minuten pin attached to a wooden applicator stick was inserted through the thorax, and the male's legs, wings and head were removed. Three to five males were prepared at one time in this manner. Females were aspirated into plastic tubes, lightly anesthetized with nitrogen gas, and placed ventral side up under a dissecting microscope. A pinned male mosquito was presented, ventral side down, at about a 45° angle to the female. After copulation was initiated, the pair was lifted off the substrate and held until the male released the female. A minimum of 5 sec mating time was required before females were considered to be inseminated. Individual males were used to mate a maximum of two females. Mated females were transferred to

clean cartons for subsequent blood feeding.

One to two days after mating, female Ae. trivittatus were divided into two groups; one group was exposed to a D. immitis-infective blood meal (dogs 02 or F5), and the other group to an uninfective blood meal (dog F6). Mosquitoes that had "fully engorged" on blood were separated and placed singly in oviposition cages on a wet cheesecloth substrate.

Eggs laid by individual mosquitoes were collected and counted daily. Attempts were made to blood feed (on an uninfected rabbit) all mosquitoes that had oviposited once, to allow production of a second egg batch. Dead mosquitoes, as well as those that failed to feed on the rabbit host, or those that oviposited a second time were dissected, and the number of eggs remaining in the ovaries was recorded. In addition, the number of D. immitis juveniles in individual mosquitoes was determined for those females exposed to a D. immitis-infective blood meal.

## Field Isolations

Seven $CO_2$-baited CDC miniature light traps were placed in a residential area of Ames, Iowa. This area was known to contain at least one dog with a microfilaremia of D. immitis. Traps were set 1/2 hr before sunset and collected at sunrise for 10 days during the period 28 July 1975 through 8 August 1975.

Mosquitoes were taken to the laboratory, lightly anesthetized with diethyl ether, and separated according to species. Twenty-one to 60

randomly selected Ae. trivittatus females were dissected daily. Other species in which a blood meal was visible or that occurred in very low numbers also were dissected. All dissections were accomplished as previously described. Those mosquitoes that could not be dissected were pooled according to species, with the maximum number of mosquitoes in any pool being 64.

Pools of mosquitoes were lightly crushed with mortar and pestle in 1 to 2 ml of Earle's solution. Lightly crushed mosquitoes were transferred to a cup-shaped screen suspended in Earle's solution in a glass funnel with clear plastic tubing and a pinch-cock at the bottom. Approximately 2-ml samples were drawn at 1-hr intervals for 3 hr and examined under a dissecting microscope at 20X and 30X magnification. This method was modified from that reported by Muller and Denham (1974).

All juveniles recovered were fixed in hot 70% glycerin alcohol and mounted in glycerin jelly. Measurements were made with an ocular micrometer. All measurements were recorded in micrometers.

## Epidemiology Studies

A survey of nearly all dogs found in that area of Ames, Iowa, where mosquitoes had been collected for field-isolations of D. immitis was conducted in August and September, 1975. All dogs negative for filariasis in 1975 were examined again in May 1976. Blood samples (1.0 ml) were drawn from the radial vein in the forelimb and examined

for microfilariae by a modified Knott's technique. After centrifugation, the bottom 0.5 ml of fluid and sediment was spread on a slide, and the wet mount was examined by using phase contrast optics at 100X. After drying, these slides were methanol fixed and stored for future reference. Differentiation of microfilariae was based on criteria reported by Lindsey (1965). Data on age, sex, breed, past history, and type of housing were recorded for each animal examined. Dogs were classified as to type of housing as follows: (1) predominantly outside, (2) predominantly inside, and (3) both inside and outside.

## BIOLOGY OF AEDES TRIVITTATUS

Aedes (Ochlerotatus) trivittatus is a medium sized, floodwater mosquito, readily identified by the following morphological characteristics: (1) a pair of submedian stripes of yellowish-white scales on the mesonotum, separated by an equally wide brown stripe; (2) 1st abdominal tergite entirely dark scaled and remaining tergites dark with basolateral patches of white scales; and (3) legs and wings all dark scaled.

Aedes trivittatus has been reported from 39 states in the continental United States, southern Canada, and parts of Mexico and Panama (Carpenter and LaCasse 1955; Carpenter 1968; 1970; Howard et al. 1917; Trimble 1972). It is found throughout Iowa (Rowe 1942; Wong et al. 1970; Pinger and Rowley 1972) and can be the most abundant species in certain areas (Wong et al. 1970). Aedes trivittatus has been reported as the 2nd most abundant mosquito collected in $CO_2$-baited CDC light traps set primarily near urban areas of Iowa (Wong et al. 1970) and the 3rd most abundant species collected in New Jersey light traps in rural Iowa (Pinger and Rowley 1972). In the Ames, Iowa area, Ae. trivittatus is often the most abundant species collected (unpublished data).

Observations on the biology of Ae. trivittatus have been made by Rowe (1942), Abdel-Malek (1948a, 1948b), Horsfall et al. (1958), Wright and Knight (1966), and Pinger and Rowley (1975). Abdel-Malek (1948a) reported that under field conditions, about 1 day was required for each of the 1st, 2nd, and 3rd instars to change from one stage to the next,

and that the 4th instar persisted for about 3-4 days before pupation. The pupal stage lasted for 1 day or less before adult emergence occurred; therefore, at 18-21° C in the field, the minimum period from egg hatching to adult emergence was reported as 8 days (Abdel-Malek 1948a). At 26.5° C in the laboratory, the minimum period from hatching of the egg to emergence of adults was 6 days (Abdel-Malek 1948a).

Larval habitats include rain pools, creek floodwater, woodland pools, semipermanent ponds, and intermittent marshes, all of which are temporary or intermittent in nature (Rowe 1942). Eggs are deposited at the water line and will hatch when the area is inundated with water, provided a drying period sufficient for completion of embryogeny has occurred (Rowe 1942; Abdel-Malek 1948a). Aedes trivittatus overwinters in the egg stage and adults first appear in the Ames area in mid- to late May (unpublished data). This species is multivoltine, producing 2, 3, or more generations each year in central Iowa (Rowe 1942; unpublished data).

Females have been reported to blood feed in the shade and in full sunlight, from early morning until 11 at night (Rowe 1942). Wright and Knight (1966), however, showed that peak biting activity occurs during the crepuscular period, from 1/2 hr before, until 45 min after sunset. Pinger and Rowley (1975) reported that Ae. trivittatus is mainly zoophilic, feeding primarily on Sylvilagus floridanus and other mammals in the area studied. Only 3.0% of the blood meals from field-collected females reacted to anti-bird or anti-amphibian-reptile antisera (Pinger and Rowley 1975).

Field-collected and laboratory-reared Ae. trivittatus used in this study yielded much additional information on the biology and laboratory maintenance of this mosquito. A total of 1,247 adult females were field-collected and blood fed on either a human or rabbit host (Table 2). An average of 62 eggs per mosquito was obtained with each blood meal type (Table 2).

Table 2. Egg production of field-collected Aedes trivittatus blood fed on a human or rabbit host

| Host | Number of mosquitoes | Number of eggs | Mean number of eggs/mosquito |
|---|---|---|---|
| Human | 699 | 43,332 | 62 |
| Rabbit | 548 | 34,419 | 62 |

Records were maintained on the number of larvae hatching during 7 different experiments involving 27,895 eggs. Percentage of eggs hatching averaged 49% and ranged from 30% to 57% (Table 3). The percentage of viable eggs hatching was probably much greater, for no attempt was made to remove damaged or unembryonated eggs. Eggs stored at 4° C in the laboratory remained viable for at least 10 months.

Mortality was determined for 12,676 larvae and 5,602 pupae using the rearing methods described for this study. In six experiments, a mean of 80% of the larvae survived, with a range of 61% to 87% (Table 4). Pupal mortality was much lower, with 97% surviving through adult

Table 3. Hatchability of Aedes trivittatus eggs obtained from field-collected adults

| Experiment number | Number eggs | Number hatching | Percent hatching | Storage condition (°C) |
|---|---|---|---|---|
| 1 | 2302 | 1175 | 51.0 | 26.5[a] |
| 2 | 2050 | 610 | 29.8 | 26.5 |
| 3 | 2349 | 981 | 41.8 | 26.5 |
| 4 | 10108 | 5223 | 51.7 | 4.0[b] |
| 5 | 4374 | 2489 | 56.9 | 4.0 |
| 6 | 2612 | 1475 | 56.5 | 4.0 |
| 7 | 4100 | 1781 | 43.4 | 4.0 |
| Total | 27895 | 13734 | 49.2 | - |

[a]Eggs stored for 14 days before hatching.

[b]Eggs maintained at 26.5° C for 14 days after removal from refrigeration.

Table 4. Larval mortality of Aedes trivittatus reared in the laboratory

| Number of larvae | Number pupating | % larval mortality |
|---|---|---|
| 361 | 309 | 14.4 |
| 522 | 320 | 38.7 |
| 2766 | 1864 | 32.6 |
| 5117 | 4472 | 12.6 |
| 2459 | 2024 | 17.7 |
| 1451 | 1130 | 22.1 |
| 12676 | 10119 | 20.2 |

emergence (Table 5).

Longevity of adult Ae. trivittatus maintained on 0.3 M sucrose solution at 25 ± 1° C and 80 ± 5% RH was determined in the laboratory (Fig. 38). At time zero, mosquitoes were actually five days old, for this

Table 5. Pupal mortality and adult sex ratios of Aedes trivittatus reared in the laboratory

| Number of pupae | Number of adults | % pupal mortality | Number of males | Number of females | Sex ratio o:o |
|---|---|---|---|---|---|
| 4472 | 4376 | 2.1 | 2198 | 2178 | 50.2:49.8 |
| 1130 | 1048 | 7.3 | 514 | 534 | 49.0:51.0 |
| 5602 | 5424 | 3.2 | 2712 | 2712 | 50.0:50.0 |

experiment was run in conjunction with longevity experiments on mosquitoes infected with D. immitis. It should be noted that more than 96% of the males had died after 27 days, but 50% of the females were still alive. Although all males were dead in 40-45 days, females survived for at least 60 days (Fig. 10).

During July and August, 1976, 194 field-collected adult female Ae. trivittatus were dissected and state of parity was determined (Table 6). Over 70% of the mosquitoes examined had oviposited one time. Ten percent had laid two egg batches. Less than 7% were nulparous. One mosquito was multiparous, having laid six egg batches.

The bionomics of Ae. trivittatus, as known at this time, suggests that this mosquito is well suited to function as a vector of D. immitis in Iowa. It occurs in high populations, is zoophilic, multivoltine, long-lived, and takes several blood meals during its lifetime. In addition, preliminary studies have shown Ae. trivittatus

Table 6. Parity of Aedes trivittatus field collected at the State Nursery, Ames, Iowa, during July and August, 1976[a]

| Date | Number | Uniparous | Biparous | Triparous | Multiparous | Gravid | Nulparous |
|---|---|---|---|---|---|---|---|
| 16 Jul | 27 | 20 | 1 | 0 | 0 | 4 | 4 |
| 19 Jul | 5 | 2 | 1 | 0 | 0 | 2 | 0 |
| 23 Jul | 43 | 29 | 7 | 1 | 0 | 1 | 5 |
| 26 Jul | 44 | 32 | 6 | 1 | 0 | 5 | 0 |
| 3 Aug | 14[b] | 12 | 0 | 0 | 0 | 0 | 2 |
| 5 Aug | 9 | 7 | 2 | 0 | 0 | 0 | 0 |
| 10 Aug | 13 | 12 | 0 | 0 | 0 | 0 | 0 |
| 11 Aug | 21 | 15 | 4 | 0 | 1 | 1 | 0 |
| 12 Aug | 6 | 3 | 1 | 0 | 0 | 0 | 2 |
| 20 Aug | 12 | 8 | 2 | 0 | 0 | 1 | 1 |
| Total | 194 | 140 | 24 | 2 | 1 | 14 | 13 |
| % of total | 72.2 | 12.4 | 1.0 | 0.5 | 7.2 | 6.7 | |

[a]Unpublished data collected by the Medical Entomology Laboratory, Iowa State University.

[b]Extremely dry weather conditions prevailed during July and August and only minimal numbers of mosquitoes were available for August collections.

to be capable of flying over 30,000 m in a 24 hr period on a flight mill system in the laboratory (unpublished data); therefore, this mosquito probably has the ability to disperse some distance to obtain a blood meal or to oviposit.

## DEVELOPMENT AND TRANSMISSION

Seventy-six percent (931/1,217) of the Ae. trivittatus blood fed on a dog with a mean microfilaremia of 347 microfilariae/20 $mm^3$. Microfilariae were active and evenly distributed in the midgut at 4 hr PE, but no microfilariae were observed in the Malpighian tubules. The peritrophic membrane had formed, and the most active microfilariae were noted between it and the midgut epithelium. Microfilariae were first recovered from the Malpighian tubules at 9 hr PE, and in one mosquito, microfilariae had reached the distal ends of the tubules. In the midgut, microfilariae were more numerous posteriorly, near the junction of hindgut and midgut. At 12 hr PE, microfilariae were in the Malpighian tubules of all mosquitoes examined.

Sixteen mosquitoes were dissected 24 hr PE. Dimensions of juveniles and percentage of infection are given in Tables 7 and 8. Microfilariae were observed actively penetrating the cells of the Malpighian tubules and in three mosquitoes, they were observed passing down the hindgut. The maximum number of microfilariae recovered from the Malpighian tubules in one mosquito was 24.

Juveniles recovered from mosquitoes 2 days PE were definitely shorter and broader (Table 7). The internal morphology, however, was similar to that of the microfilariae (Fig. 1). The G-cells were undivided (Figs. 1 and 14), and the excretory cell and excretory pore were not contiguous (Figs. 1 and 15). Microfilariae were observed passing out the hindgut in 5 of 20 mosquitoes examined. In the

largest infection, a single mosquito contained 78 juveniles in the Malpighian tubules, with 10 microfilariae in the hindgut. Most microfilariae remaining in the midgut were still active.

Changes in the development of the juveniles were pronounced by day 3 PE. Juveniles were shorter and wider (Table 7), and in most,

Table 7. Measurements (μm) of developmental stages of Dirofilaria immitis from experimentally infected Aedes trivittatus

| Days post-exposure | Number measured | Juvenile stage | Mean length ± S.D. | Mean width ± S.D. (anterior-posterior) |
|---|---|---|---|---|
| 1 | 7 | $J_1$ | 235 ± 26.4 | 5.6 ± 0.51 |
| 2 | 25 | $J_1$ | 196 ± 17.6 | 6.3 ± 0.76 - 8.9 ± 1.19 |
| 3 | 25 | $J_1$ | 154 ± 7.5 | 11.2 ± 1.64 - 15.7 ± 1.95 |
| 4 | 25 | $J_1$ | 150 ± 6.8 | 17.5 ± 2.34 - 21.6 ± 2.41 |
| 5 | 21 | $J_1$ | 205 ± 7.3 | 20.8 ± 1.72 - 24.0 ± 2.05 |
| 6 | 25 | $J_1$ | 249 ± 17.9 | 23.3 ± 2.39 ± 27.2 ± 2.31 |
| 7 | 7 | $J_2$ | 259 ± 8.8 | 27.7 ± 1.60 - 30.6 ± 1.13 |
| 8 | 31 | $J_2$ | 433 ± 33.4 | 31.1 ± 2.15 - 33.7 ± 1.92 |
| 9 | 20 | $J_2$ | 559 ± 41.6 | 30.5 ± 2.13 - 36.0 ± 1.78 |
| 11 | 10 | $J_3$ | 854 ± 29.0 | 28.3 ± 3.42 |
| 12 | 13 | $J_3$ | 964 ± 33.3 | 26.0 ± 1.65 |
| 14 | 25 | $J_3$ | 956 ± 63.1 | 23.4 ± 2.91 |

G-cells 1-3 had divided (Fig. 2). The excretory cell was closer to the excretory pore, and the muscle or hypodermal cells were differentiated under certain areas of the body wall (Fig. 2). Seventeen of 18 Ae. trivittatus dissected had blood in the midgut, and all contained microfilariae, many of them still active. Active microfilariae were also noted in the hindgut of five mosquitoes and in the lumen of the proximal portion of the Malpighian tubules of three of these. Active microfilariae, in the Malpighian tubules, were noted through developmental day six and seemed to have emerged from the digested blood as it passed out the hindgut.

First-stage juveniles, by 4 days PE, had acquired their minimum length and were "sausage-shaped" (Table 7; Fig. 3). G-cells 1-3 had further subdivided, and G-4 cell had divided and extended to form the anal plug (Figs. 3 and 16). Muscle cells were well delineated from other cells, and the excretory cell was in close proximity to the excretory pore (Fig. 3). Ten of 20 mosquitoes had remnants of a blood meal in the midgut, but nine of these contained microfilariae (Table 8), and three had active microfilariae in the hindgut. The only negative mosquito found during this study was dissected at 4 days PE, and although no blood was evident in the midgut, eggs were almost fully developed.

At 5 days PE, $J_1$ were increasing in length (Table 7). Formation of the pseudocoele, esophagus, intestine, and rectum was evident, and

cells of the nerve ring were less conspicuous (Figs. 4 and 17) and the cuticle was easily distinguishable (Fig. 4). A portion of the blood meal persisted in six mosquitoes and all contained some active microfilariae (Table 8). Increased size and further structural differentiation of $J_1$ was evident at day 6 PE (Table 7; Fig. 5). Juveniles were similar in length as those observed at day 1 PE, but were more robust. The esophageal lumen was evident, as was a beginning of the intestinal lumen. At this time the nerve ring appeared as a fibrous band, just anterior to the excretory complex. Infected Malpighian tubules were swollen and clear, in contrast to uninfected tubules (Fig. 10). Juveniles within Malpighian tubules at day 6 PE are shown in section in Fig. 24. Although two mosquitoes had remnants of a blood meal (Table 8), microfilariae present were not active, but active microfilariae were observed in the lumen of the Malpighian tubules.

At 7 days PE juveniles were noticeably wider (Table 7). In a few specimens, the buccal cavity was visible with an open stoma (Fig. 19). The long cuticular tail extension was no longer evident (Fig. 6). Juveniles with these characteristics were judged to have molted (Fig. 18). They were not included in Table 8 because they occurred in very low numbers. Several juveniles were observed in the process of molting on the 7th day PE.

At days 8-10 PE, most mosquitoes contained $J_2$ (Table 8), which had greatly increased in length in contrast with those seen on day 7 PE (Table 7). By day 9 PE, juveniles had reached their maximum width

Table 8. Mean number of Dirofilaria immitis juveniles recovered from experimentally infected Aedes trivittatus at various days postexposure (PE)

| Days PE | $\bar{X}$ No. $J_1$/ mosquito | $\bar{X}$ No. $J_2$/ mosquito | $\bar{X}$ No. $J_3$/ mosquito | $\bar{X}$ No. total J's/mosquito | $\bar{X}$ No. microfilariae/ midgut |
|---|---|---|---|---|---|
| 1 | 6.9( 81)[a] | | | 6.9( 81) | 20.9(16)[b] |
| 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) | |

[a]Percent mosquitoes infected.

[b]Number of midguts examined.

(Table 7). Morphological detail was better delineated. The lumen of the intestine was complete and the esophagus showed beginnings of an anterior muscular and posterior glandular region (Fig. 7). The anal plug was still present, and the rectum was not completely differentiated (Fig. 7). At this stage of development, juveniles were active, and the Malpighan tubules showed considerable damage (Fig. 11). Large numbers of $J_1$ were still present on days 8-10 PE, but the mean number per mosquito had decreased by day 10 PE (Table 8). Different stages ($J_1$ and $J_2$) commonly occurred at the same time in individual mosquitoes throughout the study (Fig. 11). A mosquito dissected at 10 days PE contained 53 $J_2$ (Fig. 12), but was very active.

Day 11 PE was the first time $J_3$ were seen (Table 8). Second-stage juveniles at various stages of molting were often seen at this time (Figs. 20-22). Early $J_3$ exceeded 800 μm in length and were noticeably narrower (Table 7: Fig. 13). Of those mosquitoes harboring $J_3$ on day 11 PE, 40% had $J_3$ outside the Malpighian tubules, and three had $J_3$ in the head and labium (Fig. 25). At 12 days PE, juveniles had increased in length (Table 7), but thereafter little change in morphology occurred. A typical $J_3$, at 12 days PE, is illustrated in Figs. 8 and 9. The gut was complete, the anus open, rectum differentiated, and the esophagus divided into anterior muscular and posterior glandular regions. The nerve ring was clearly visible, but the excretory complex was indistinct in most instances. The caudal end with three small papillae was the typical "cigar-shape", characteristic of the genus Dirofilaria (Fig. 23). All mosquitoes examined after 12 days contained "infective-

stage" juveniles outside the Malpighian tubules.

Throughout the developmental period, the mean number of juveniles recovered from Ae. trivittatus remained relatively constant (Table 8). Mosquitoes dissected as late as 20, 27, and 35 days PE contained an average of 12-13 $J_3$. This number did not seemingly impair the vital activities of the mosquito.

The dog (F4) exposed to 100 $J_3$ by needle inoculation showed no microfilaremia in any of 9 blood samples examined. The last sample was taken at 276 days PE. At this time, the dog was euthanized. Heart and pulmonary arteries were examined, and seven (5 males and 2 females) D. immitis were recovered. The dog exposed to the bite of 49 infective Ae. trivittatus showed a patent infection of D. immitis by 210 days PE (24 microfilariae/20 $mm^3$). The microfilaremia had increased to 85 microfilariae/20 $mm^3$ by 261 days PE.

The development of D. immitis in Ae. trivittatus, relative to tissue differentiation, is similar to that reported by Taylor (1960) for D. immitis in Ae. aegypti. Developmental times and the sizes of the developmental stages, however, are markedly different. The first molt of D. immitis in Ae. trivittatus occurs at 7-8 days, and the second molt at 10-11 days PE. In Ae. aegypti, the first molt does not occur until about 10 days, and the second molt at 13 days PE (Taylor 1960). Infective-stage juveniles were recovered from the cephalic spaces and labium of Ae. trivittatus at 11 days PE. The migration of $J_3$ to the head of Ae. aegypti, however, did not begin until 15 days PE (Taylor

1960). The temperature at which mosquitoes were maintained was 0.5° C higher in this study than in Taylor's study (26.5 ± 1° C versus 26.0° C). Previous studies (Beam 1967; Kutz and Dobson 1974) on the effects of temperature on the development of D. immitis in Ae. sollicitans and An. quadrimaculatus suggest that this slight temperature difference should not account for the greatly reduced developmental times in Ae. trivittatus. It is interesting to contemplate the possible adaptive significance of a reduced developmental time in a temperate mosquito vector as opposed to that in a tropical one. Detailed studies should be undertaken of different temperatures and humidities as they may affect the development of D. immitis in Ae. trivittatus.

Measurements of juvenile stages obtained in this study 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 glycerin-alcohol fixed without coverslip pressure. Taylor (1960), however, 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. Nelson (1959) reported a mean length of 975 µm for $J_3$ D. immitis from experimentally infected Cx. fatigans and a range of 800-1040 µm for $J_3$ recovered from experimentally infected Ae. aegypti, Ae. pembaensus, and Cx. fatigans. Intermill (1973) recorded measurements of about 800-900 µm for $J_3$ D. immitis from experimentally infected Ae. triseriatus.

Other investigators (Kutz and Dobson 1974; Bemrick and Sandholm

1966) have used Taylor's data as an aid in determining the developmental stages of D. immitis in mosquitoes. Jankowski and Bickley (1976) referred to Bemrick and Sandholm (1966) when establishing four stages of D. immitis development in Ae. canadensis and Ae. vexans. Bemrick and Sandholm (1966) also used Taylor's data, and did not establish any criteria for the differentiation of developmental stages. The disparity in measurements between previous studies (Taylor 1960) and this study, emphasizes the necessity for determining dimensions and characteristics of D. immitis developmental stages in each vector-D. immitis system studied.

Relatively large numbers of microfilariae were retained within the peritrophic membrane and blood clot of exposed Ae. trivittatus and often were observed passing out the hindgut. This seems to have a beneficial effect on this vector-parasite system. Dirofilaria immitis commonly produces extremely high microfilaremias in dogs; therefore, mosquitoes may be exposed to detrimentally heavy infections. This study indicates that at least the same number of microfilariae may be retained in the midgut as infect the Malpighian tubules. Kershaw et al. (1955) suggested 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 could be made concerning worm burden as it may affect mosquito longevity. Results of vector mortality studies, designed to

determine the effect of D. immitis on Ae. trivittatus mortality, will be discussed in a later section.

Different stages of juvenile development occurring at the same time in a mosquito species exposed to a single infective blood meal often have been reported (Nayar and Sauerman 1975; Kutz and Dobson 1974; Intermill 1973; Kartman 1953a). Explanations for this phenomenon 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, usually when active microfilariae are in the digested blood as it is excreted. At such times, microfilariae were most often seen in the lumen of the tubules. These data become important when investigators attempt to determine the presence of multiple infections in field-collected mosquitoes.

The ability of Ae. trivittatus to transmit D. immitis by bite, as demonstrated in this study, fulfills one of the criteria Ludlam et al. (1970) considered essential in establishing a mosquito as a natural vector of D. immitis.

## ENCAPSULATION-MELANIZATION

Throughout the developmental study, observations were made on the defense reactions of Ae. trivittatus against developing stages of D. immitis. An encapsulation and/or melanization response was noted in 15% (41/281) of the mosquitoes examined (Table 9). In no instance, however, were all juveniles encapsulated in a single mosquito.

Microfilariae constituted the developmental stage most often melanized (Table 9). Although the Malpighian tubules were the site where most encapsulated-melanized microfilariae were found (Fig. 28), several mosquitoes contained melanized microfilariae in the hemocoele (Figs. 29 and 31). All microfilariae found in the hemocoele of any Ae. trivittatus examined were completely melanized.

Two encapsulated microfilariae and one melanized microfilaria were first observed in one mosquito at 12 hr PE. A microfilaria recovered from a mosquito at day 2 PE showed melanization beginning in the area of the excretory pore (Figs. 26 and 27), suggesting a reaction to excretory products.

Seventeen Ae. trivittatus harbored encapsulated $J_1$ (Fig. 30), and in one mosquito several encapsulated $J_1$ appeared to be spotted with melanin (Fig. 35). Only two mosquitoes showed a reaction against $J_2$. This was manifested in one dissected at day 10 PE by a melanization of the tail region, excretory pore, and stoma of a $J_2$ (Figs. 32-34). Two mosquitoes also showed a reaction against $J_3$ D. immitis. In both cases, melanized $J_3$ were observed in the hemocoele of the abdomen (Figs. 36 and

Table 9. Encapsulation response of Aedes trivittatus to developing juveniles of Dirofilaria immitis

| Days post-exposure | Percent encapsulation[a] | Juvenile encapsulated[b] | Location of encapsulation[c] |
|---|---|---|---|
| 1 | 0 (0/16) | - | - |
| 2 | 20 (4/20) | microfilaria | MT |
| 3 | 11 (2/18) | microfilaria | MT |
| 4 | 10 (2/20) | microfilaria | MT |
| 5 | 10 (2/20) | microfilaria | MT, HG |
| 6 | 15 (3/20) | microfilaria | MT, HG |
| 7 | 5 (1/20) | microfilaria | HE |
| 8 | 0 (0/20) | - | - |
| 9 | 20 (5/20) | microfilaria, $J_1$ | MT, HG |
| 10 | 40 (8/20) | microfilaria, $J_1$ | MT, HG, HE |
| 11 | 20 (3/15) | $J_1$ | MT |
| 12 | 21 (4/19) | microfilaria, $J_1$,$J_2$,$J_3$ | MT, HE |
| 13 | 5 (1/20) | microfilaria | HE |
| 14 | 30 (6/20) | microfilaria, $J_1$,$J_2$ | MT, HE |
| 20 | 0 (0/5) | - | - |
| 27 | 0 (0/5) | - | - |
| 35 | 0 (0/3) | - | - |

[a]Number of mosquitoes encapsulating/number of mosquitoes examined.

[b]$J_1$, 1st stage juvenile; $J_2$, 2nd stage juvenile; $J_3$, 3rd stage juvenile.

[c]MT, Malpighian tubules; HG, hindgut; HE, hemocoele.

37).

An encapsulation-melanization reaction seemed to occur more often in heavily infected mosquitoes. Mosquitoes exhibiting this defense reaction harbored an average of 22 D. immitis per mosquito (917/41), whereas those showing no reaction harbored an average of 15 (3,251/233). An average of 4 encapsulated microfilariae and/or juveniles was recovered from each mosquito examined.

Encapsulation-melanization of filarial juveniles has been suggested as a response occurring in refractive mosquitoes, or one that occurs so seldom that it is of no consequence (Lavoipierre 1958; Nayar and Sauerman 1975). In the Ae. trivittatus-D. immitis system, however, it may function as an additional means of reducing the parasite load.

## FIELD ISOLATIONS

Aedes trivittatus constituted nearly 60% of the mosquitoes collected (Table 10). The population of this species was below normal because of drought conditions prevailing in the Ames area through July and the first nine days of August, 1975. On the first two collection days, 72% of the total number of mosquitoes collected were Ae. trivittatus, but only 23% were of this species on the last two study days. Anopheles punctipennis, the next most abundant species (Table 10), increased from 10.5% on the first two days to 35.5% of the light-trap collections on the last two days. Aedes trivittatus decreased from a mean number of 242 mosquitoes per light-trap night in the first five days to 55 per night in the last five days; however, An. punctipennis also decreased from 55 to 38 mosquitoes per light-trap night. It seems evident that the percentage increase in An. punctipennis was not due to increased numbers, but rather to a decrease in the total population of Ae. trivittatus. The number of all other mosquito species taken in CDC light traps remained constant.

The Ae. triseriatus population may be greater than indicated because these mosquitoes feed in late afternoon and are not readily attracted to light traps (Love and Smith 1957; Rowley et al. 1973). This mosquito has been reported as a potential vector of D. immitis (Intermill 1973) and probably warrants further examination by a more suitable collecting method.

Natural infections of Dirofilaria were recovered from 18 of 393 Ae.

Table 10. Species composition of mosquitoes collected in $CO_2$-baited CDC light traps in Ames, Iowa, 1975

| Mosquito species | Total collected | % of Total |
|---|---|---|
| Aedes stimulans | 1 | 0.05 |
| Ae. triseriatus | 6 | 0.20 |
| Ae. trivittatus | 1,471 | 58.70 |
| Ae. vexans | 124 | 4.90 |
| Anopheles punctipennis | 468 | 18.70 |
| Coquilletidia perturbans | 45 | 1.80 |
| Culex pipiens pipiens | 297 | 11.90 |
| Cx. tarsalis | 91 | 3.60 |
| Orthopodomyia signifera | 1 | 0.05 |
| Uranotaenia sapphirina | 2 | 0.10 |

trivittatus dissected and from 7 of 31 pools (Tables 11 and 12). First- and second-stage juveniles were readily identified as Dirofilaria because of their location in the Malpighian tubules. No other genus of filarial parasite is known to develop in this location in mosquitoes. Third-stage juveniles were identified as Dirofilaria on the basis of several characteristics. In dissected mosquitoes harboring $J_3$, damage to the Malpighian tubules was evident, indicating development at this location. Distal ends of the tubules were inflated and transparent because of an absence of cellular material. Those $J_3$ recovered from

Table 11. Number and location of developmental stages of *Dirofilaria immitis* recovered from dissection of 393 *Aedes trivittatus* and 2 *Anopheles punctipennis*

| Mosquito species | Mf | $J_1$ | $J_2$ | $J_3$ | | | |
|---|---|---|---|---|---|---|---|
| | | Malpighian tubules | | Malpighian tubules | Abdomen | Thorax | Head |
| *Ae. trivittatus* | | | | | | | |
| 1 | + | 1 | - | - | - | - | - |
| 2 | - | 3 | - | - | - | - | - |
| 3 | + | 16 | - | - | - | - | - |
| 4 | - | - | 21 | - | - | - | - |
| 5 | - | - | - | - | 2 | 4 | 6 |
| 6 | + | 2 | - | - | - | - | - |
| 7 | - | - | 3 | - | 6 | 13 | 8 |
| 8 | - | - | 6 | - | - | - | - |
| 9 | + | - | - | - | - | - | - |
| 10 | - | - | 7 | - | - | - | - |
| 11 | - | - | - | 2 | 1 | - | - |
| 12 | - | - | - | - | - | - | - |
| 13 | - | 31 | - | - | - | - | - |
| 14 | - | - | - | - | - | - | - |
| 15 | - | - | 1 | 1 | - | - | 3 |
| 16 | - | 5 | - | - | - | - | - |
| 17 | - | 3 | - | - | - | - | - |
| 18 | - | 7 | - | - | - | - | - |
| *An. punctipennis* | | | | | | | |
| 1 | + | 52 | - | - | - | - | - |

Table 12. Positive pools and infection rates for 6 species of mosquitoes pooled for Dirofilaria immitis isolations

| Species | Total No. pooled | No. of pools | Positive pools | Infection rate |
|---|---|---|---|---|
| Ae. trivittatus | 911 | 31 | 7 ($J_3$) | 1/130 |
| Ae. vexans | 88 | 5 | 0 | 0 |
| An. punctipennis | 466 | 11 | 1 ($J_1$) | 1/466 |
| Cq. perturbans | 43 | 2 | 0 | 0 |
| Cx. pipiens pipiens | 295 | 10 | 0 | 0 |
| Cx. tarsalis | 86 | 6 | 0 | 0 |

pooled Ae. trivittatus were identified by length, breadth, distance of the anus from the caudal extremity, anal ratio (Nelson 1959), and by the presence of very small terminal papillae. These measurements were compared with those from juveniles recovered in experimentally infected Ae. trivittatus (Table 13).

Mosquitoes were captured in an urban residential area where the population of wild mammals was minimal. Thus, on the basis of the geographical area from which mosquitoes were trapped and considering the comparable measurements obtained, it is suggested, but not proven, that the developing stages were D. immitis.

Aedes trivittatus carries very large numbers of parasites, yet is able to fly to an attractant. Thirty-one late $J_1$ were found in one mosquito, and 27 $J_3$ and 3 $J_2$ in another (Table 13), suggesting consider-

Table 13. Measurements (μm) of third-stage juveniles of Dirofilaria immitis from field-collected and experimentally infected Aedes trivittatus

| Mosquito source | Number | Mean length | Mean width | Mean distance anus to caudal extremity | Mean width halfway anus to caudal | Mean anal ratio |
|---|---|---|---|---|---|---|
| Field: | | | | | | |
| Isolation 1 | 2 | 875 | 26.6 | 33.3 | 15.5 | 2.1 |
| Isolation 2 | 1 | 1020 | 24.4 | 35.5 | 14.4 | 2.5 |
| Isolation 3 | 10 | 933 | 23.1 | 33.2 | 16.7 | 2.0 |
| Isolation 4 | 1 | 980 | 22.2 | 36.6 | 17.8 | 2.1 |
| Isolation 5 | 2 | 890 | 20.6 | 35.5 | 15.5 | 2.3 |
| Isolation 6 | 5 | 936 | 22.0 | 33.3 | 16.7 | 2.1 |
| Isolation 7 | 1 | 990 | 21.1 | 35.5 | 16.7 | 2.1 |
| Total | 22 | 933 | 23.0 | 33.7 | 16.4 | 2.1 |
| Experimental | 25 | 956 | 23.4 | 34.6 | 16.3 | 2.1 |

able tolerance by this mosquito to the damage done by the developing juveniles, even under field conditions.

Infective juveniles were recovered in seven of 31 pools of Ae. trivittatus (Table 14). If the assumption is made that there was only one infected mosquito in each pool, the infection rate was 0.77%. In comparison, the infection rate for $J_3$ dissected from mosquitoes was 1.01%. These figures compare favorably when one considers the possibility of a pool containing more than one infected mosquito. The

pooling method was not designed to recover the relatively inactive $J_2$ and $J_3$, although 3 $J_1$ were obtained from a pool of 47 An. punctipennis. This method seems practical for the examination of large numbers of mosquitoes. Between 1,500 and 2,000 mosquitoes could have been handled per day had the mosquito population warranted.

Other than Ae. trivittatus, the only mosquito with developing juveniles was An. punctipennis (Tables 13 and 14). This species has been reported as supporting the complete development of D. immitis by many investigators (Hu 1931; Yen 1938; Phillips 1939). The apparent absence of infective juveniles in 466 mosquitoes indicates that An. punctipennis may not serve as a vector for D. immitis in this area. It is possible that the parasite is unable to develop to the infective stage under natural conditions or may cause a high degree of mortality with the central Iowa strain of An. punctipennis. There is a paucity of information on the host preference of this mosquito, and it is therefore possible that it does not often feed on dogs.

Naturally infected Ae. vexans were recovered by Bemrick and Sandholm (1966) in Minnesota and incriminated as the major vector of D. immitis in that area. No infected Ae. vexans were recovered in our study, but this may have been a result of the small number examined. Only 5% of the mosquito population examined was Ae. vexans; therefore, the role played by this mosquito in the maintenance of D. immitis in this study area could not be determined.

Culex pipiens pipiens and Cx. tarsalis accounted for more than 15%

of the population collected (Table 12), and both have been reported as potential vectors of dog heartworm (Hu 1931; Kartman 1953a; Yen 1938). Their true potentials under natural conditions probably are minimal because of their preference for avian hosts.

All Coquillettidia perturbans examined were negative. This species has been reported to be a poor vector for D. immitis even though individual mosquitoes readily feed on dogs (Bemrick and Sandholm 1966). The remaining mosquitoes examined constituted less than 1% of those collected and cannot be considered significant potential vectors in this area.

The high rate of infection in Ae. trivittatus in conjunction with the numbers of these mosquitoes collected suggests that this mosquito functions very well as a natural vector of D. immitis in this area.

## VECTOR MORTALITY

In the first longevity experiment (LE-I), 399 Ae. trivittatus fed on a dog (02) with a microfilaremia of 347 microfilariae/20 $mm^3$, and 429 fed on an uninfected dog (F6). A 50% cumulative mortality occurred at 15-16 and 24-25 days in infected and control mosquitoes, respectively (Fig. 39). This nine day difference in longevity between the two groups was evident from 10% through 90% cumulative mortality readings. Data were plotted in a different way to determine if these differences were significant. The natural log of the proportion living was plotted against time to make the longevity curves more homogenous (Fig. 40). Regression lines were estimated for both infected and uninfected Ae. trivittatus, and the slopes were compared using a Student's t-test (Steel and Torrie 1960). The difference between slopes was highly significant ($P<0.001$).

Dissection of dead or dying infected mosquitoes revealed that nearly 50% of those examined contained 10 or fewer juveniles per mosquito (Fig. 41). About 30% harbored 11-20, 15% contained 20-35, and only 5% had more than 35 juveniles per individual mosquito (Fig. 41). The maximum number of juveniles recovered from a single Ae. trivittatus was 99 at day 3 PE. No uninfected mosquitoes were found. A total of 133 mosquitoes was dissected throughout LE-I, and the mean number of juveniles recovered per mosquito on each day was recorded (Table 14).

An attempt was made to determine what relationship existed between the longevity of a mosquito and the number of developing juveniles it

Table 14. Mean number of juvenile *Dirofilaria immitis* recovered from individual *Aedes trivittatus* exposed to dogs with high (LE-I) or low (LE-II) microfilaremias. Mosquitoes were dissected at time of death

| Days post-exposure | Mean No. juveniles | | Days post-exposure | Mean No. juveniles | |
|---|---|---|---|---|---|
| | LE-I | LE-II | | LE-I | LE-II |
| 1 | 56.0(2)[a] | 7.0(2) | 25 | 4.5(2) | 1.0(1) |
| 2 | - | 6.2(5) | 26 | 2.3(3) | - |
| 3 | 41.7(6) | 5.8(5) | 27 | 3.5(2) | 1.5(6) |
| 4 | 22.9(9) | 6.2(5) | 28 | 3.0(3) | 2.7(3) |
| 5 | 18.3(6) | 3.0(3) | 29 | 15.0(1) | 1.7(3) |
| 6 | 15.1(7) | 5.7(2) | 30 | - | 3.0(3) |
| 7 | 25.3(4) | 6.3(4) | 31 | - | 1.3(4) |
| 8 | 13.5(8) | 1.0(2) | 32 | - | 1.0(2) |
| 9 | 19.0(5) | 3.5(2) | 33 | - | 1.5(2) |
| 10 | 21.7(3) | - | 34 | - | 3.0(2) |
| 11 | 7.2(5) | 4.7(3) | 35 | - | 3.0(3) |
| 12 | 14.7(6) | 1.3(3) | 36 | 2.0(1) | 2.5(2) |
| 13 | 15.1(7) | 2.3(3) | 37 | - | 2.7(3) |
| 14 | 11.3(4) | 1.8(4) | 38 | - | 2.0(2) |
| 15 | 11.8(4) | 7.0(3) | 39 | - | 1.5(2) |
| 16 | 8.3(10) | 1.7(3) | 40 | 1.0(1) | 1.5(2) |
| 17 | 12.8(4) | 2.2(5) | 41 | - | 4.0(1) |
| 18 | 14.9(7) | 5.0(5) | 42 | - | 2.0(2) |
| 19 | 7.2(5) | 4.0(1) | 44 | - | 1.0(1) |
| 20 | 6.3(6) | 1.8(6) | 48 | - | 1.0(1) |
| 21 | 8.8(4) | 2.3(3) | 49 | - | 1.0(1) |
| 22 | - | 2.0(2) | 50 | 2.0(1) | - |
| 23 | 7.0(5) | 1.3(3) | 60 | - | 1.0(1) |
| 24 | 7.3(4) | 2.0(2) | - | - | - |

[a]Number of mosquitoes dissected.

contained. Mean parasite burdens were calculated for five day periods up to 30 days PE, and from day 31 PE to the time the last mosquito died (Fig. 42). Mosquitoes dying from day 1 to 5 PE harbored a mean of 29.5 juvenile D. immitis per mosquito, whereas those surviving until day 30 to 50 PE contained an average of only 1.7 juveniles. Between days 1 to 5 and 11 to 15 PE, there was a decrease in means of 17.1 juveniles per mosquito (58%). From days 11 to 15 until 21 to 25 PE, however, a decrease of only 5.2 juveniles per mosquito occurred (42%). No Ae. trivittatus harboring more than two D. immitis survived beyond day 30 PE, although one mosquito containing 15 juveniles lived for 29 days PE (Table 10).

When maximum longevity, (Y), was plotted arithmetically against mean number of juveniles (X), the relationship was parabolic; therefore, X values were plotted on a logarithmic scale and Y values on an equal-interval scale so that the points (X,Y) could be analyzed by linear regression (Steel and Torrie 1960; Mack 1967). Increases in the parasite burden showed a strong negative linear correlation with maximum longevity (Fig. 43). The regression equation was determined as Y = 37.60 - 21.90 Log X, where Y represents the number of days maximum longevity, X the number of juveniles per mosquito, - 21.90 the slope, and 37.60 the intercept. The correlation coefficient, r, was calculated to be - 0.903.

In the second longevity experiment, (LE-II), 448 Ae. trivittatus fed on a dog (F5) with a microfilaremia of 62 microfilariae/ 20 $mm^3$, and 307 fed on an uninfected dog (F6). In contrast with results

obtained in LE-I, a 50% cumulative mortality of infected mosquitoes occurred only four days before control mosquitoes (Fig. 44). Fifty percent of the infected population was dead at days 18-19 PE and with control mosquitoes at days 22-23 PE (Fig. 44). A 50% cumulative mortality occurred two days earlier in control and three days earlier in infected mosquitoes in LE-I as compared with LE-II.

The greatest differences between infected and uninfected mosquitoes was 4-5 days, and occurred between 35% and 60% cumulative mortality. On both sides of these points, differences decreased (Fig. 44). The method used in LE-I was employed in LE-II to determine if differences were significant (Fig. 45). A comparison of slopes showed no significant difference between infected and uninfected mosquitoes.

Dissections of dead and dying infected mosquitoes in LE-II, showed that 83.6% contained 1-5 juveniles per mosquito and 13.9% harbored 6-10 (Fig. 46). Only 2.5% harbored more than 10 D. immitis per mosquito and none harbored over 15 (Fig. 46). Only 3 of 125 mosquitoes dissected were uninfected. This reduced frequency distribution due to the limited number of juveniles developing in individual mosquitoes was evident when the effect of parasite burden on vector longevity was analyzed. The mean number of juveniles per mosquito at time of death was determined (Table 10) and data were analyzed by the same method employed in LE-I (Fig. 42). An analysis of relationships by linear regression was not done because of the restricted frequency distribution and the resulting similarity between infected and uninfected mosquitoes.

High burdens of D. immitis have a detrimental effect on Ae. trivittatus. The density of microfilariae in the definitive host's peripheral blood determines to a large extent the number of juveniles invading the Malpighian tubules. In LE-I, a total of 1935 juveniles was recovered from 133 mosquitoes for an average of 14.5 juveniles per mosquito, and in LE-II, 383 juveniles were recovered from 122 Ae. trivittatus for an average of 3.1 juveniles per mosquito. These averages were calculated as 4.2% (14.5/347) and 5.0% (3.1/62) of the microfilaremias of dogs used to expose mosquitoes in LE-I and LE-II, respectively. No attempt was made to determine the number of microfilariae actually ingested by Ae. trivittatus in relation to the microfilarial density of the host.

Kershaw et al. (1953) reviewed the early literature indicating that mosquito vectors of filarial worms ingested more micrifolariae than what was expected, or that occasionally mosquitoes took in surprisingly large numbers of microfilariae. In their own work on Ae. aegypti and D. immitis, however, they found that most Ae. aegypti ingested fewer microfilariae than expected, and that ingestion of large numbers of microfilariae was the exception. Similar results have been obtained for D. aethiops and Ae. aegypti by Webber (1955), W. bancrofti and Ae. polynesiensis by Rosen (1955), and B.malayi and M. longipalpus by Wharton (1957a). Gordon and Lumsden (1939) observed directly the ingestion by Ae. aegypti, of microfilariae of Foleyella dolichoptera in frogs and noted that Ae. aegypti feed either directly from a capillary or

from a "pool" of blood formed by laceration of a capillary. They also reported that there were many more microfilariae in some capillaries than in others. These findings help explain variations reported in the literature concerning uptake of microfilariae by mosquito vectors. It is evident, however, that a high microfilarial density in a host produces a greater parasite burden in the vector.

Increased mortality of mosquitoes infected with filarial worms has often been reported (Lavoipierre 1958; Beam 1966; Weiner and Bradley 1970; Kutz and Dobson 1974). That vector mortality increases as the microfilarial density in the blood of their host increases has also been suggested by Kartman (1953b), Kershaw et al. (1953, 1955), Wharton (1957b), and Beam (1966), but no quantitative reports are available on the number of developing juveniles in relation to maximum longevity of the vector. In Ae. trivittatus, not all ingested D. immitis microfilariae reach the Malpighian tubules, and vector mortality data suggest that mosquitoes harboring five or fewer juveniles have as good a chance of surviving as do uninfected mosquitoes. Even mosquitoes harboring 5-15 juveniles will likely survive long enough to transmit "infective-stage" juveniles. Heavy infections (16+ juveniles) are detrimental to Ae. trivittatus, but occur less frequently than do lower parasite burdens, even when mosquitoes feed on a host with a high microfilaremia.

## VECTOR FECUNDITY

An evaluation of the effects of D. immitis infection on the fecundity of Ae. trivittatus was accomplished in two studies. In the first, (FE-I), all mated, blood-fed females were used, regardless of the quantity of blood ingested. The mean egg production was 52.6 ± 20.6 eggs per mosquito in those having fed on an infected dog (337 microfilariae/20 $mm^3$), and 69.6 ± 2.12 eggs per mosquito in those having fed on an uninfected dog (Table 5). This difference, however, was not significant when means were compared with a Student's t-test.

Table 15. Egg production in Aedes trivittatus blood fed on a Dirofilaria immitis-infected or uninfected dog

| Blood meal | Host | Number fed | Total eggs produced | Mean number eggs/mosquito |
|---|---|---|---|---|
| 1st | uninfected[a] | 68 | 4730 | 69.6 ± 21.2 |
| 1st | infected[b] | 75 | 3947 | 52.6 ± 20.6 |
| 1st | uninfected[c] | 80 | 5165 | 64.6 ± 25.8 |
| 1st | infected[d] | 117 | 7010 | 59.9 ± 26.8 |
| 2nd | rabbit[e] | 13 | 649 | 49.9 ± 19.5 |
| 2nd | rabbit[f] | 15 | 643 | 42.9 ± 21.8 |

[a] FE-I.

[b] FE-I (337 microfilariae/20 $mm^3$).

[c] FE-II.

[d] FE-II (60 microfilariae/20 $mm^3$).

[e] FE-II, uninfected mosquitoes.

[f] FE-II, infected mosquitoes.

It was thought that three factors could have accounted for these results. First, the variability in egg production within groups could have been influenced by the different quantities of blood ingested by individual mosquitoes. Woke et al. (1956) noted an upward trend in egg production in Ae. aegypti correlated with successively larger quantities of ingested blood up to 2.5-2.9 mg. As the amount of ingested blood increased above this point, no significant increase in egg production occurred. A second factor considered was that the presence of young, developing juveniles did not influence egg production, whereas older and larger D. immitis juveniles might. Such was the situation reported for B. pahangi developing in Ae. aegypti (Javadian and Macdonald 1974). A third consideration was that D. immitis had no detrimental effects on the fecundity of Ae. trivittatus.

In a second study (FE-II) undertaken to clarify the results obtained in FE-I, only mosquitoes that appeared "fully engorged" were used. In addition, all mosquitoes having oviposited following the first blood meal were exposed to a rabbit for a second blood meal, so that any effects larger juveniles might have on egg production could be analyzed. Unfortunately, a dog with a microfilaremia similar to the one used in FE-I was not available. The microfilaremia of the FE-II dog was substantially lower (60 microfilariae/20 $mm^3$).

The difference in mean total egg production between infected and uninfected mosquitoes was 4.7 eggs for the first oviposition and 7.0 eggs for the second (Table 11). The differences were not significant in

either case.

Infected mosquitoes were dissected in both studies and the number of juveniles recovered was correlated with the number of eggs produced. No relationship between parasite burden and egg production could be determined in either FE-I ($r = -0.006$) or FE-II ($r = -0.112$).

It seems from these data that D. immitis has no detrimental effects on the fecundity of Ae. trivittatus. It must be noted, however, that egg production by individual mosquitoes within experimental groups was extremely variable, thus restricting the ability to detect differences between groups. More information is needed on the reproductive biology of Ae. trivittatus before complete studies such as this can be conducted.

## EPIDEMIOLOGY

Surveys to determine the incidence of canine filariasis have been conducted in many parts of the United States (Graham 1974; McGreevy et al. 1970; Zydeck et al. 1970; Keegan et al. 1968; Gubler 1966; Hirth et al. 1966; Marquardt and Fabian 1966; Groves and Koutz 1964; Schlotthauer and Griffiths 1964). Such surveys have provided needed information on overall incidence, sex-specific attack rates, and age-specific attack rates of D. immitis in dogs. Each of these studies, however, involved only a single sample and provided no indication on the number of new infections acquired each season. Furthermore, they have not provided data on vector infection rates relative to the acquisition of new infections in susceptible dogs. An attempt was made in this study to determine the number of new infections acquired in susceptible dogs when the infection rate in the vector was known.

Seventy-three dogs were located within the study area, but 26 (35.6%) were on prophylactic medication for D. immitis and were not examined. Of the 47 dogs examined, 27 (57.4%) were classified as predominantly inside, 13 (27.7%) as predominantly outside, and seven (14.9%) as both inside and outside.

Infection rates for all types of dogs, for both years, are given in Table 16. Of seven infected dogs observed in 1975-76, six were housed outside. The other infected animal, maintained both inside and outside, had a patent infection in 1976 and resided at a household in which another dog had a D. immitis infection in 1975 (Fig. 47). No infections

Table 16. Dirofilaria immitis in dogs from an area of Ames, Iowa, in 1975 and 1976

| Year | Number of individuals examined | Location and number of infected dogs (% infected) | | | |
|---|---|---|---|---|---|
| | | Inside | Outside | Inside & Outside | Total |
| 1975 | 47 | 0 (0) | 4 (2) | 0 (0) | 4 (9) |
| 1976 | 43 | 0 (0) | 2 (22) | 1 (14) | 3 (7) |
| Both | 47 | 0 (0) | 6 (46) | 1 (14) | 7 (5) |

were found in animals housed predominantly inside.

The three infections acquired in 1975 (patent in 1976) were in dogs located in close proximity to a dog that had a microfilaremia of D. immitis in 1975 (Fig. 47).

No infections of Dipetalonema reconditum were detected in any dogs examined.

Field collections in 1975 showed that of the Ae. trivittatus examined, 4.6% contained developmental stages of D. immitis, and approximately 1.0% harbored $J_3$. Mosquito populations were extremely high in the Ames area in 1975 (unpublished data), and during July of that year, as many as 14 Ae. trivittatus were observed feeding concurrently on the muzzle and ears of a dog in the study area; therefore, a much higher rate of infection was expected than actually occurred.

Several factors may have accounted for this reduced infection rate. First, the population of susceptible animals was quite small. Dogs housed predominantly inside could not be considered part of the

susceptible population. During the limited time these animals were outside, they usually were being exercised and therefore were not sedentary hosts serving to attract mosquitoes. Dogs classified as both inside and outside generally were outside during the day and inside during the evening and night; therefore, these animals were not readily available during crepuscular periods when Ae. trivittatus feeds most actively (Wright and Knight 1966). Dogs housed entirely outside constituted only 21% (9/43) of the population sampled in 1976.

A second factor may have been an impairment of flight in infected mosquitoes. Data from this study suggest that mosquito flight ability is adversely affected by D. immitis infection. The only animals to acquire infections in 1975 were housed in close proximity to a dog that was infective that year. Susceptible dogs housed outside, but located some distance from infective animals, did not contract heartworm.

A third contributing factor may have been the effect of D. immitis on the longevity of Ae. trivittatus. Increased mortality in Ae. trivittatus harboring more than 10-15 juveniles has been noted in laboratory infections, but the parasite load necessary to cause an increased mortality may be lower under field conditions.

It is apparent, however, that when 1.0% of the vector population harbors infective-stage D. immitis, a substantial percentage of the susceptible dog population will become infected.

## SUMMARY AND CONCLUSIONS

1. Aedes trivittatus supports the complete development of D. immitis 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 PE, respectively. Third-stage juveniles were recovered from the labium and cephalic spaces of Ae. trivittatus as early as 11 days PE.
2. Large numbers of microfilariae were retained within the blood clot and peritrophic membrane of exposed Ae. trivittatus. This probably increases the chances for survival of mosquitoes feeding on hosts with high microfilaremias.
3. Nearly 15% of the Ae. trivittatus examined showed an encapsulation-melanization response to microfilariae or developing juveniles of D. immitis. In no instance, however, were all juveniles in a single mosquito encapsulated. This reduction in parasite load also may aid in decreasing vector mortality.
4. Some microfilariae retained in the midgut of Ae. trivittatus invaded the Malpighian tubules as digested blood was being excreted. This invasion of the Malpighian tubules occurred as late as six days PE, thus explaining the presence of various developmental stages of D. immitis in a single Ae. trivittatus exposed to only one infective meal.

5. Aedes trivittatus is capable of transmitting D. immitis to susceptible hosts by bite. A susceptible dog, exposed to the bite of 49 mosquitoes having fed on a D. immitis-infective dog 16 days earlier, developed a patent infection by 210 days PE.
6. Juveniles identified as D. immitis were recovered from Ae. trivittatus collected with $CO_2$-baited CDC miniature light traps. Dirofilaria immitis was recovered from 18 of 393 (4.6%) dissected and from seven of 31 pools (911 mosquitoes; 0.77%). Approximately 1.0% of the Ae. trivittatus collected harbored infective-stage juveniles. Anopheles punctipennis was the only other mosquito species infected, with two of 468 harboring $J_1$.
7. A survey of dogs from the area containing naturally infected Ae. trivittatus indicated that only dogs housed predominantly outside could be considered susceptible to D. immitis. Approximately 22% (2/9) of this population sampled became infected when 1.0% of the Ae. trivittatus collected contained $J_3$. These data suggest that natural transmission of D. immitis by Ae. trivittatus occurs in the field.
8. Aedes trivittatus exposed to dogs with high microfilaremias of D. immitis show a significantly increased mortality in contrast to uninfected controls, and there is a negative correlation between parasite load and longevity. Aedes trivittatus exposed to dogs with low microfilaremias of D. immitis do not show an increased mortality.

9. Aedes trivittatus showed considerable tolerance to D. immitis infection. Seventy percent of the mosquitoes harboring fewer than 15 D. immitis juveniles lived long enough in the laboratory to allow complete development of the parasite. Seventy-two percent of the mosquitoes containing more than 15 juveniles died before juveniles developed to the infective stage.
10. No significant decrease in fecundity of D. immitis-infected Ae. trivittatus was detected. Egg production was not significantly lower in infected compared to uninfected mosquitoes in either the first or second oviposition. No correlation was noted between parasite burden and egg production.
11. The bionomics of Ae. trivittatus is such that this mosquito has the capability to function as a good vector of D. immitis in central Iowa. It occurs in high populations, is zoophilic, multivoltine, long-lived, and takes several blood meals during its lifetime.
12. Aedes trivittatus should be considered the principal vector of D. immitis in central Iowa.

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# APPENDIX: PLATES AND FIGURES

## Abbreviations

| | |
|---|---|
| A | Anus |
| AP | Anal plug |
| BC | Buccal cavity |
| C | Cuticle |
| E | Esophagus |
| EC | Excretory cell |
| EG | Egg |
| EP | Excretory pore |
| G1 | G-cell 1 |
| G2 | G-cell 2 |
| G3 | G-cell 3 |
| G4 | G-cell 4 |
| GE | Glandular esophagus |
| H | Hemocyte |
| HG | Hindgut |
| I | Intestine |
| it | Infected Malpighian tubule |
| $J_1$ | First-stage juvenile |
| $J_2$ | Second-stage juvenile |
| $J_3$ | Third-stage juvenile |
| ME | Muscular esophagus |
| MG | Midgut |
| MT | Malpighian tubule |
| NR | Nerve ring |
| P | Papillae |
| R | Rectum |
| T | Trachea |
| ut | Uninfected Malpighian tubule |

## Plate I

Figures 1-4. First-stage juvenile *Dirofilaria immitis*

Figure 1. Developmental day 2

Figure 2. Developmental day 3

Figure 3. Developmental day 4

Figure 4. Developmental day 5

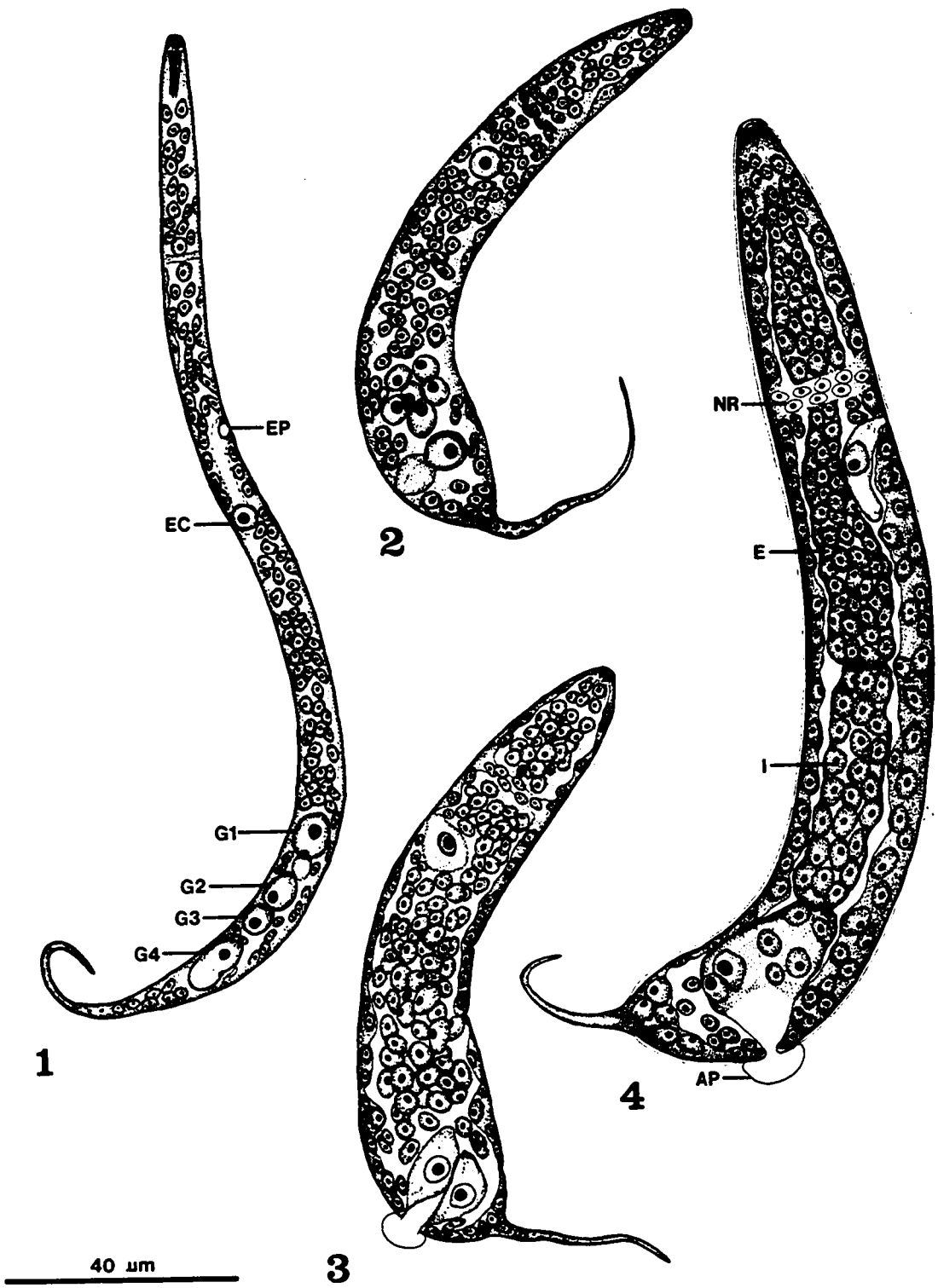
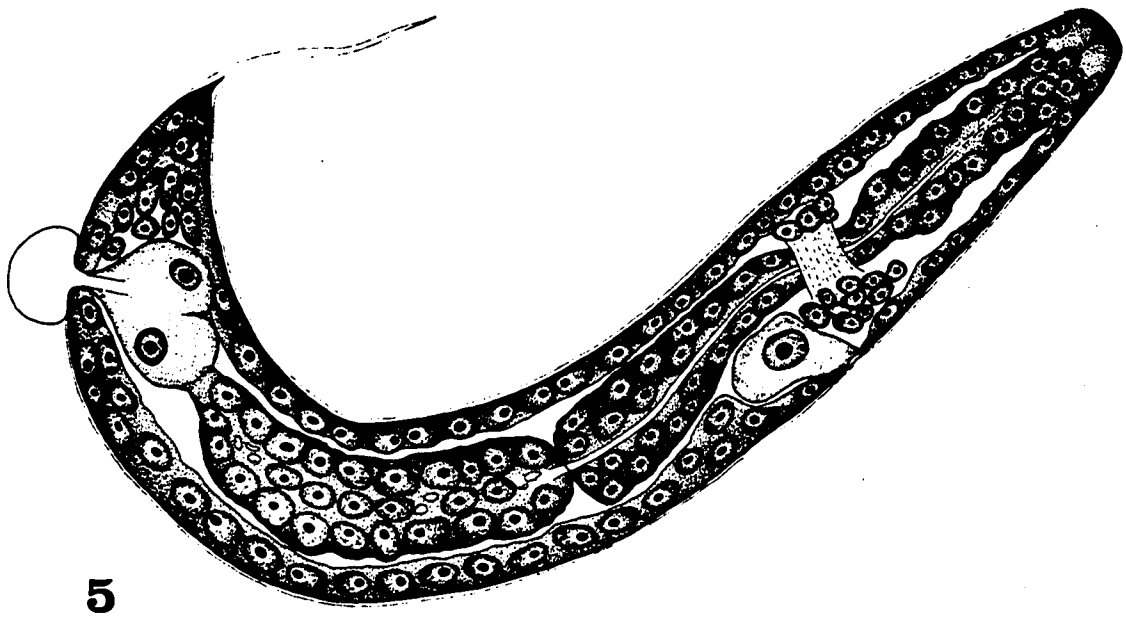

EP
EC
G1
G2
G3
G4
1
2
NR
E
I
AP
4
3
40 µm

Plate II

Figures 5-6. First- and second-stage juvenile Dirofilaria immitis

Figure 5. First-stage juvenile, developmental day 6

Figure 6. Early 2nd-stage juvenile, developmental day 7



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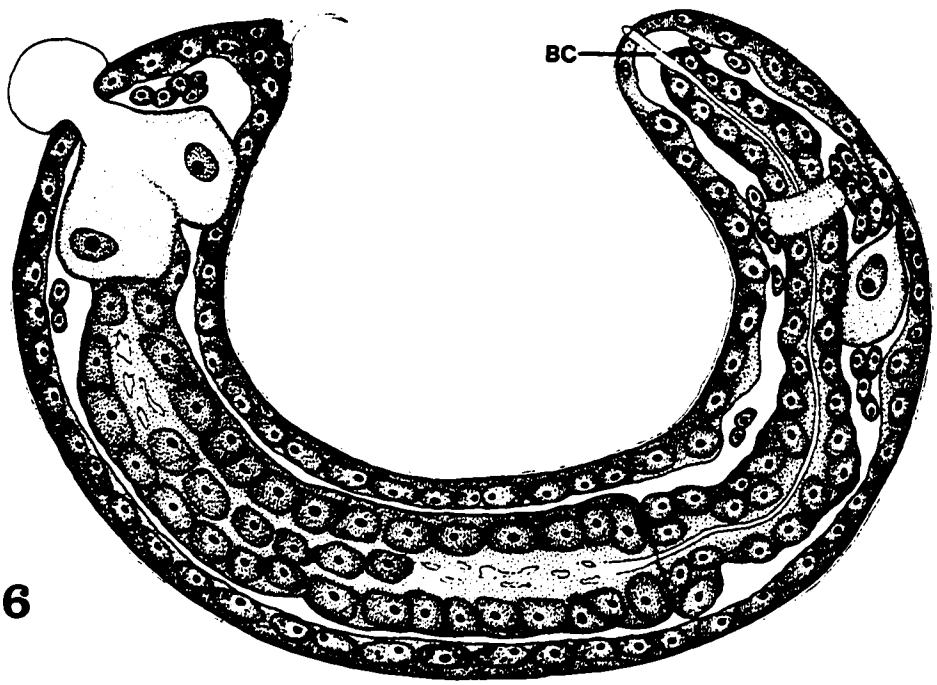



6

40 µm

## Plate III

Figures 7-9. Second- and third-stage juvenile Dirofilaria immitis

Figure 7. Late 2nd-stage juvenile, developmental day 9

Figure 8. Anterior end of 3rd-stage juvenile, developmental day 12

Figure 9. Posterior end of 3rd-stage juvenile, developmental day 12

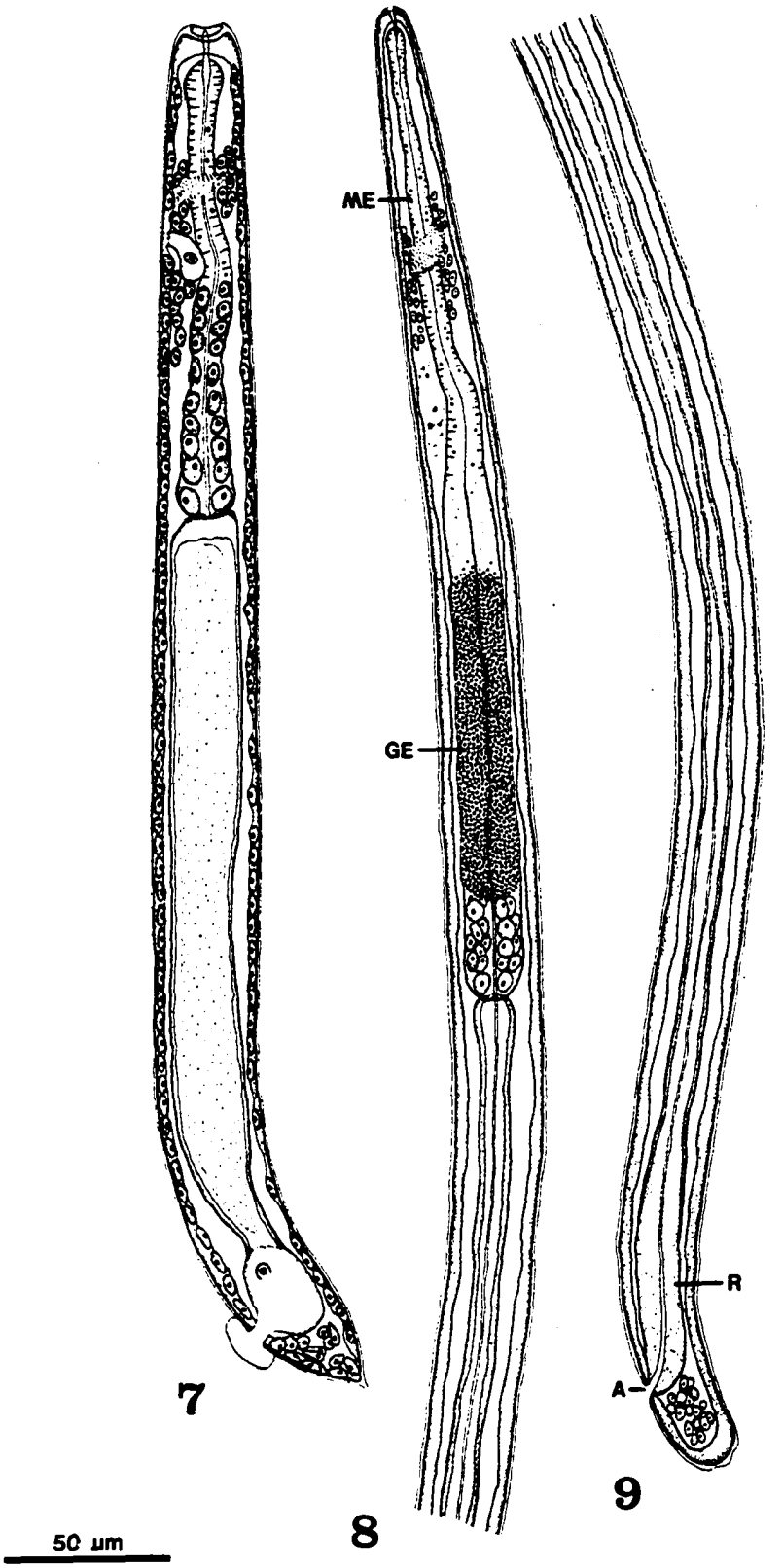

ME
GE
R
A
7
8
9
50 µm

Plate IV

Figures 10-13. Malpighian tubules of *Aedes trivittatus* infected with *Dirofilaria immitis*

Figure 10. Infected and uninfected Malpighian tubules at day 6 PE (X 80)

Figure 11. Juvenile *D. immitis* in different stages of development in the same mosquito at day 9 PE (X 205)

Figure 12. Malpighian tubules from a mosquito containing 53 $J_2$ (dissected at day 10 PE) (X 170)

Figure 13. Third-stage juvenile (arrow) at day 12 PE (X 260)

10
ut
it
11
J1
J2
13

Plate V

Figures 14-17. First-stage juvenile *Dirofilaria immitis* from *Aedes trivittatus*

Figure 14. Undivided G-cells in a $J_1$ at day 2 PE (X 2040)

Figure 15. Excretory cell and excretory pore connected by a fibrous band (arrow) in a $J_1$ at day 2 PE (X 1920)

Figure 16. First-stage juvenile at day 4 PE showing G4 divided and anal plug (X 2250)

Figure 17. Juvenile showing esophagus and intestine evident by day 5 PE (X 1615)

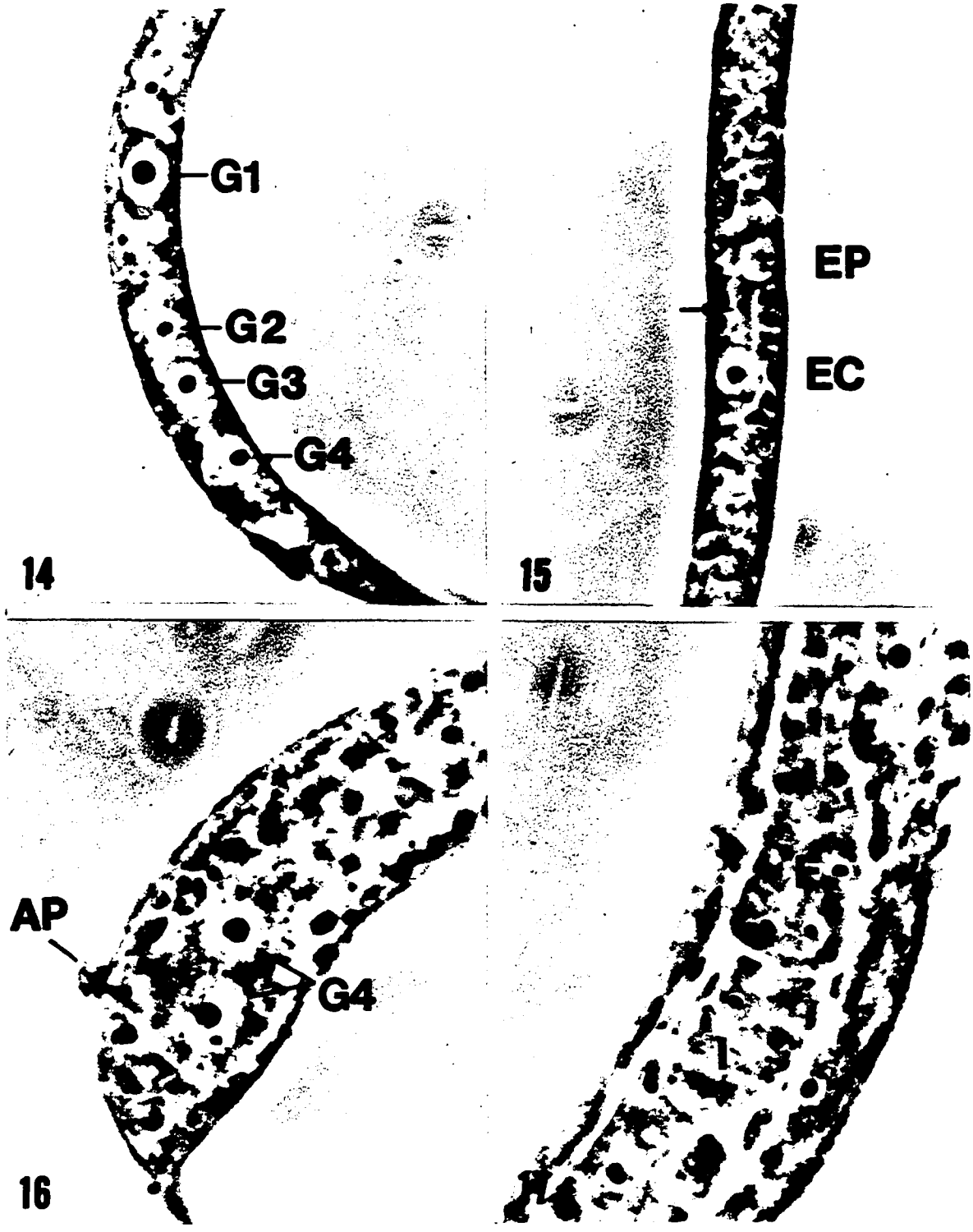

G1
G2
G3
G4
14
EP
EC
15
AP
G4
16

Plate VI

Figure 18-23. *Dirofilaria immitis* juveniles from *Aedes trivittatus*

Figure 18. Second-stage juvenile (X 150)

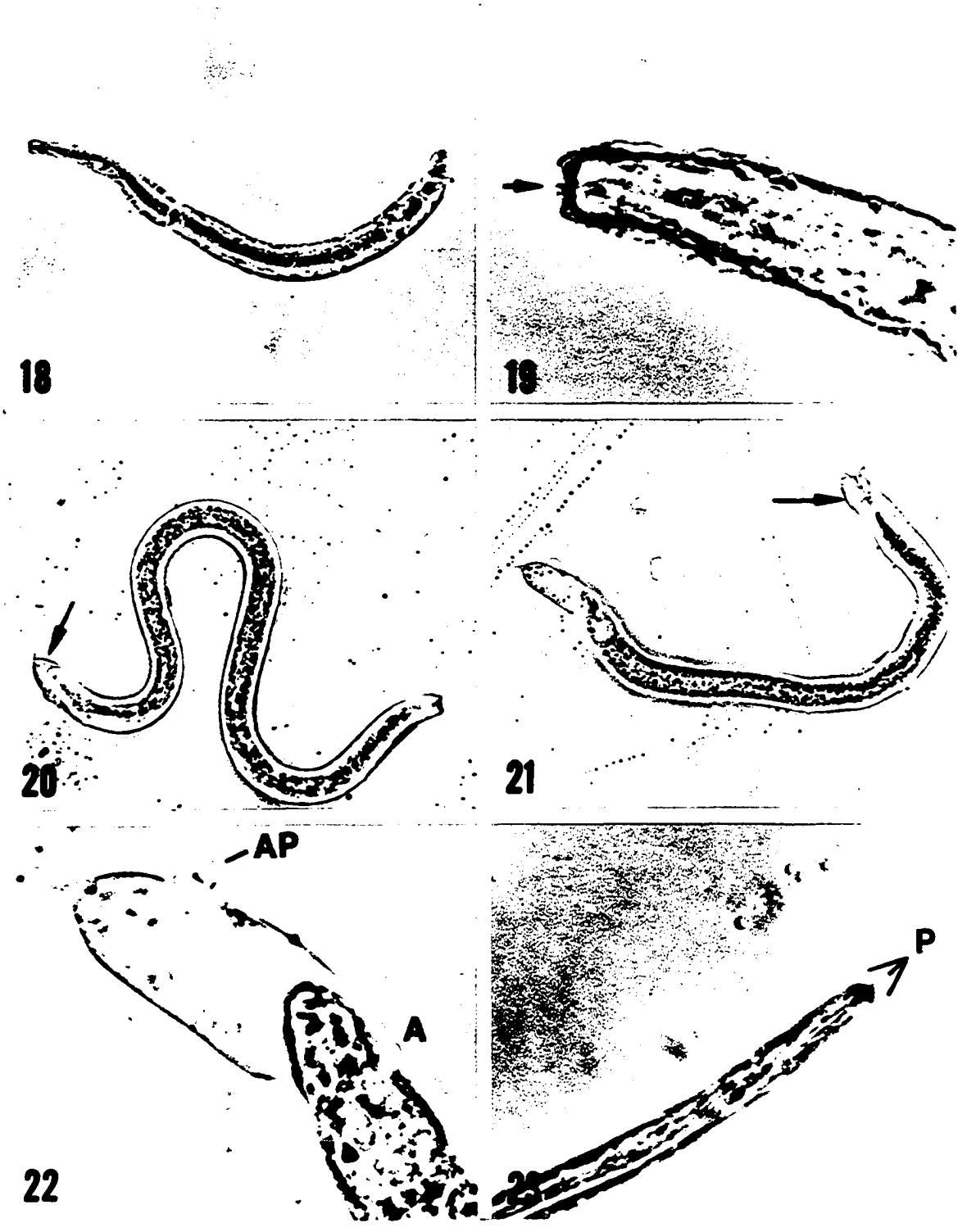
Figure 19. Anterior of $J_2$ showing stoma open to the exterior (arrow) (X 730)

Figure 20. Early molting of $J_2$ to $J_3$. Note the loosened cuticle (arrow) (X 185)

Figure 21. Later stage in molt from $J_2$ to $J_3$ showing a greatly loosened cuticle (arrow) (X 150)

Figure 22. Anal plug being sloughed with the cuticle, resulting in an opening of the anus to the exterior (X 650)

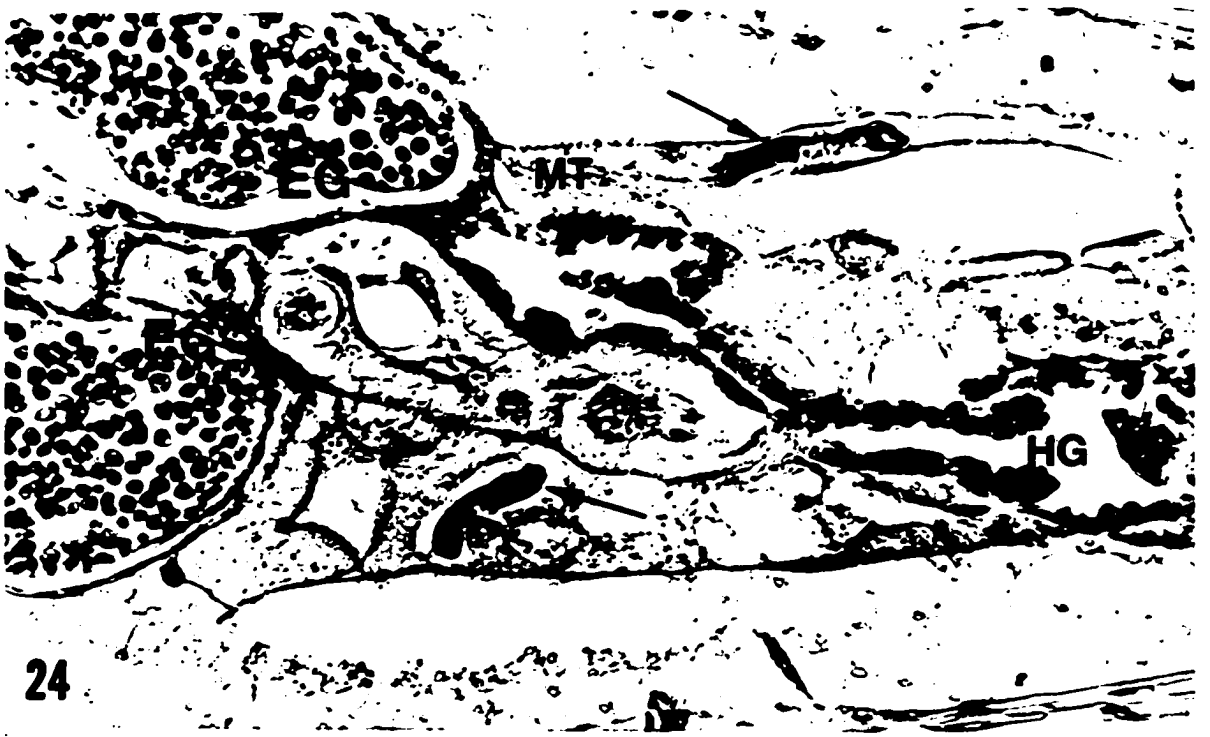
Figure 23. Three small papillae on the caudal end of a $J_3$ (X 435)


18
19
20
21
AP
A
22
P

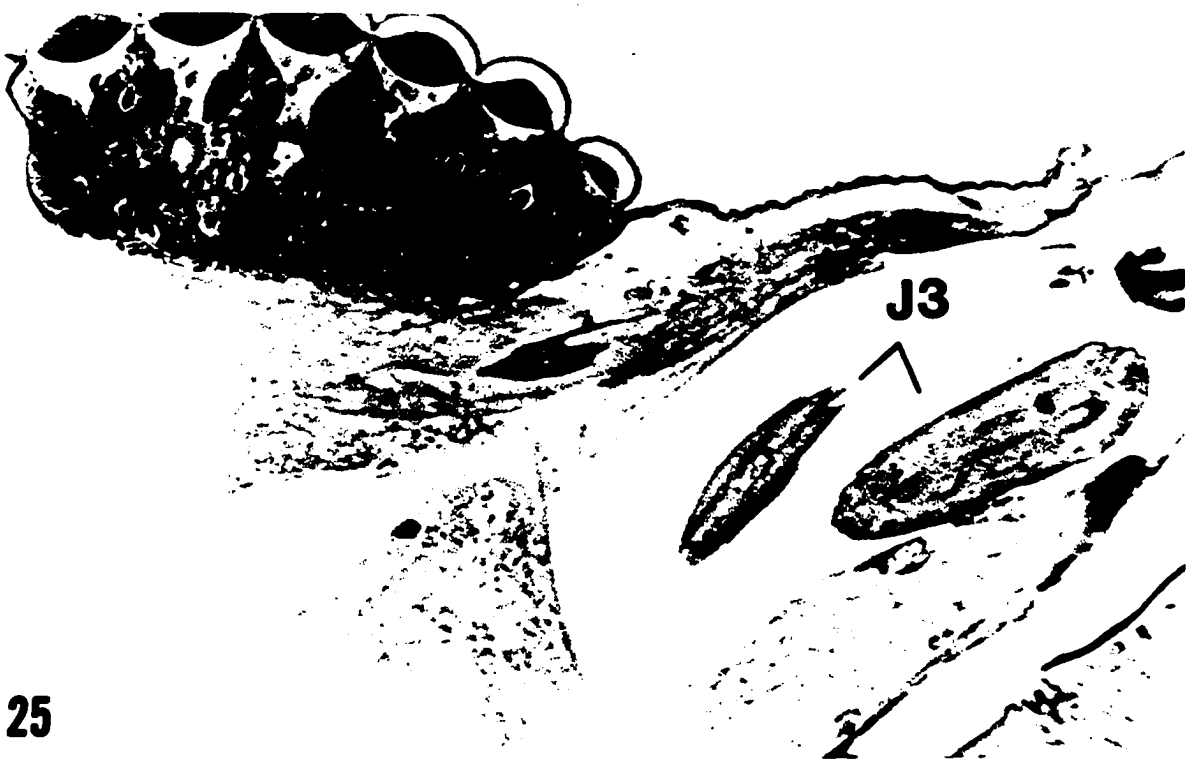
## Plate VII

Figure 24. Section through the abdomen of Aedes trivittatus at day 6 PE to Dirofilaria immitis showing $J_1$ (arrows) within the cells of the Malpighian tubules (X 230)

Figure 25. One µm section through the head of Aedes trivittatus at day 11 PE to Dirofilaria immitis showing $J_3$ in the cephalic spaces at the base of the labium (X 1235)




24




25

## Plate VIII

Figure 26. Dirofilaria immitis $J_1$ from Aedes trivittatus at day 2 PE showing excretory cell (X 1530)

Figure 27. Same juvenile as in Fig. 26, but focus is on the surface of the juvenile over the excretory cell. Note the early deposition of melanin (arrow) (X 1530)

Figure 28. Melanized microfilaria (arrow) of Dirofilaria immitis within a Malpighian tubule of Aedes trivittatus at day 3 PE (X 310)

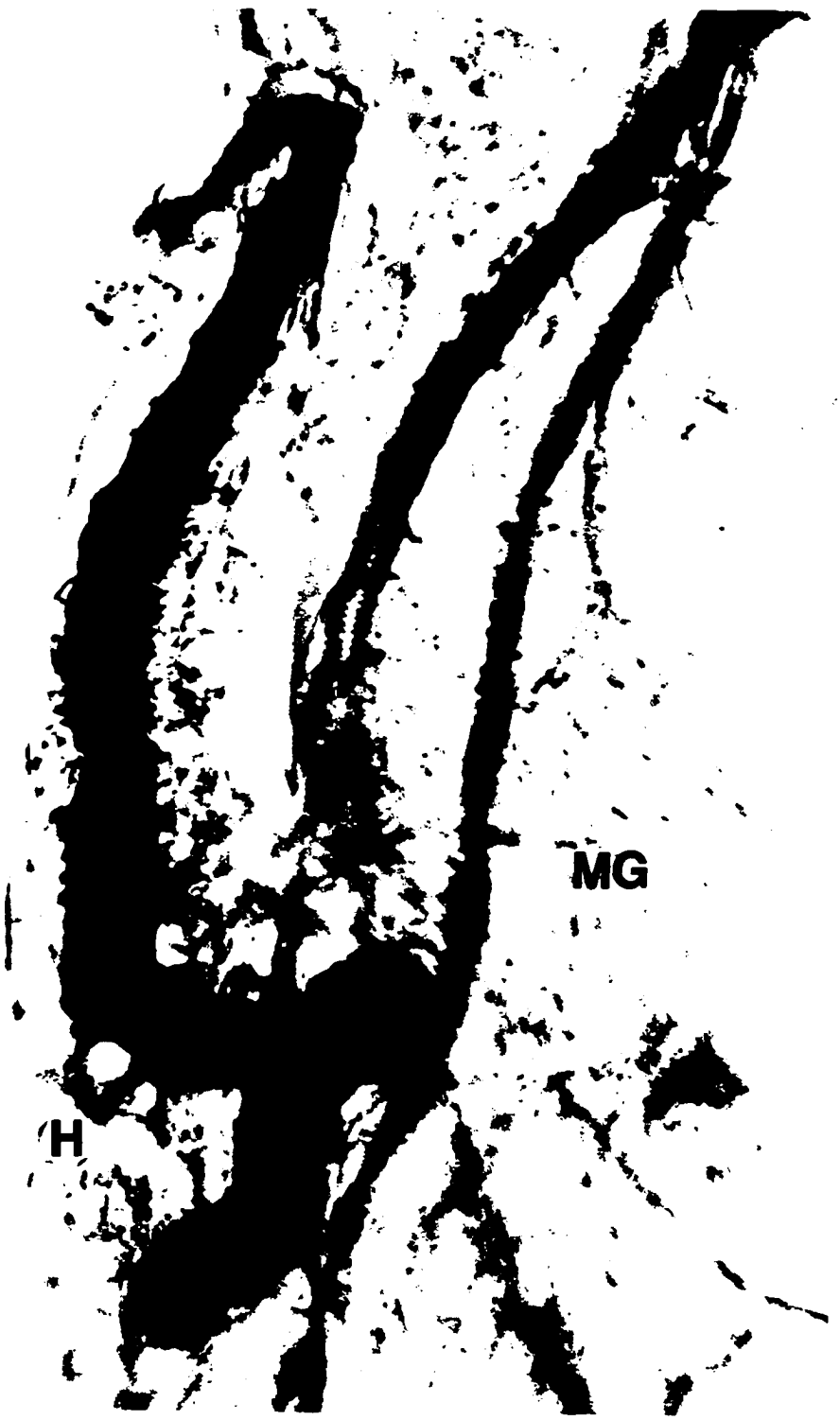
Figure 29. Melanized microfilariae (arrows) of Dirofilaria immitis in the hemocoele of Aedes trivittatus at day 9 PE (X 180)

Figure 30. Melanized $J_1$ (arrows) of Dirofilaria immitis within a Malpighian tubule of Aedes trivittatus at day 7 PE (X 180)


EC
26
27
28
MT
30

Plate IX

Figure 31. Encapsulation and melanization of a microfilaria of Dirofilaria immitis lying on the hemocoelomic surface of the midgut of Aedes trivittatus at day 7 PE. Note the accumulation of hemocytes around the microfilariae (X 1470)




31

Plate X

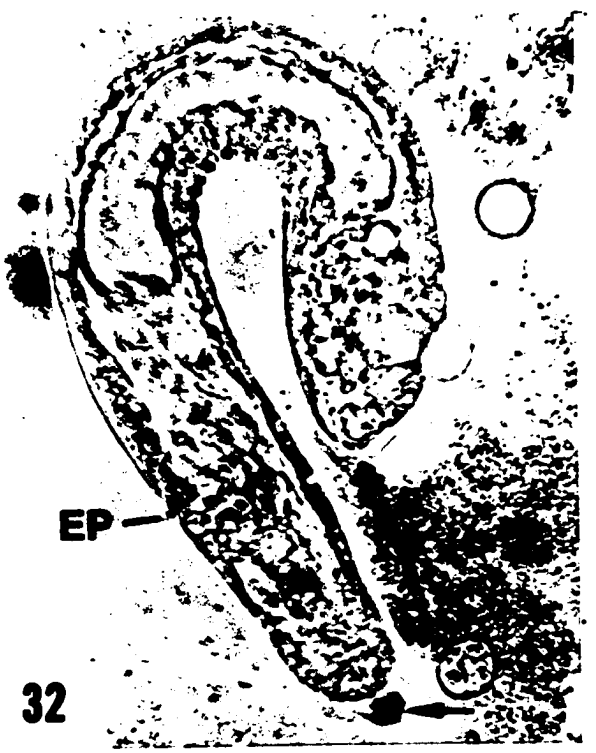
Figures 32-34. Melanization of $J_2$ *Dirofilaria immitis* from *Aedes trivittatus* at day 10 PE

Figure 32. Melanization evident only in the areas of the excretory pore, stoma (arrow), and cuticular tail extension (X 760)

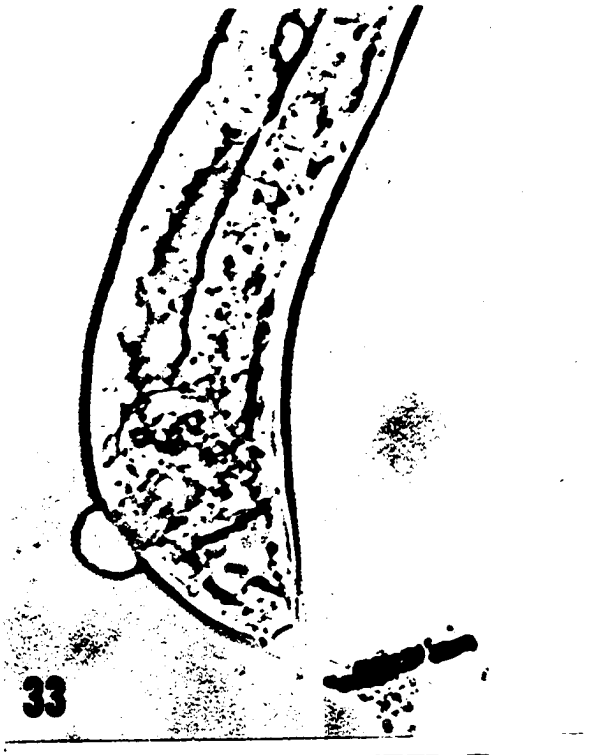
Figure 33. Slight melanization of the cuticular tail extension (X 1005)

Figure 34. Melanized cuticular tail extension being sloughed with the shed cuticle during molting (X 1020)

Figure 35. "Spotty" melanization of $J_1$ *Dirofilaria immitis* within a Malpighian tubule of *Aedes trivittatus* at day 9 PE (X 690)




32



33

34

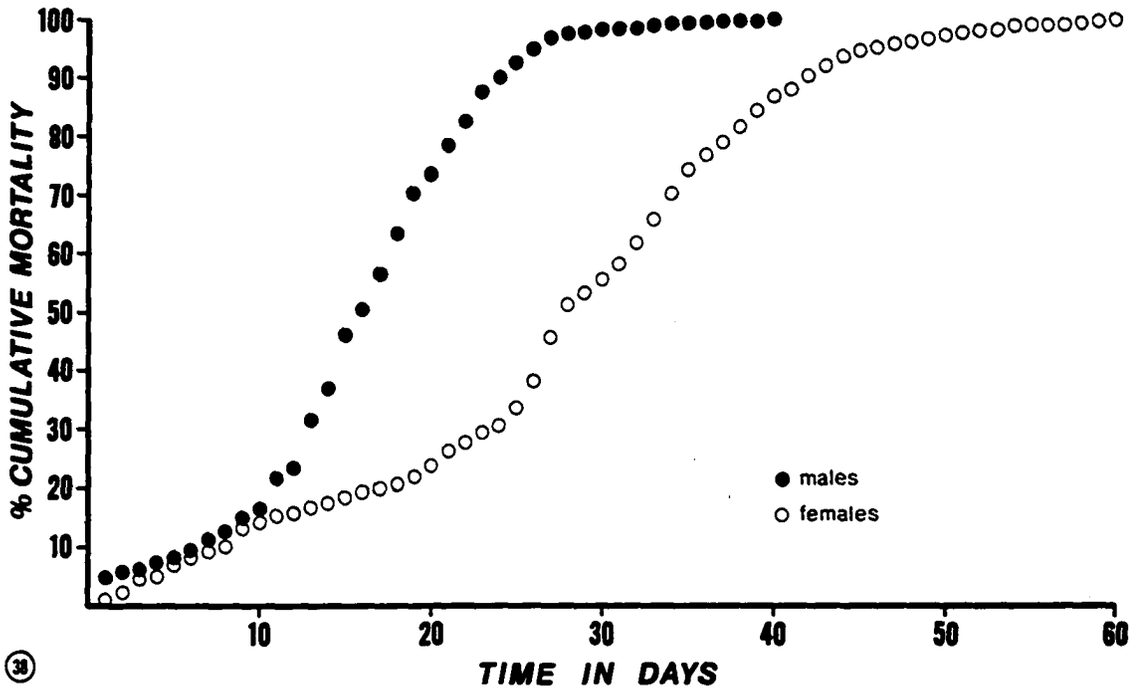
35

Table XI

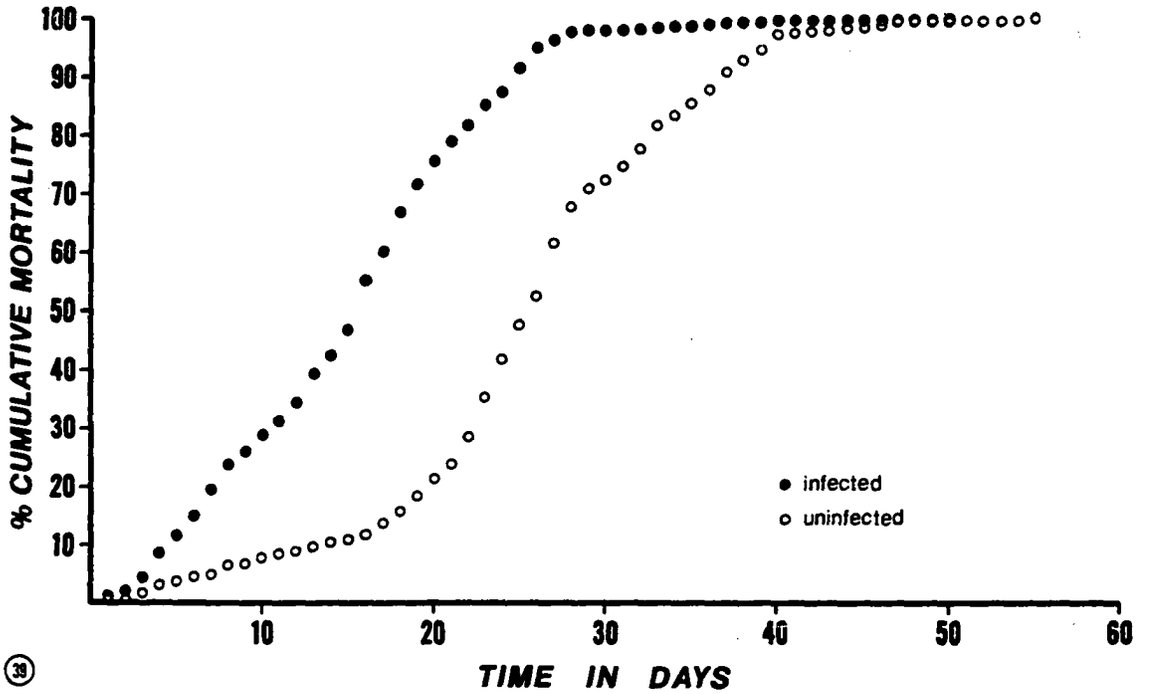
Figures 36-37. Melanized $J_3$ Dirofilaria immitis (arrows) in the hemocoele of Aedes trivittatus (X 260 and 220, respectively)

Figure 38. Longevity curves for male and female Aedes trivittatus maintained on 0.3 M sucrose solution in the laboratory at 26.5 ± 1° C and 80 ± 5% RH

Figure 39. Longevity curves for Aedes trivittatus exposed to a Dirofilaria immitis infected (347 microfilariae/20 $mm^3$) or uninfected dog


% CUMULATIVE MORTALITY
100
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
TIME IN DAYS
males
females

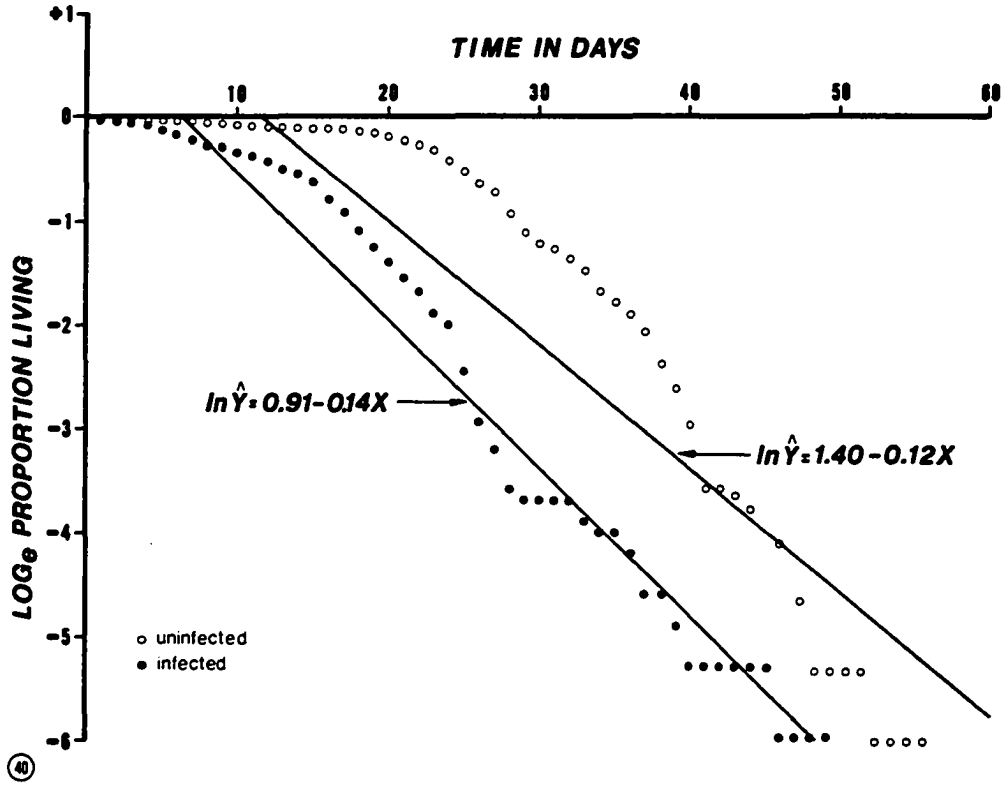
(38)


% CUMULATIVE MORTALITY
100
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
TIME IN DAYS
infected
uninfected

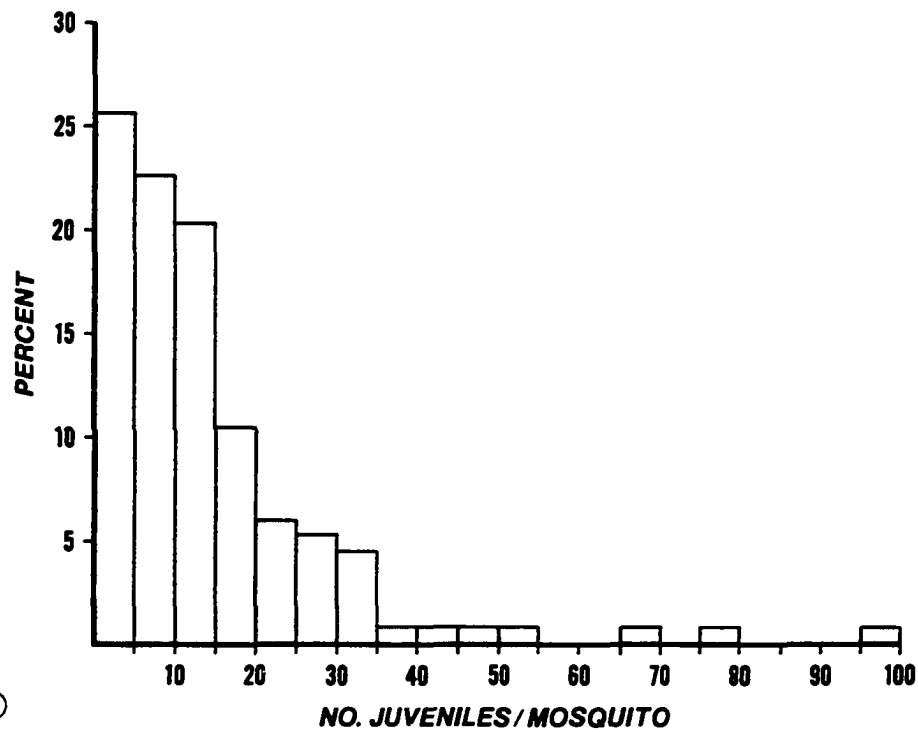
(39)

Figure 40. Relationship between proportion living and time in Dirofilaria immitis infected (347 microfilariae/20 $mm^3$) and uninfected Aedes trivittatus, with estimated regression lines

Figure 41. Frequency distribution of Dirofilaria immitis juveniles in Aedes trivittatus exposed to a dog with a high microfilaremia (347 microfilariae/20 $mm^3$)

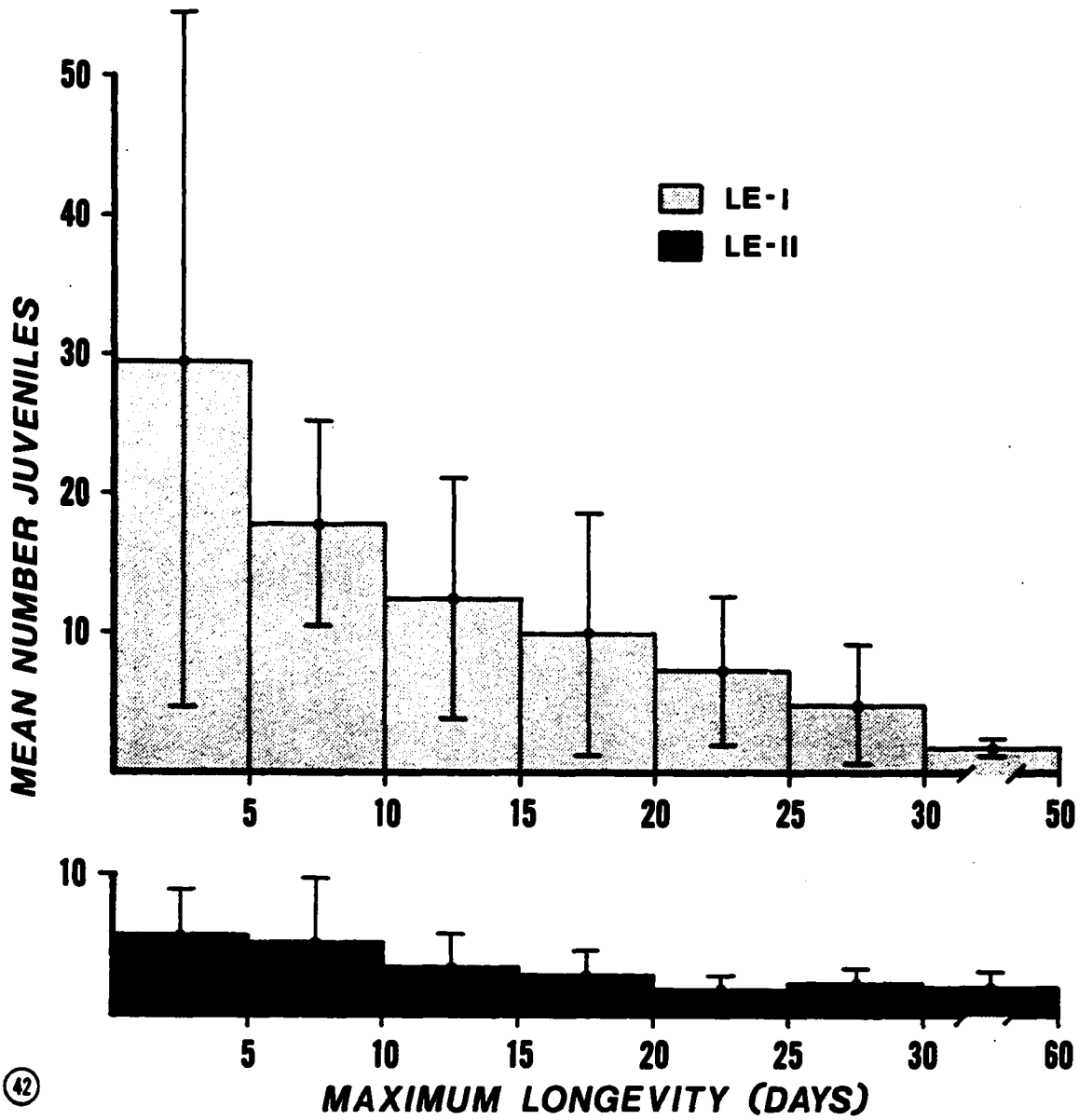

TIME IN DAYS
10
20
30
40
50
60
+1
0
-1
-2
-3
-4
-5
-6
LOGe PROPORTION LIVING
ln Ŷ = 0.91 - 0.14X
ln Ŷ = 1.40 - 0.12X
o uninfected
• infected

(40)


30
25
20
15
10
5
PERCENT
10
20
30
40
50
60
70
80
90
100
NO. JUVENILES / MOSQUITO

(41)

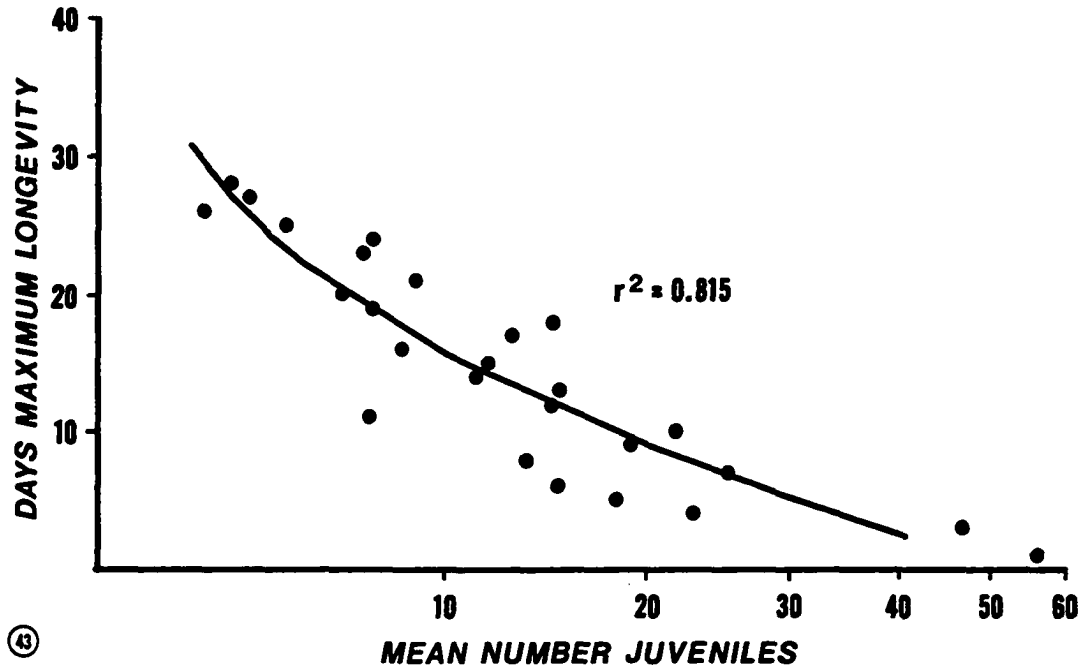
Figure 42. Mean numbers of juveniles recovered from Aedes trivittatus at time of death following exposure to a dog with a high (LE-I; 347 microfilariae/20 $mm^3$) or low (LE-II; 62 microfilariae/20 $mm^3$) microfilaremia of Dirofilaria immitis


LE-I
LE-II
MEAN NUMBER JUVENILES
50
40
30
20
10
5
10
15
20
25
30
50
10
5
10
15
20
25
30
60
MAXIMUM LONGEVITY (DAYS)

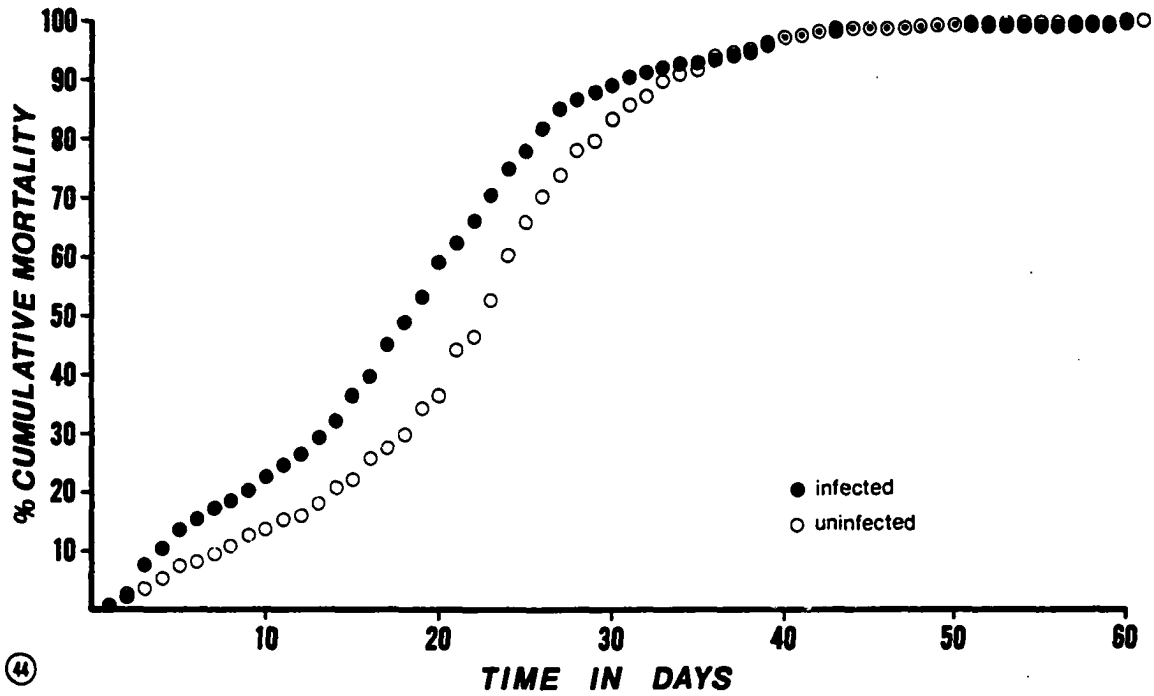
(42)

Figure 43. Relationship between parasite load and maximum longevity in Aedes trivittatus exposed to a dog with a high microfilaremia (347 microfilariae/20 $mm^3$) of Dirofilaria immitis

Figure 44. Longevity curves for Aedes trivittatus exposed to a Dirofilaria immitis infected (62 microfilariae/20 $mm^3$) or uninfected dog


40
30
20
10
DAYS MAXIMUM LONGEVITY
r² = 0.815
10
20
30
40
50
60
MEAN NUMBER JUVENILES

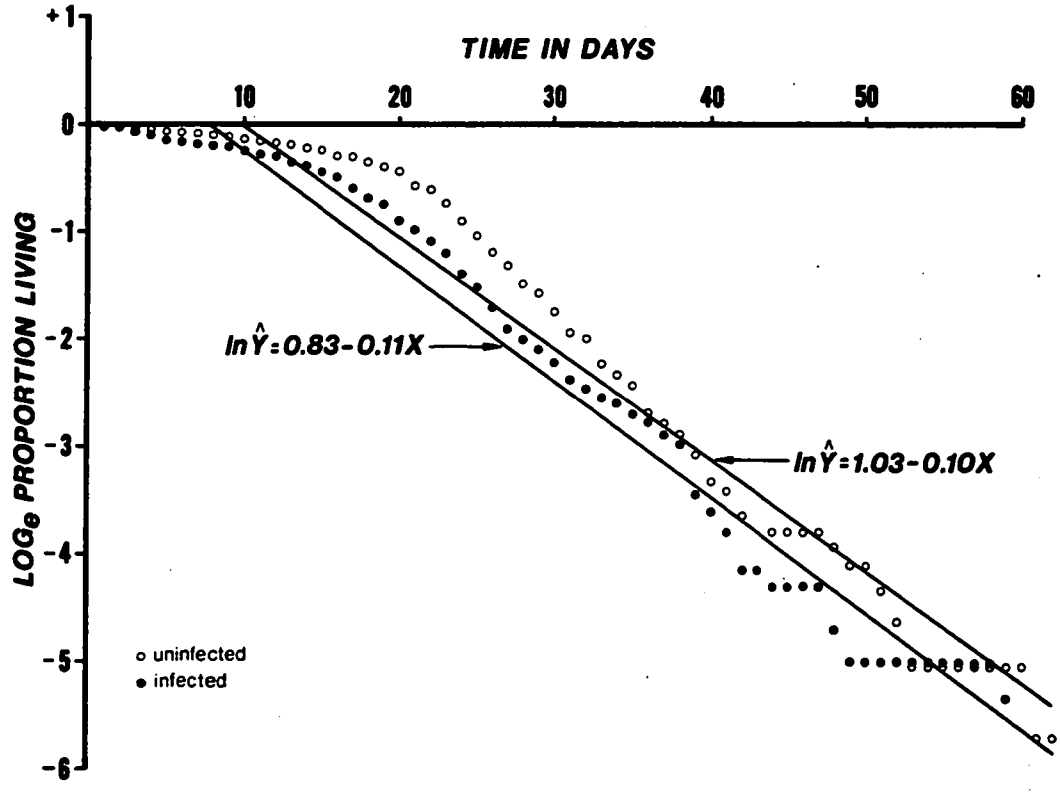
(43)


100
90
80
70
60
50
40
30
20
10
% CUMULATIVE MORTALITY
infected
uninfected
10
20
30
40
50
60
TIME IN DAYS

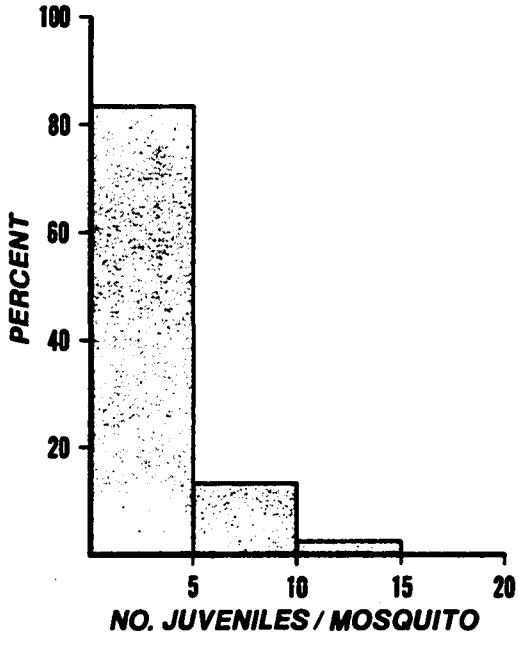
(44)

Figure 45. Relationship between proportion living and time in *Dirofilaria immitis* infected (62 microfilariae/20 $mm^3$) and uninfected *Aedes trivittatus*, with estimated regression lines

Figure 46. Frequency distribution of *Dirofilaria immitis* juveniles in *Aedes trivittatus* exposed to a dog with a low microfilaremia (62 microfilariae/20 $mm^3$)

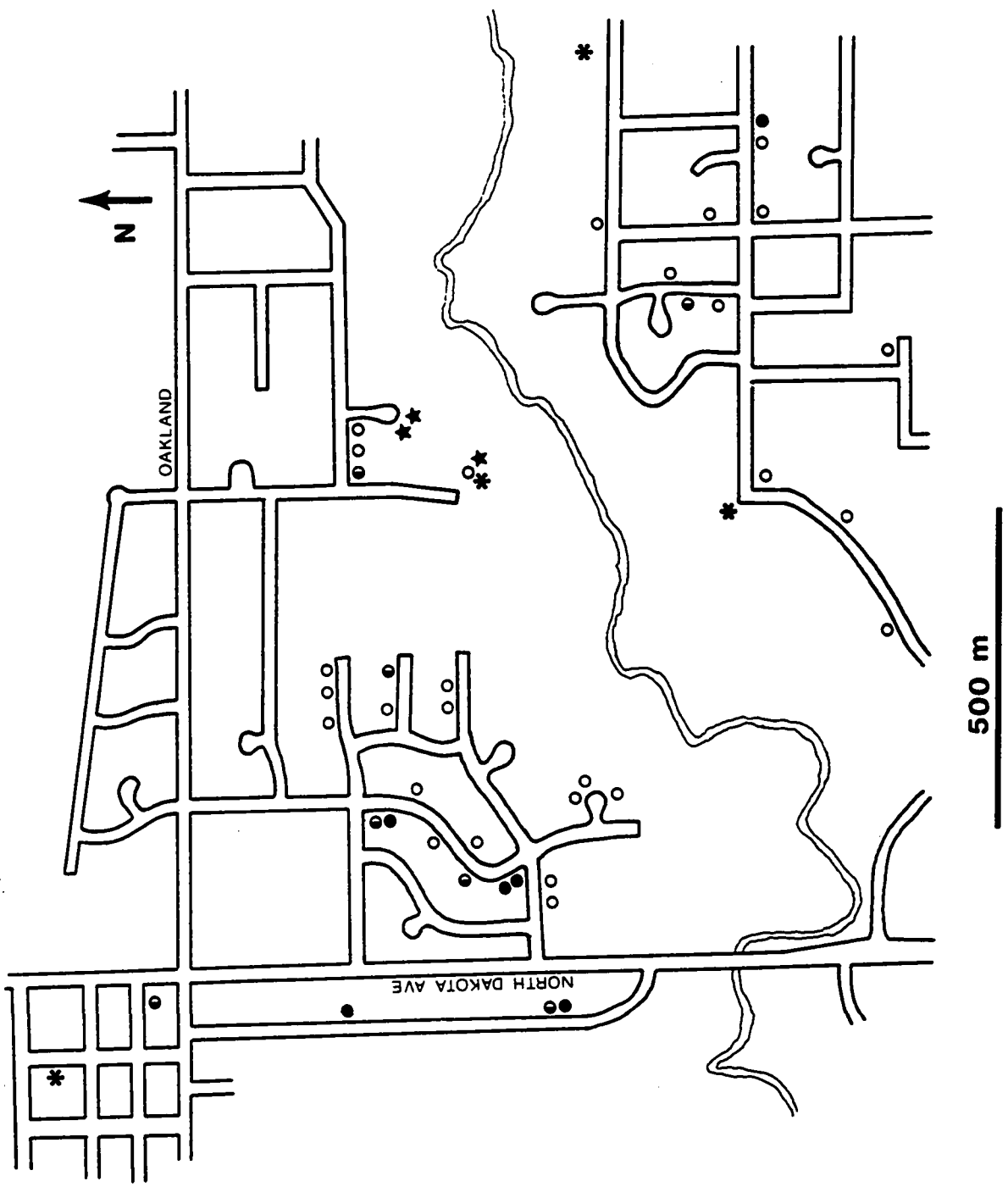

TIME IN DAYS
10
20
30
40
50
60
+1
0
-1
-2
-3
-4
-5
-6
LOG$_e$ PROPORTION LIVING
$\ln \hat{Y} = 0.83 - 0.11X$
$\ln \hat{Y} = 1.03 - 0.10X$
o uninfected
• infected

(45)


100
80
60
40
20
PERCENT
5
10
15
20
NO. JUVENILES / MOSQUITO

(46)

Figure 47. Map of an area of Ames, Iowa, surveyed for Dirofilaria immitis in 1975 and 1976 (Symbols: white circle, uninfected dog housed inside; black and white circle, uninfected dog housed inside and outside; black circle, uninfected dog housed outside; asterisk, patent infection in 1975; star, patent infection in 1976)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