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EVALUATION OF AEDES HENDERSONI AND

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JAMES S. ROGERS

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EVALUATION OF *AEDES HENDERSONI* AND *AEDES TRISERIATUS* AS POTENTIAL VECTORS OF *DIROFILARIA IMMITIS*

By

James Speed Rogers

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ABSTRACT

EVALUATION OF AEDES HENDERSONI AND AEDES TRISERIATUS AS POTENTIAL VECTORS OF DIROFILARIA IMMITIS

By

James Speed Rogers

The tree-hole breeding mosquitoes Ae. hendersoni and Ae. triseriatus were separately evaluated as potential vectors of D. immitis (dog heartworm) in Michigan. Significantly fewer D. immitis developed to the infective stage in Ae. triseriatus than Ae. hendersoni. Infection with D. immitis caused significantly greater mortality in Ae. hendersoni than Ae. triseriatus. Paired vertical ovitrapping in a 10 acre beech-maple woodlot indicated that Ae. hendersoni slightly outnumbered Ae. triseriatus. Ae. hendersoni and Ae. triseriatus were respectively the fourth and fifth most abundant species caught in the dog-baited traps. Paired vertical ovitrapping indicated that both Ae. hendersoni and Ae. triseriatus are primarily ground-level feeders. Ae. hendersoni and Ae. triseriatus are reviewed in relation to breeding habitat, flight range, relative abundance, feeding habits, longevity, and susceptibility to infection. It is concluded that both mosquitoes are potential vectors of D. immitis in wooded areas of Michigan.

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INTRODUCTION

In recent years dog heartworm, Dirofilaria immitis (Leidy), has become a serious veterinary problem in Michigan and other parts of the midwest (Lewandowski, 1977; Otto, 1969). Aedes triseriatus (Say), a tree-hole breeding mosquito, has been incriminated as a potential vector of D. immitis in Michigan (Lewandowski, 1977), Massachusetts (Phillips, 1939), Texas (Keegan et al., 1968), and Mississippi (Intermill, 1973). These last four studies failed to distinguish between Ae. triseriatus and a closely related species, Ae. hendersoni Cockerell, which is the most widely distributed tree-hole breeding mosquito in North America (Zavortink, 1972). Ae. hendersoni has never been specifically evaluated as a potential vector of D. immitis, although it is known that Ae. triseriatus and Ae. hendersoni have very different vector potentials for another disease, California encephalitis (Watts et al., 1975). The purpose of performing the field and laboratory studies reported here was to separately consider Ae. hendersoni and Ae. triseriatus in relation to the following factors which are relevant to D. immitis vector determination (Ludlam et al., 1970): flight range; breeding habitat; relative population density; feeding habits; longevity; and susceptibility to infection.

LITERATURE REVIEW

Taxonomy and Morphology

Ae. triseriatus var. hendersoni was described by Cockerell in 1918. Dyar (1919) placed hendersoni in synonymy with triseriatus, and Breland (1960) resurrected hendersoni to full specific rank on the basis of morphological and chromosomal studies. Breland's action has been fully substantiated by subsequent reports of morphological and ecological differences between Ae. hendersoni and Ae. triseriatus. According to Zavortink (1972), Ae. hendersoni and Ae. triseriatus are sibling species (closely related species which are reproductively isolated but morphologically identical or nearly so; Mayr, 1963).

Morphological differences between Ae. hendersoni and Ae. triseriatus are subtle; however, Zavortink (1972) listed five characteristics of adults, six of the male genitalia, six of the larvae, and two of the pupae, which may be used to differentiate between the species. In addition, Zaim et al. (1977) described differences in the egg morphology of Ae. hendersoni and Ae. triseriatus, using light and electron microscopy.

Apparently, both behavioral and morphological differences between Ae. hendersoni and Ae. triseriatus ensure reproductive isolation. In small laboratory cages, Ae. triseriatus mates freely, while Ae.

hendersoni will not. Laboratory colonies of Ae. hendersoni must be propagated by the forced copulation technique of McDaniel and Horsfall (1957). Truman and Craig (1968) used this technique to produce hybrids of Ae. hendersoni and Ae. triseriatus, and found the larval and adult hybrids to be morphologically intermediate between the two species. The F_1 hybrid males produced by mating male Ae. hendersoni to female Ae. triseriatus, however, had deformed genitalia. Grimstad et al. (1974) reported that less than .5% of field-collected larvae were hybrids.

Geographical and Ecological Distribution

Ae. hendersoni is the most widespread tree-hole breeding mosquito in North America. Ae. triseriatus is nearly as widespread, and is sympatric with Ae. hendersoni in all states and provinces east of the Great Plains (Zavortink, 1972). According to Lunt and Peters (1976), the range of Ae. hendersoni extends further west because Ae. hendersoni is more tolerant of arid conditions. Hanson and Hanson (1970) described how past land management has inadvertently increased the number of tree-holes in the midwest.

Although water-filled rot holes in trees are the usual larval habitat of Ae. hendersoni and Ae. triseriatus, these mosquitoes have been collected from artificial containers such as tin cans and discarded tires. Ovitraps made of jars or cans are attractive to gravid females and make useful field samplers for Ae. hendersoni and Ae. triseriatus (Furlow and Young, 1970). The utility of ovitrapping was greatly enhanced by the report by Zaim et al. (1977) that the eggs of Ae. hendersoni and Ae. triseriatus could easily be distinguished

morphologically.

The use of ovitraps has permitted study of ovipositioning in the canopy of woods, and differences between the oviposition sites of Ae. hendersoni and Ae. triseriatus have been described. Scholl and De-Foliart (1977) in Wisconsin, and Sinsko and Grimstad (1977) in Indiana, reported that Ae. triseriatus oviposits primarily in ground-level ovitraps, while Ae. hendersoni oviposits primarily in elevated ovitraps. However, in a special problems project designed to replicate these ovitrapping studies, Rogers (1978) in Michigan found that Ae. hendersoni preferred elevated ovitraps, but Ae. triseriatus utilized ovitraps at both levels equally.

Epizootiology

According to Otto (1972), D. immitis was originally enzootic in areas within 83-125 km of the Atlantic coast, from New Jersey to Texas, and this situation prevailed as recently as 30 years ago. Since then, there have been reports of D. immitis infections in several inland states and provinces, both north and west of the original enzootic foci. In the North, D. immitis has been known to be enzootic in Minnesota for the last two decades (Otto, 1972), and Slocombe (1978) reported enzootic D. immitis infections in Ontario and Manitoba. In the West, D. immitis is an increasing problem in California (Weinmann and Garcia, 1974) and Oklahoma (Kocan and Laubach, 1976).

It is of particular interest that the incidence of D. immitis is increasing in many, but not all, parts of the midwest (Otto, 1972). In Ohio, Streitel et al. (1977) found 11 of 500 (2.2%) dogs necropsied in a Columbus humane shelter to be infected with D. immitis.

Groves and Kutz (1964) found 7 of 380 (1.8%) necropsied dogs in Ohio to be infected with D. immitis. In the 13 year period between those studies, the incidence of D. immitis had not significantly increased, and Streitel et al. (1977) concluded that a highly enzootic area had not developed in Ohio. Kazacos (1978) reported that 17 of 112 (15%) dogs necropsied in Lafayette, Indiana were infected with D. immitis. Marquardt and Fabian (1966) reported that D. immitis infection rates in southern, central, and northern Illinois were 35%, 22%, and 10%, respectively.

D. immitis has received considerable attention in Michigan. Like most other midwestern states, Michigan has experienced an increase in the prevalence of D. immitis. Leash and Hanson (1961) reported that 4 of 192 (2.1%) blood smears seen at the Michigan State University Veterinary Clinic were positive for D. immitis. Zydeck et al. (1970) found 4 of 248 (1.6%) blood smears taken from dogs in the Detroit dog pound positive for D. immitis. Worley (1964) found 6 of 123 (5.4%) dogs from southeastern Michigan dog pounds positive for D. immitis when necropsied. Prouty (1972) reported that 195 of 880 (22%) Belleville dogs, 22 of 399 (6%) Detroit dogs, and 41 of 698 (6%) Farmington dogs had D. immitis microfilariae. Prouty found that older dogs and dogs kenneled outdoors had a higher probability of being infected with D. immitis.

The most comprehensive data on the epizootiology of D. immitis in Michigan has been collected by Dr. H. D. Newson, who sent out questionnaires to veterinarians throughout Michigan. The data of Dr. Newson show that there were at least 14,525 confirmed cases of D. immitis in 54 counties of Michigan from pre-1951 to May, 1972. Since 1972, D.

immitis infections have been reported from six additional counties in the state. The majority of D. immitis infections have been reported from urban counties in the lower peninsula.

The reported distribution of D. immitis in the midwest is not uniform (Otto, 1972), and the different methods of making D. immitis surveys probably accentuate this apparent uneven distribution. Streitel et al. (1977) reported that 13 of 24 dogs with D. immitis in their hearts did not have a microfilaremia, illustrating a bias in surveys based on microfilarial findings. Further, the various methods of examining blood for microfilariae that were used in reported studies are not equally sensitive. Dogs screened at a veterinary clinic would presumably show a higher infection rate than dogs randomly chosen at a dog pound, because infected dogs often present symptoms that would elicit medical attention. Also, increasing awareness of D. immitis among veterinarians in recent years has resulted in more efficient diagnosis. An additional complication in comparing reported data on D. immitis incidence is that microfilarial surveys conducted before 1956 must be questioned. In that year Newton and Wright described a new species of microfilariae in dogs, Dipetalonema reconditum (Grassi), that is morphologically very close to D. immitis. It is probable that earlier surveys would have mistaken D. reconditum microfilariae for those of D. immitis. D. reconditum is transmitted by fleas, and produces no pathology in the dog.

In Michigan, there are two reports of D. reconditum infections. Leash and Hanson (1961) reported that 8 of 192 (4.2%) dogs examined at the Michigan State University Veterinary Clinic were positive for D. reconditum. Zydeck et al. (1970) reported that 7 of 248 (2.8%) dogs

from Detroit were positive for D. reconditum.

Pathogenesis

According to McGreevy et al. (1974), the third-stage D. immitis larvae in the labium of an infective mosquito are stimulated to escape from the tip of the labium when it is bent in the feeding process. A tiny droplet, probably mosquito haemolymph, is deposited on the skin along with the larvae. This fluid protects the larvae from desiccation, and allows them to penetrate the skin at the fasicle puncture site.

Next, the larvae enter the bloodstream and migrate to submuscular membranes and subcutaneous tissues (Kume and Itagaki, 1955), where they molt at 9-12 days and 60-70 days after inoculation. After 3-4 months, the larvae migrate to the heart via the veins. Once in the heart, males and females attain adult lengths of 15-18 cm and 25-30 cm, respectively. Sexual maturation is reached about 8 months after inoculation, as indicated by the release of microfilariae into the bloodstream.

According to Garlick (1975), each female D. immitis releases about 30,000 microfilariae daily into the bloodstream, and the microfilariae live up to 2 years. The microfilariae of D. immitis exhibit both daily and seasonal periodicity. According to Otto (1969), in a 24 hour period the maximum microfilaremia is from 5 to 50 times the minimum microfilaremia. Church et al. (1976) described D. immitis microfilarial fluctuations as "subperiodic" and could not even characterize the fluctuations as diurnal or nocturnal. Eyles et al. (1954), Kume (1974), and Sawyer (1974) reported that the microfilaremia is lower in colder months. According to James and Harwood (1969), circadian periodicity is a response of the parasite to its vectors, the maximum

microfilaremia coinciding with the chief biting period of the vector. According to Ansari (1970), during the peak microfilaremia, the microfilariae are evenly distributed throughout the circulation, and at low microfilaremia, the microfilariae are sequestered in the lungs. Hawking (1967) reported that oxygen tension in the blood is the factor which determines the distribution of the microfilariae.

A dog infected with D. immitis may be symptomless except for the presence of circulating microfilariae (Soulsby, 1968). Commonly, dogs infected with D. immitis have a persistent cough and lack stamina. In severe cases, there are a variety of other symptoms which can be fatal. According to Soulsby (1968), the severity of the symptoms is proportional to the number of adult worms in the heart. Fowler et al. (1973), however, found no correlation between the microfilaremia and the number of adult worms in the heart.

Abnormal Hosts

Although D. immitis adults have been found in many types of wild animals, Otto (1972) reported that there is no evidence of any reservoir host. Lewandowdki (1977) listed the following animals as hosts of adult D. immitis: fox, beaver, coyote, wolf, dingo, cat, seal, gibbon, tiger, jaguar, and sea lion. Goble (1942) reported the muskrat, Williams and Dade (1976) the wolverine, and Johnson (1975) the black bear, as having D. immitis infections.

Human dirofilariasis has been reported with increasing frequency in the southern and eastern United States and Canada (Blecka, 1978). According to Schlotthauer et al. (1969), most cases of human dirofilariasis are not caused by D. immitis, but by Dirofilaria tenuis

Chandler, a raccoon parasite. Schlotthauer et al. (1969), however, attributed 14 cases of human dirofilariasis to D. immitis. Twelve of these cases produced "coin" lesions of the lung. The lesions may be detected by routine chest X-rays, or X-rays may be prompted by cough and chest pain (Schlotthauer et al., 1969). The chief importance of human dirofilariasis is its diagnostic interpretation, because the lesions produced by D. immitis may be confused with a malignancy.

Vectors

Mosquitoes are the only known vectors of D. immitis (Ludlam et al., 1970), and become infected by ingesting circulating microfilariae along with the blood of the dog. Kershaw et al. (1955) found that fewer microfilariae are ingested by the mosquito than would be expected on the basis of the microfilaremia and volume of blood ingested.

Taylor (1960) reported that at 24.4^{o} C, the microfilariae leave the midgut of Ae. aegypti (Linnaeus) after 24 hours and enter the cells of the malpighian tubules. Once in the cells of the malpighian tubules, the microfilariae become less motile. On the sixth or seventh day, the larvae enter the lumen of the malpighian tubules, and by the tenth day molt to the second-stage. The 2nd-stage larvae, which are senentary, molt to become the highly motile 3rd-stage beginning on the 13th day after ingestion. On the 15th day after ingestion, 3rd-stage larvae leave the malpighian tubules, and migrate to the mouthparts of the mosquito via the haemocoel.

Kutz and Dobson (1974) described how temperature affects the rate of development of D. immitis in the mosquito, and how the geographical range of D. immitis is influenced by climate. Ho et al. (1974)

described how third-stage D. immitis larvae in mosquito mouthparts may spontaneously escape. According to Bemrick and Bemrick (1969), third-stage D. immitis larvae do not escape from the mouthparts when the mosquito takes a blood meal.

Laboratory studies have shown that different mosquito species (and strains) vary greatly in their susceptibility to D. immitis infection (Hu, 1931; Kartman, 1953a). In refractory hosts, blood clotting in the midgut may mechanically impede the microfilariae, or they may be passed to the hindgut and excreted (Kartman, 1953a, 1953b). Some hosts resist infection by forming a chitinous capsule around the developing larvae, and in others, larval development does not procede even in the absence of encapsulation (Kartman, 1953a, 1953b).

Death of the vector is also a physiological response to D. immitis infection, and according to Hamilton and Bradley (1979), is the most salient problem attending experimental attempts to transmit dirofilarias through laboratory mosquitoes. These authors found that early death of D. immitis-infected mosquitoes was due to active, living microfilariae, and not to dead, intact, or homogenized larvae, whole mosquito bodies, or body fractions. Although Intermill (1973) reported that D. immitis larvae in Ae. triseriatus did not cause apparent histological damage to the gut or malpighian tubules, Hamilton and Bradley (1979) judged that the malpighian tubules could be damaged without visible changes.

Ludlam et al. (1970) listed 63 species of mosquitoes in which complete larval development of D. immitis has been reported. Despite this long list, these authors wrote that "the principal mosquito vectors of D. immitis have not been identified in any area of the world."

According to these authors, the weakness of most reports on potential D. immitis vectors is that they go no further than reporting development of D. immitis to the infective third-stage under laboratory conditions. Ludlam et al. (1970) judged that the ability of microfilariae to develop to the infective stage in the laboratory does not indicate that the mosquito in question is an efficient vector in nature. These authors noted that data on field-collected mosquitoes harboring infective D. immitis larvae are an important step in vector determination, but are found in only a few reports. Christensen (1977) pointed out that dog-to-dog transmission of D. immitis has been reported for only three species of North American mosquitoes. Ludlam et al. (1970) also mentioned other factors which are relevant to vector determination, but which are often ignored: breeding habitat; flight range; relative population density; feeding habits; longevity; and genetics of different strains which affect susceptibility to D. immitis infection. Michigan is one of the few regions where a detailed study, giving due consideration to the above factors has been made. Lewandowski (1977) concluded that Ae. vexans (Meigen), An. quadrimaculatus Say, and An. walkeri Theobald are D. immitis vectors of primary importance in Michigan, and that Ae. canadensis (Theobald), Ae. cinereus Meigen, and Ae. triseriatus are vectors of secondary importance.

Ae. hendersoni and Ae. triseriatus as Vectors

There is some relevant information on the D. immitis vector potential of Ae. triseriatus, but until this study there was none on Ae. hendersoni. Benach et al. (1971) in the laboratory, and Wright and DeFoliart (1970) in the field, found that Ae. triseriatus is a general

feeder, feeding on man, other mammals, birds and turtles. According to Loor and DeFoliart (1970), Ae. triseriatus is a daytime feeder, with chief biting activity in the late afternoon and evening. Morris and DeFoliart (1971) found Ae. triseriatus to have the highest parous rate (32-45%) of any Wisconsin woodland mosquito. This finding enhances the vector potential of Ae. triseriatus (for any disease) because it indicates that this species lives a long time and takes repeated blood meals.

Phillips (1939) in Massachusetts, was the first to study Ae. triseriatus as a vector of D. immitis. (The study of Phillips was made before Ae. hendersoni was elevated to specific rank.) Phillips found that Ae. triseriatus fed avidly on dogs in the field and laboratory, and that D. immitis readily developed to the infective stage in Ae. triseriatus in the laboratory. Keegan et al. (1968) in Texas also found that D. immitis developed to the infective stage in Ae. triseriatus in the laboratory. No mention is made in their study of Ae. hendersoni, and it is not possible to know if proper care was taken to differentiate Ae. hendersoni from Ae. triseriatus. Intermill (1973) made the most detailed study on the ability of D. immitis to develop in Ae. triseriatus, and judged Ae. triseriatus to be an efficient vector. He noted that host mortality was low, and that a high proportion of the mosquitoes which ingested D. immitis developed infective larvae, although some encapsulation of developing larvae took place. Intermill collected larvae for his study from tree-holes in Mississippi, and notably, made no mention of any effort to differentiate between Ae. hendersoni and Ae. triseriatus, although both species occur in Mississippi.

Lewandowski (1977) made field studies on the D. immitis vector potential of Ae. triseriatus in Michigan. He found that Ae. triseriatus was locally abundant (i.e., occurring in woodlots), and was attracted to dogs in dog-baited mosquito traps. He judged that Ae. triseriatus was an excellent host and potential vector of D. immitis, but due to its local distribution, may have only secondary importance in the natural maintenance of this disease. Lewandowski made no attempt to differentiate between Ae. hendersoni and Ae. triseriatus, and in view of the known mixed populations of these two species that are present in his study area, the "Ae. triseriatus" in his study can reasonably be considered a mixture of Ae. hendersoni and Ae. triseriatus.

MATERIALS AND METHODS

Colony Development and Maintenance

The insectary, where all stages of the mosquitoes were maintained, had a 16 hour photoperiod, including ½ hour crepuscular periods of diminished light. The temperature in the insectary ranged from 21.1-26.7° C and the relative humidity from 80-100%. Larvae were reared in white enamel pans, and each pan was fed daily a pinch of Tetramin^{R} fish food. Cages of adult mosquitoes were provided with a 7% sucrose solution in a flask from which a cotton wick protruded. Cages were placed on corks in petri dishes filled with mineral oil to protect the mosquitoes from predacious ants. Eggs were stored in the insectary for at least one week before hatching in water deoxygenated with autolyzed yeast (Difco Laboratories, Detroit).

The colonies of both *Ae. hendersoni* and *Ae. triseriatus* were founded by eggs taken in ovitraps in the summer of 1977. Larvae were not added to their respective colonies until all the egg shells on the tongue blade of an ovitrap were checked under a light microscope by the method of Zaim *et al.* (1977), and found to belong to one species. If a tongue blade had eggs of both species, all larvae which hatched from that tongue blade were discarded. Despite this check, several months after being colonized, the *Ae. hendersoni* colony was found to contain *Ae. hendersoni*-*Ae. triseriatus* hybrids, as determined by the

criteria of Grimstad et al. (1974). The hybrids were excluded from the colony by individually segregating gravid adult female Ae. hendersoni and collecting all the eggs produced by each one. For this, each gravid female Ae. hendersoni was placed in a 472 ml paper cup, which was covered with mosquito netting and contained a sucrose source and an oviposition beaker made from a 118 ml paper cup lined on the inside with paper toweling. The entire egg batch produced by each female, after maturation, was hatched in an enamel larval pan, and the larvae reared to the fourth-stage. At least ten fourth-stage larvae of each egg batch were examined and determined to be Ae. hendersoni, Ae. triseriatus, or hybrids, according to the criteria of Grimstad et al. (1974). If any hybrid or Ae. triseriatus larvae were found, the entire egg batch was discarded. The Ae. hendersoni obtained by this method were placed in a cage, and after one generation, a pure colony was obtained, as determined by subsequent larval spot checks.

The Ae. triseriatus mated freely in laboratory cages, but the Ae. hendersoni had to be propagated by a forced copulation technique similar to that of McDaniel and Horsfall (1957). The thorax of the male was firmly pinched by a pair of forceps. This served as a handle, and also severed any connection to the brain (which inhibits copulation), eliminating the need to decapitate the male. The female then was held by "suction forceps" powered by a water-driven aspirator (Carolina Biological Supply Co.) (Figure 1). The glass tip of the "suction forceps" was made by drawing out a fine glass tube in a bunsen burner.

The mosquitoes were anesthetized for forced copulation by carbon

Figure 1. Suction forceps for mosquito forced copulation.

a. Threads for faucet attachment.

b. Water exhaust.

c. Rubber tubing (1 m long).

d. Thumb hole, used to make and break vacume.

e. Glass tip, which contacts female mesoscutum.

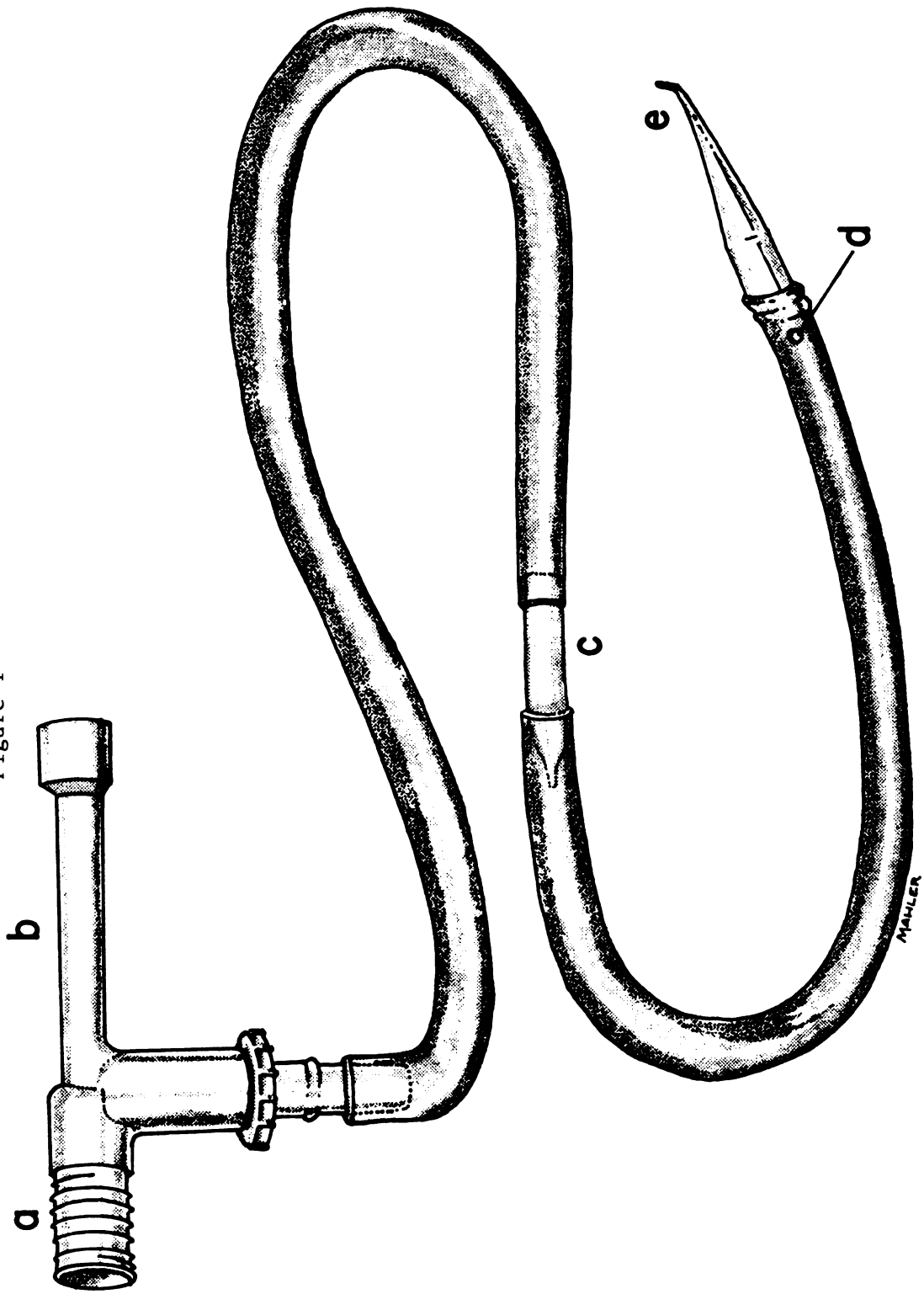


Figure 1

dioxide evolved from dry ice. It was convenient to anesthetize the mosquitoes in the aspirator which was used to transfer them from the holding cage. Males were exposed to carbon dioxide until they collapsed (about ten seconds), and were immediately pinched by the forceps. Females were exposed for about 20 seconds, because they had to remain anesthetized for at least 1 minute for the procedure to be successful. (Females which revived during copulation would kick away their partner.) The forced copulation technique was very time consuming, and even after a year of trial and error variations, the success rate never rose above 20%. The highest rate of insemination was achieved when the abdomens of the copulating pair formed an angle of 90-120 degrees, and males were 1-3 weeks old.

Dr. G. B. Craig (personal communication) suggested that if given a large enough cage, the Ae. hendersoni might swarm and mate freely, obviating the forced copulation technique. A 2.3 m^3 cage was built and several thousand Ae. hendersoni put into it. The cage had a 16 hour photoperiod, including crepuscular periods created with a rheostat controlling light intensity. Unfortunately, the Ae. hendersoni did not mate freely, and the forced copulation technique had to be used.

Laboratory Infection of Mosquitoes

In the laboratory experiments, a dog served as the infective blood source for D. immitis. All dogs used in these studies were obtained through the Michigan State University Laboratory Animal Care Service. Originally, attempts were made to use the canvas dog-restraining

harness described by Lewandowski (1977), to allow the mosquitoes to feed on the dog. In later experiments, this apparatus was discarded in favor of making the dog lie quietly on its side, with a shaved paw extended into the mosquito cage. The dog tolerated the mosquito-feeding very well, and no tranquilizer or restraining apparatus was needed. For experimental purposes, mosquitoes were 4-7 days old at the time of blood feeding. The order in which the two treatment groups in each replicate were fed (since they could not be fed simultaneously) was determined by flipping a coin.

For microfilaremia determination, blood samples were obtained at 9:00 a. m., the time at which each infective feeding replicate was begun. Blood was taken from the cephalic vein of the infected dog in a syringe containing a pinch of ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, and 20 mm^3 of the sample was transferred from the syringe and divided between 2 slides. A 20 X 60 mm coverslip was then placed on each slide, and the microfilariae were counted under a compound microscope.

Susceptibility to D. immitis

Mosquito susceptibility to D. immitis infection was studied in two ways. In study 1, several thousand Ae. hendersoni were fed on the infected dog after determining its microfilaremia. For 15 days after the infective blood meal, from 11 to 26 mosquitoes were dissected daily, and the number of larvae, their location, and observations on their developmental stage recorded. The dissection technique was that of Jones (1967), in which the entire gut and ovaries were drawn out into a drop of saline with the use of instruments made of minuten pins

embedded in applicator sticks.

In study 2, 300 Ae. hendersoni and 300 Ae. triseriatus were fed on the infected dog, in each of 10 replicates. Sixteen days after the infective blood meal, 12 heads of each species were dissected under a microscope, and the number of D. immitis larvae present was recorded.

Longevity

Three studies addressed the subject of mosquito longevity. In study 3, 100 infected Ae. hendersoni were randomly assigned to a cage lacking an oviposition beaker and 100 infected Ae. hendersoni were likewise assigned to a cage with a beaker, in each of ten replicates. The number of surviving mosquitoes in each cage was recorded 16 days after feeding on the infected dog.

To begin study 4, several hundred Ae. hendersoni were allowed to engorge on the infected dog immediately after its microfilaremia had been determined. Several hundred other Ae. hendersoni were allowed to engorge simultaneously on the uninfected dog. Immediately after feeding, 70 engorged females within each treatment group were randomly assigned to each of six cages. For 17 days, the number of survivors in each cage was recorded daily.

The mosquitoes used in study 5 were the same as those in study 2. Sixteen days after the infective blood meal, the number of survivors of each species was recorded.

Relative Abundance

Study 6 concerned the relative abundance of Ae. hendersoni and Ae. triseriatus in a ten acre beech-maple woodlot (Hudson's Woods)

on the campus of Michigan State University. This woodlot was chosen because it was within bicycling distance, barking dogs would not disturb people there, it was inaccessible to the public, and because preseason reconnoitering indicated that it was well endowed with treeholes.

Twenty-one pairs of ovitraps were widely dispersed within the woodlot (Figure 2). The ovitraps were made by removing the tops from 354 ml beer cans and spray painting the cans black. The ovitraps were always paired, one placed at 6 m, and the other at ground level. The upper traps were attached to a cord, and could be raised and lowered for servicing. The cords were placed by using a ladder, and looped either over a convenient limb or small nail. Each trap had a wooden tongue blade wrapped with paper toweling clipped into it with a large paper clip. At weekly intervals, the old tongue blades were collected and replaced with new ones, and the traps were refilled with water. The tongue blades with attached eggs were stored for at least one week in the insectary to allow them to maturate. Maturated eggs were hatched by placing the tongue blades containing the eggs in a beaker of water, adding a pinch of autolyzed yeast, and leaving them overnight. Hatched egg cases were cleared by the technique of Zaim et al. (1977) and examined under a light microscope. A hand counter was used to tally the number of Ae. hendersoni and Ae. triseriatus eggs.

Feeding Habits

The 10 rat-baited mosquito traps used in study 7 were the same as those used by Shaw (1976) as chicken-baited traps. These traps were paired, one at ground level and the other at 10 m, and were widely

Figure 2. Distribution of mosquito traps in Hudson's Woods

■ = dog-baited trap

● = pair of vertical ovitraps

▲ = pair of rat-baited traps

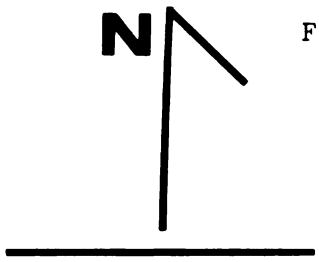
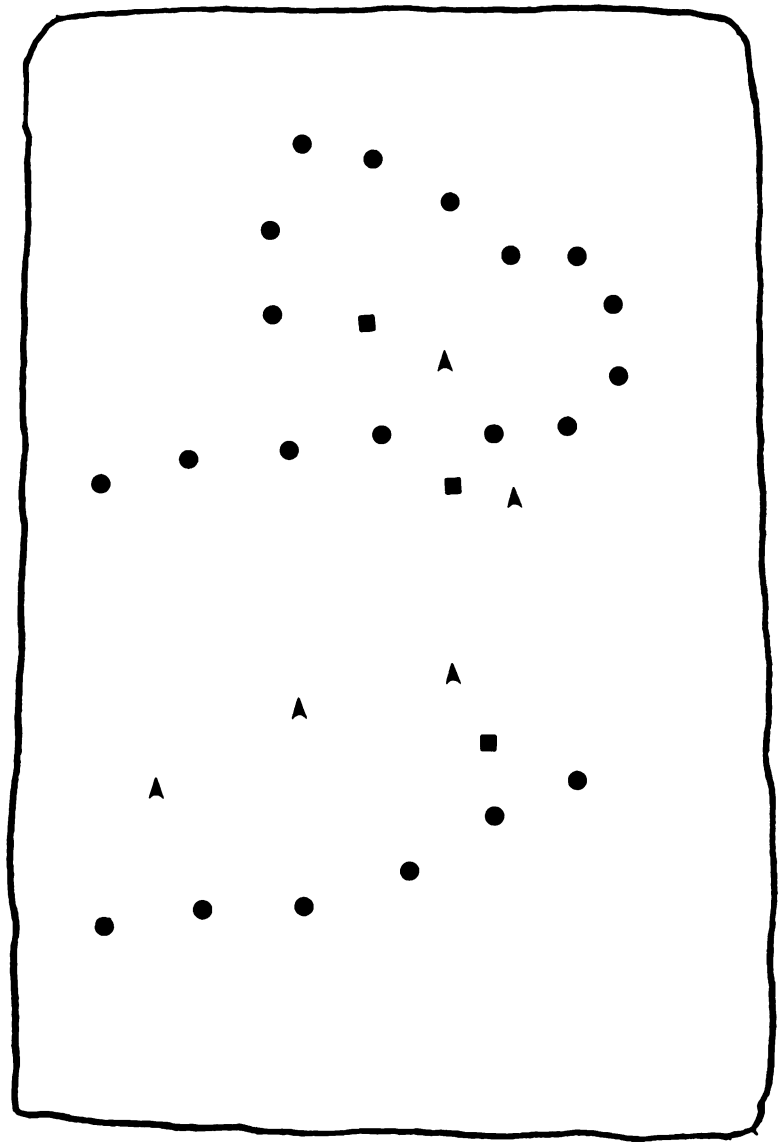


Figure 2.

100 m



Bennett Rd.

dispersed in Hudson's Woods (Figure 2). The captured mosquitoes were killed in the evening with chloroform.

The three dog-baited mosquito traps used in study 8 were widely dispersed in Hudson's Woods (Figure 2). The traps were described by Lewandowski (1977). The dogs remained in the traps 24 hours per day. The louvered side panels containing mosquitoes were emptied twice daily, at approximately 10 a. m. and 7 p. m., by placing the panels in a large plastic bag and killing the mosquitoes with chloroform.

Adult Ae. hendersoni and Ae. triseriatus collected in the animal-baited traps were identified on the basis of their mesoscutal scale patterns (Grimstad et al., 1974), and their tarsal claws (Harmston, 1969). The possibility of encountering hybrids was judged to be negligible, since Zaim (1978) in the same locality in Michigan found 400 Ae. hendersoni and 1,100 Ae. triseriatus, and no hybrids among 4th-stage larvae derived from eggs caught in ovitraps.

RESULTS

Susceptibility to D. immitis

The microfilaremia of the dog used in study 1 was 25,000 per cm^3. Developing D. immitis larvae were recovered from the malpighian tubules of every mosquito dissected during the extrinsic incubation period (Appendix A). The 1st 3rd-stage D. immitis reached the head and mouthparts of Ae. hendersoni on the 16th day after the blood meal (Table 1). No encapsulation or other resistance to the development of D. immitis was observed. The development of D. immitis in Ae. hendersoni (Table 1) was similar to that reported by Intermill (1973) in Ae. triseriatus. The decreasing mean and standard deviation (Table 1) of the number of larvae found in the dissections on successive days suggests that the mosquitoes with a heavier parasite load tended to die earlier, leaving as survivors those mosquitoes with fewer parasites. (Although several thousand mosquitoes fed on the infected dog, none were alive 18 days later.)

Study 2 addressed the relative susceptibility of Ae. hendersoni and Ae. triseriatus to D. immitis by comparing the number of third-stage larvae in the heads and mouthparts of these mosquitoes after the extrinsic incubation period (16 days). Dissection results are shown in Appendix B, and the computed mean number of larvae for each treatment within each replicate are in Table 2. The ten replicates in this experiment

Table 1. Development of *D. immitis* in *Ae. hendersoni*.

Days after blood meal	Mean no. worms in malpighian tubules	Standard deviation	Mean no. worms in head & mouthparts	Observations
0	-	-	-	At 12 h after feeding, most microfilariae are in the malpighian tubes.
1	62.2 (24)*	46.3	0	
2	51.8 (24)	19.1	0	
3	54.1 (19)	35.1	0	
4	55.6 (20)	32.4	0	
5	46.0 (21)	20.3	0	
6	49.3 (26)	27.1	0	Remaining microfilariae in the midgut are not motile.
7	41.6 (20)	26.6	0	Midgut is empty.
8	33.8 (21)	17.9	0	
9	40.8 (20)	15.2	0	Stage II larvae first seen.

*no. dissections

Table 1 (cont'd).

Days after blood meal	Mean no. worms in malpighian tubules	Standard deviation	Mean no. worms in head & mouthparts	Observations
10	37.1 (21)	20.6	0	
11	33.1 (20)	12.9	0	Nearly all larvae are stage II.
12	34.0 (20)	15.7	0	
13	25.4 (11)	12.4	0	
14	-	-	-	Stage III larvae are first seen.
15	-	-	-	All stage III larvae are within the malpighian tubules.
16	-	-	9.5 (12)	Stage III larvae are first seen in the head.
17	-	-	14.4 (11)	Most larvae are stage III.
18	-	-	-	No mosquitoes are alive.

Table 2. Third-stage *D. immitis* in mosquito heads.

Species	Replicate									
	1	2	3	4	5	6	7	8	9	10
Ae. hendersoni	6.7*	4.7	3.7	8.4	3.7	8.1	10.2	8.5	13.0	5.4
Ae. triseriatus	4.4	6.4	3.0	4.7	2.7	4.9	4.3	6.1	4.8	1.8

*each entry is the mean number of third-stage *D. immitis* larvae found in dissections of 12 randomly chosen mosquitoes on post-infection day 16.

were performed over a 15 week period, during which the microfilaremia of the dog varied from 13,000 to 20,000 per cm^3. Ae. hendersoni supported the development of a significantly greater number of D. immitis to the third-stage than Ae. triseriatus ($p < .01$).

Longevity

Study 3 was preliminary, designed to determine if ovipositing by Ae. hendersoni infected with D. immitis affected longevity. The results of this study had practical significance, since if ovipositing did affect longevity, oviposition beakers would have to be supplied to cages in subsequent studies. (Because presumably mosquitoes have the opportunity to oviposit in the field.) The number of surviving mosquitoes in each cage was recorded 16 days after feeding on the infected dog (Table 3). There was no significant difference between the longevity of those mosquitoes which oviposited and those which did not ($p > .2$). Therefore, oviposition beakers were not used in the other studies.

In study 4, cummulative longevity curves were constructed for Ae. hendersoni which had fed on an uninfected dog, and on a dog which had a D. immitis microfilaremia of 15,000 per cm^3. For 17 days after the blood meal, the number of survivors in each treatment group was recorded daily (Appendix C). The mortality of the uninfected mosquitoes was small and gradual (Figure 3), with 89% of the mosquitoes surviving 17 days after feeding. The mortality of the infected mosquitoes was much larger (Figure 3), with only 18% of the original mosquitoes surviving 17 days after the blood meal.

Study 5 compared the longevity of Ae. hendersoni and Ae.

Table 3. Effect of oviposition on survival of *D. immitis*-infected *Ae. hendersoni*.

Treatment	Surviving *Ae. hendersoni* (%) 16 days post-infection								
	Replicate								
	1	2	3	4	5	6	7	8	9
Oviposition beakers supplied	38	30	7	14	44	25	31	8	12
Oviposition beakers not supplied	32	38	28	14	26	30	33	6	8

Figure 3. Mortality of *D. immitis*-infected and uninfected *Ae. hendersoni*. (Vertical bars indicate 95% confidence intervals.)

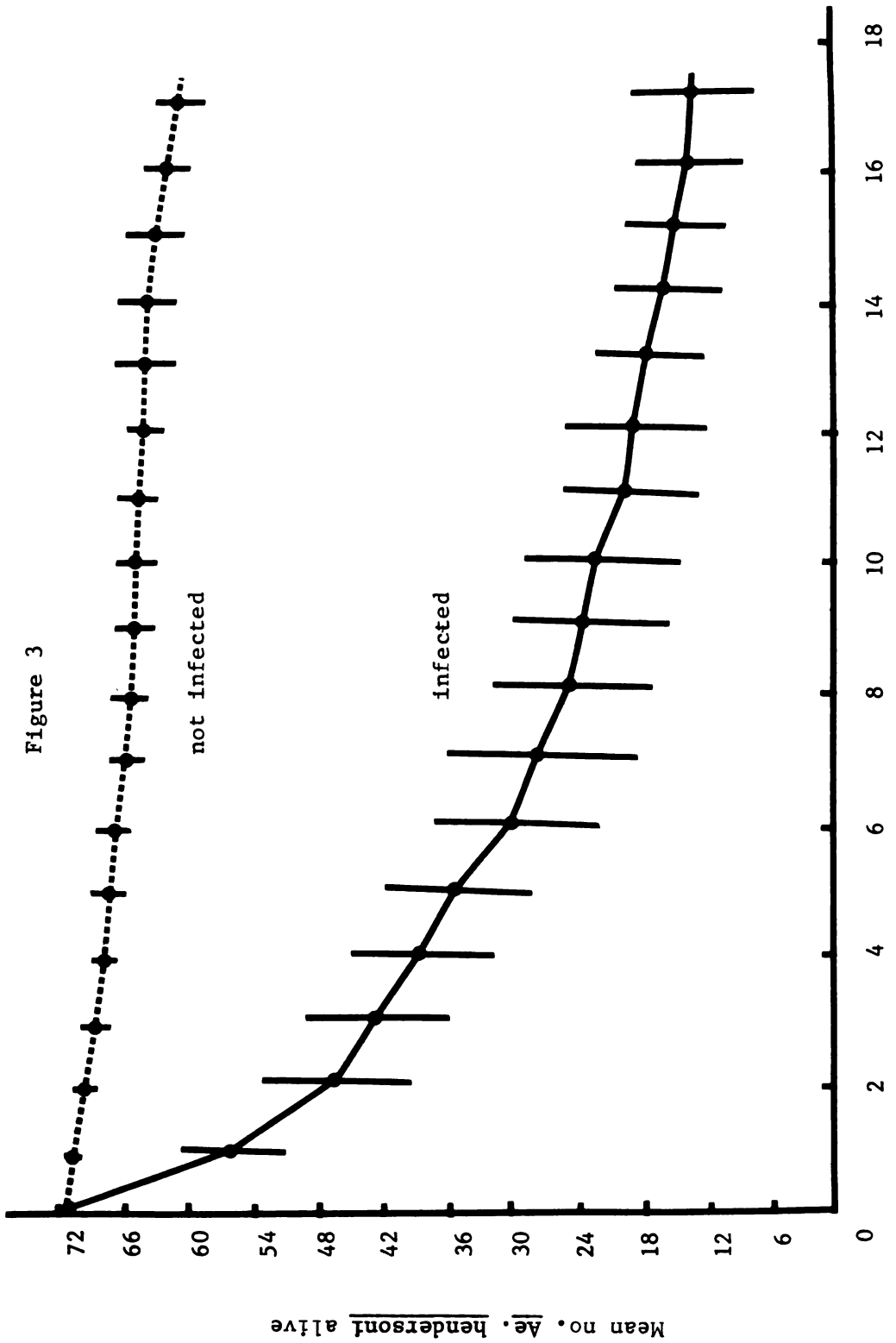


Figure 3
not infected
infected
Mean no. Ae. hendersoni alive
72
66
60
54
48
42
36
30
24
18
12
6
0
2
4
6
8
10
12
14
16
18
Days after blood meal

triseriatus which were infected with D. immitis. Sixteen days after feeding, the number of survivors in each replicate was recorded (Table 4). Ae. triseriatus had significantly greater longevity than Ae. hendersoni when both were infected with D. immitis ($p < .05$).

Relative Abundance

Study 6 was relevant to the relative abundance of Ae. hendersoni and Ae. triseriatus, and incidentally, to the vertical ovipositioning preferences of these mosquitoes. From June 13 to August 22 (1979) all eggs collected in the ovitraps were identified to species (Appendix D), and weekly results summarized (Table 5). Apparently, Ae. hendersoni slightly outnumbered Ae. triseriatus in Hudson's Woods during the trapping period because 6,653 total Ae. hendersoni eggs and 5,136 Ae. triseriatus eggs were captured. Ae. hendersoni, as expected, displayed a strong preference for the upper traps (Table 5). Ae. triseriatus, however, utilized traps at both levels equally (Table 5).

Feeding Habits

Paired vertical rat-baited mosquito traps were used in study 7 to determine whether Ae. hendersoni and Ae. triseriatus fed at different heights. Both Ae. hendersoni and Ae. triseriatus fed at 0 and 10 m, but greater numbers of both species were caught in the traps at 0 m, (Table 6). The number of mosquitoes of all species caught in the rat-baited traps was small (Appendix E); either white rats do not make good bait, or the traps were poorly designed.

Dog-baited mosquito traps were used in study 8 to determine the extent to which Ae. hendersoni and Ae. triseriatus feed on dogs. From

Table 4. Survival rate of D. immitis-infected mosquitoes.

Species	Survivors/300 infected mosquitoes									
	Replicate									
	1	2	3	4	5	6	7	8	9	10
Ae. hendersoni	33*	16	15	17	12	13	37	26	45	16
Ae. triseriatus	50	73	21	16	40	24	55	134	116	31

*survivors were counted on post-infection day 16

Table 5. Paired vertical ovitrapping summary.

End of trapping week	Ae. hendersoni		Ae. triseriatus	
	0 m	6 m	0 m	6 m
June 20	0*	181	0	0
27	60	459	0	33
July 4	64	964	106	166
11	120	566	547	1083
18	153	559	721	525
25	105	543	673	549
Aug. 1	195	528	106	245
8	815	734	141	34
15	0	400	77	62
22	20	187	68	0
Total:	1532	5121	2439	2697

*no. eggs

Table 6. Rat-baited trapping summary.

Species	Trap height 0 m	9 m
Ae. hendersoni	9*	4
Ae. triseriatus	15	1

*no. mosquitoes

June 19 to August 2 (1979), 1,537 mosquitoes were taken in the 3 dog-baited traps. The traps were emptied twice daily on 33 days during this period, and all mosquitoes identified to species, or species complex (Appendix F). Ae. hendersoni and Ae. triseriatus were caught in nearly equal numbers, being respectively the fourth and fifth most abundant species taken in the dog-baited traps (Table 7).

Table 7. Dog-baited trapping summary.*

Species	Number
C. restuans-pipiens	565
Ae. vexans	500
Ae. stimulans	174
Ae. hendersoni	115
Ae. triseriatus	110
Ae. trivitattus	43
An. quadrimaculatus	18
C. tarsalis	6
Ae. sticticus	4
Ae. cinereus	2

*mosquitoes were trapped from June 19 to August 2.

DISCUSSION

Barnett (1960) lists four criteria which should be met to incriminate an arthropod as a vector:

1. demonstration of feeding or other effective contact with the host under natural conditions,
2. demonstration of a convincing biological association in time and/or space of the suspected arthropod species and occurence of clinical or subclinical infection in the host.
3. repeated demonstrations that the arthropod, under natural conditions, harbors the infective stage,
4. and, transmission of the agent under controlled conditions.

In the case of D. immitis, these four criteria are hard to meet, and in fact, no mosquito has ever met all four of them. Strictly speaking, then, every suspected mosquito species should be referred to as a "potential" vector of D. immitis. Of course, "potential" mosquito vectors do transmit D. immitis, because there is no recent report suggesting that other insects or arthropods transmit D. immitis.

Only a few attempts have been made to meet the third criterion of Barnett, and none have been successful. Christensen (1977) isolated third-stage nematodes from Ae. trivitattus in Iowa, but it is possible that these nematodes were not D. immitis. Lewandowski (1977)

encountered similar difficulties in Michigan. He also isolated third-stage nematodes from several mosquito species (but not Ae. triseriatus) and could not identify the nematodes to species. Until a method is devised for positively identifying third-stage D. immitis larvae, further attempts to meet Barnett's third criterion cannot be conclusive. Other difficulties also prevent application of this criterion to Ae. hendersoni and Ae. triseriatus. First, effective trapping methods for Ae. hendersoni and Ae. triseriatus do not exist (Zaim, 1978). Since, even in an important vector, D. immitis infection rates would be very low (Dr. H. D. Newson, personal communication), many thousands of a mosquito species, from several locations, must be examined to make reasonable inferences. Presently, it is not possible to collect such large numbers of Ae. triseriatus or Ae. hendersoni. Secondly, even if it were possible to collect large numbers of these two species, it would not be possible to differentiate between them on a large scale. Differentiation of Ae. hendersoni and Ae. triseriatus is very time consuming, and is particularly difficult for old and flight-worn individuals (which are the only individuals which would harbor infective third-stage larvae). D. immitis isolation attempts require fast handling of large numbers of mosquitoes, and in such attempts, Ae. hendersoni and Ae. triseriatus would have to be pooled, defeating the purpose of the study.

Only three North American mosquitoes have met the fourth criterion of Barnett. Newton (1957) transmitted D. immitis from one dog to another in the laboratory with An. quadrimaculatus, Bickley et al. (1977) transmitted D. immitis with Ae. canadensis, and Christensen (1977) accomplished this with Ae. trivitattus. According to Dr. J. F. Williams (personal communication), D. immitis transmission attempts

using only one or a few dogs are not meaningful, and I did not even attempt to meet the fourth criterion of Barnett with Ae. hendersoni.

The first criterion of Barnett is relatively easy to satisfy, and this has been accomplished for the Ae. triseriatus-Ae. hendersoni complex by Phillips (1939) and Lewandowski (1977). (Neither author distinguished between Ae. hendersoni and Ae. triseriatus.) In study 8 I have explicitly satisfied the first criterion of Barnett for both Ae. hendersoni and Ae. triseriatus.

Even though it is not feasible to meet all of the criteria of Barnett, it is both reasonable and necessary to follow other lines of evidence in order to make inferences about potential vectors of D. immitis. Ludlam et al. (1970) discussed the difficulties involved with meeting the third and fourth criteria of Barnett, and listed complementary factors which are relevant to D. immitis vector determination: 1) breeding habitat, 2) flight range, 3) relative population density, 4) feeding habits, 5) longevity, and 6) genetics of different strains which affect susceptibility to D. immitis infection.

I could not improve on the studies of Lewandowski (1977), Phillips (1939), Intermill (1973), or Keegan et al. (1968) in the sense of satisfying more of the criteria of Barnett. However, unlike the authors of these previous "Ae. triseriatus" vector potential studies, I did consider the sixth D. immitis vector determination factor of Ludlam et al., by recognizing the genetic difference between Ae. hendersoni and Ae. triseriatus. The fact that Ae. hendersoni and Ae. triseriatus are actually different species (instead of strains of "Ae. triseriatus") makes it even more important to consider these mosquitoes separately in

relation to D. immitis. Of course, potential species differences between Ae. hendersoni and Ae. triseriatus need to be considered not only for the sixth D. immitis vector determination factor of Ludlam et al., but for the first five as well.

In relation to the first and second D. immitis vector determination factors of Ludlam et al. (breeding habitat and flight range, respectively), Ae. hendersoni and Ae. triseriatus are similar; both mosquitoes could play a role in the transmission of D. immitis in wooded areas. Tree-holes, the breeding habitat of Ae. hendersoni and Ae. triseriatus, occur in significant numbers only in wooded areas. The different oviposition altitude preferences shown by Ae. hendersoni and Ae. triseriatus (study 6), while ecologically interesting, have no obvious bearing on the D. immitis vector potential of these mosquitoes. The short flight range of Ae. hendersoni and Ae. triseriatus confines these mosquitoes to the woodlot of their origin. During three summers of field work, I never encountered either Ae. hendersoni or Ae. triseriatus outside of a woodlot. Nonetheless, both Ae. hendersoni and Ae. triseriatus could be important D. immitis vectors, because dogs often frequent wooded regions such as campgrounds, parks, suburban subdivisions, and farm woodlots.

Data on the third vector determination factor of Ludlam et al., relative population density, supports the conclusion that both Ae. hendersoni and Ae. triseriatus are important woodland potential vectors of D. immitis. Gorton (1973) sampled mosquito populations in an Owosso, Michigan woodlot by means of human biting collections. He found that "Ae. triseriatus" was the most common mosquito in the woodlot. Gorton made no mention of efforts to differentiate between Ae.

hendersoni and Ae. triseriatus, and it is reasonable to regard the "Ae. triseriatus" in his study as a mixture of Ae. hendersoni and Ae. triseriatus. As measured by dog-baited mosquito trapping during the summer of 1979 (study 8), Ae. hendersoni and Ae. triseriatus were respectively the fourth and fifth most abundant mosquitoes in Hudson's Woods. Morris and DeFoliart (1971) noted that Ae. hendersoni and Ae. triseriatus are reluctant (compared to other mosquitoes) to enter animal-baited traps, and will be underestimated in studies using such traps. In a special problems project (Rogers, 1978) eggs of both Ae. hendersoni and Ae. triseriatus were captured in all seven East Lansing, Michigan woodlots in which paired vertical ovitraps were placed. It is concluded that Ae. hendersoni and Ae. triseriatus are common Michigan woodland mosquitoes. Ecological factors which would favor one species over the other are not known.

Feeding habits, the fourth D. immitis vector determination factor of Ludlam et al., was addressed by using rat- and dog-baited mosquito traps (studies 7 and 8, respectively). The results of the dog-baited mosquito trapping are consistent with the conclusion that both Ae. triseriatus and Ae. hendersoni are potential vectors of D. immitis. Both species were attracted to dogs in the field, with the total catch of Ae. hendersoni slightly exceeding that of Ae. triseriatus (115 and 110, respectively). In view of the fact that the concurrent paired vertical ovitrapping (study 6) indicated that Ae. hendersoni slightly outnumbered Ae. triseriatus in Hudson's Woods, it appears that these mosquitoes are equally attracted to dogs in the field. Consistent with this conclusion are the results of the vertical rat-baited trapping, which found that neither Ae. hendersoni nor Ae. triseriatus are

primarily canopy feeders.

The fifth D. immitis vector determination factor of Ludlam et al., longevity, was investigated for the Ae. triseriatus-Ae. hendersoni complex in Wisconsin by Morris and DeFoliart (1971). They found that mosquitoes of this complex had the highest parous rate of any Wisconsin woodland mosquitoes. This finding supports the conclusion that Ae. hendersoni and Ae. triseriatus are potential vectors of D. immitis, because it indicates that these mosquitoes live a long time and take repeated blood meals. Study 5 demonstrated that relative to Ae. triseriatus, Ae. hendersoni suffers more mortality when infected with D. immitis. The great mortality experienced by D. immitis-infected Ae. hendersoni in study 4 does not necessarily indicate that infected Ae. hendersoni would suffer similar mortality in the field. Hamilton and Bradley (1979) judged that the high mosquito mortality which has occurred in most laboratory infections with D. immitis was an artifact.

The sixth vector determination factor of Ludlam et al., genetics of different strains which affect susceptibility to D. immitis infection, was addressed by studies 1 and 5. The daily dissections of Ae. hendersoni during the extrinsic incubation period of D. immitis demonstrated that Ae. hendersoni is an excellent intermediate host of D. immitis in the laboratory. In all cases, mosquitoes which lived long enough (17 days) supported the development of D. immitis to the infective stage. No encapsulation or any other refractory influence was encountered in the development of D. immitis. Intermill (1973) on the basis of a similar daily dissection study of D. immitis-infected Ae. triseriatus concluded that Ae. triseriatus was an excellent intermediate host of D. immitis. The results of Intermill on Ae.

triseriatus differed from the results of study 1 on Ae. hendersoni mainly in that he encountered some encapsulation of D. immitis. This discrepency suggested that Ae. hendersoni has greater susceptibility to D. immitis than Ae. triseriatus. Accordingly, study 2 tested this hypothesis, by comparing the number of third-stage D. immitis larvae in the heads and mouthparts of Ae. hendersoni and Ae. triseriatus after the extrinsic incubation period. A greater number of D. immitis larvae reached the heads and mouthparts in Ae. hendersoni than in Ae. triseriatus, so Ae. hendersoni should be considered the more susceptible species. (It should be kept in mind, however, that both Ae. hendersoni and Ae. triseriatus supported the development of D. immitis to the infective stage.)

Whether the greater D. immitis-susceptibility of Ae. hendersoni is outweighed by the greater longevity of Ae. triseriatus is impossible to judge. The resolution of vector potential studies based on the six D. immitis vector determination factors of Ludlam et al. is not keen enough to allow precise rating of vector potential. Vector potential is an elusive quality because it is based on a variety of uncontrollable factors which have complex interactions. After reviewing Ae. hendersoni and Ae. triseriatus in relation to the six vector determination factors of Ludlam et al., it is concluded that both Ae. hendersoni and Ae. triseriatus should be considered important potential vectors of D. immitis in wooded areas of Michigan.

SUMMARY AND CONCLUSIONS

1. In the laboratory, D. immitis readily develops to the infective third-stage in Ae. hendersoni (study 1).
2. Ovipositing by Ae. hendersoni infected with D. immitis has no influence on mosquito mortality (study 3).
3. Heavy infection with D. immitis greatly reduces the longevity of Ae. hendersoni in the laboratory (study 4).
4. In the laboratory, Ae. hendersoni supports the development of more D. immitis in the heads and mouthparts than Ae. triseriatus (study 2).
5. Greater numbers of both Ae. hendersoni and Ae. triseriatus were caught in rat-baited traps at 0 m than 10 m (study 7), suggesting that neither species is primarily a canopy feeder.
6. Ae. hendersoni has a strong preference for elevated ovitraps but Ae. triseriatus uses both basal and elevated ovitraps equally (study 6).
7. As indicated by paired vertical ovitrapping (study 6), Ae. hendersoni slightly outnumbered Ae. triseriatus in Hudson's Woods.
8. Slightly greater numbers of Ae. hendersoni were caught in the dog-baited trapping (study 8) than Ae. triseriatus. Ae. hendersoni and Ae. triseriatus appear to be equally attracted to dogs in the field.

9. On the basis of the studies reported in this dissertation, and studies in the literature, Ae. hendersoni and Ae. triseriatus were reviewed in relation to the six D. immitis vector determination factors of Ludlam et al. (1970) (breeding habitat, flight range, longevity, relative population density, feeding habits, and genetics of different strains which affect susceptibility to D. immitis). It is concluded that both Ae. hendersoni and Ae. triseriatus are important potential vectors of D. immitis in wooded areas of Michigan.

APPENDICES

APPENDIX A

Table 8. *D. immitis*-infected *Ae. hendersoni* dissections.

Days after blood meal	Mosquito number	No. larvae in malpighian tubules	No. larvae in head and mouthparts
1	1	65	0
	2	76	0
	3	32	0
	4	133	0
	5	142	0
	6	13	0
	7	22	0
	8	107	0
	9	132	0
	10	164	0
	11	41	0
	12	27	0
	13	30	0
	14	9	0
	15	11	0
	16	101	0
	17	68	0
	18	69	0
	19	76	0
	20	21	0
	21	11	0
	22	50	0
	23	29	0
	24	63	0
2	1	27	0
	2	59	0
	3	21	0
	4	66	0
	5	57	0
	6	32	0
	7	50	0
	8	35	0
	9	100	0

Table 8 (cont'd).

Days after blood meal	Mosquito number	No. larvae in malpighian tubules	No. larvae in head and mouthparts
2	10	49	0
	11	48	0
	12	40	0
	13	32	0
	14	39	0
	15	78	0
	16	58	0
	17	36	0
	18	36	0
	19	61	0
	20	60	0
	21	79	0
	22	65	0
	23	71	0
	24	39	0
3	1	19	0
	2	86	0
	3	36	0
	4	132	0
	5	43	0
	6	33	0
	7	36	0
	8	60	0
	9	19	0
	10	19	0
	11	73	0
	12	101	0
	13	71	0
	14	120	0
	15	89	0
	16	22	0
	17	40	0
	18	72	0
	19	17	0
4	1	34	0
	2	100	0
	3	31	0
	4	35	0
	5	4	0
	6	90	0
	7	58	0
	8	65	0
	9	50	0

Table 8 (cont'd).

Days after blood meal	Mosquito number	No. larvae in malpighian tubules	No. larvae in head and mouthparts
4	10	31	0
	11	34	0
	12	115	0
	13	24	0
	14	52	0
	15	112	0
	16	49	0
	17	106	0
	18	46	0
	19	24	0
	20	51	0
5	1	85	0
	2	39	0
	3	22	0
	4	41	0
	5	39	0
	6	47	0
	7	55	0
	8	57	0
	9	53	0
	10	49	0
	11	100	0
	12	27	0
	13	9	0
	14	53	0
	15	29	0
	16	59	0
	17	29	0
	18	36	0
	19	59	0
	20	40	0
	21	39	0
6	1	22	0
	2	78	0
	3	64	0
	4	87	0
	5	83	0
	6	43	0
	7	12	0
	8	22	0
	9	69	0
	10	64	0

Table 8 (cont'd).

Days after blood meal	Mosquito number	No. larvae in malpighian tubules	No. larvae in head and mouthparts
6	11	7	0
	12	75	0
	13	13	0
	14	20	0
	15	75	0
	16	54	0
	17	59	0
	18	53	0
	19	13	0
	20	26	0
	21	74	0
	22	10	0
	23	83	0
	24	73	0
	25	54	0
	26	55	0
7	1	60	0
	2	11	0
	3	68	0
	4	68	0
	5	31	0
	6	61	0
	7	4	0
	8	53	0
	9	17	0
	10	95	0
	11	47	0
	12	19	0
	13	22	0
	14	64	0
	15	36	0
	16	81	0
	17	7	0
	18	20	0
	19	18	0
	20	47	0
8	1	12	0
	2	35	0
	3	36	0
	4	13	0
	5	35	0
	6	34	0
	7	48	0

Table 8 (cont'd).

Days after blood meal	Mosquito number	No. larvae in malpighian tubules	No. larvae in head and mouthparts
8	8	21	0
	9	19	0
	10	29	0
	11	30	0
	12	41	0
	13	82	0
	14	21	0
	15	38	0
	16	47	0
	17	8	0
	18	65	0
	19	36	0
	20	45	0
	21	15	0
9	1	22	0
	2	55	0
	3	23	0
	4	35	0
	5	23	0
	6	45	0
	7	46	0
	8	31	0
	9	18	0
	10	53	0
	11	65	0
	12	26	0
	13	40	0
	14	30	0
	15	43	0
	16	49	0
	17	68	0
	18	53	0
	19	30	0
	20	61	0
10	1	41	0
	2	56	0
	3	41	0
	4	62	0
	5	32	0
	6	25	0
	7	15	0

Table 8 (cont'd).

Days after blood meal	Mosquito number	No. larvae in malpighian tubules	No. larvae in head and mouthparts
10	8	15	0
	9	13	0
	10	81	0
	11	28	0
	12	23	0
	13	84	0
	14	16	0
	15	34	0
	16	32	0
	17	36	0
	18	19	0
	19	48	0
	20	19	0
	21	60	0
11	1	52	0
	2	7	0
	3	66	0
	4	35	0
	5	29	0
	6	46	0
	7	29	0
	8	26	0
	9	20	0
	10	24	0
	11	42	0
	12	25	0
	13	21	0
	14	30	0
	15	26	0
	16	40	0
	17	39	0
	18	41	0
	19	28	0
	20	36	0
12	1	33	0
	2	28	0
	3	59	0
	4	7	0
	5	62	0
	6	34	0
	7	18	0
	8	48	0

Table 8 (cont'd).

Days after blood meal	Mosquito number	No. larvae in malpighian tubules	No. larvae in head and mouthparts
12	9	39	0
	10	7	0
	11	36	0
	12	14	0
	13	43	0
	14	53	0
	15	24	0
	16	33	0
	17	34	0
	18	52	0
	19	33	0
	20	28	0
13	1	42	0
	2	43	0
	3	41	0
	4	15	0
	5	13	0
	6	14	0
	7	19	0
	8	24	0
	9	28	0
	10	10	0
	11	30	0
16	1	undetermined	17
	2	"	23
	3	"	23
	4	"	10
	5	"	0
	6	"	14
	7	"	11
	8	"	0
	9	"	3
	10	"	3
	11	"	0
	12	"	10
17	1	undetermined	14
	2	"	19
	3	"	14
	4	"	6
	5	"	28
	6	"	30
	7	"	6

Table 8 (cont'd).

Days after blood meal	Mosquito number	No. larvae in malpighian tubules	No. larvae in head and mouthparts
17	8	undetermined	8
	9	"	12
	10	"	17
	11	"	5

APPENDIX B

Table 9. Dissections of mosquito heads and mouthparts.

Replicate 1		
Mosquito No.	Ae. hendersoni	Ae. triseriatus
	*	
1	6	1
2	1	2
3	6	0
4	16	5
5	0	0
6	4	6
7	11	12
8	3	3
9	1	1
10	3	1
11	3	13
12	27	9

Replicate 2		
Mosquito No.	Ae. hendersoni	Ae. triseriatus
1	8	18
2	10	7
3	6	1
4	11	7
5	0	0
6	5	13
7	2	0
8	0	7
9	3	11
10	3	0
11	0	13
12	8	0

* No. D. immitis larvae

Table 9 (cont'd).

Replicate 3		
Mosquito No.	Ae. hendersoni	Ae. triseriatus
1	3	4
2	7	2
3	0	0
4	0	5
5	8	1
6	2	1
7	0	9
8	7	1
9	8	3
10	3	2
11	1	8
12	5	0

Replicate 4		
Mosquito No.	Ae. hendersoni	Ae. triseriatus
1	12	2
2	14	0
3	10	12
4	7	19
5	3	4
6	6	1
7	5	1
8	14	2
9	7	0
10	16	2
11	5	13
12	2	0

Replicate 5		
Mosquito No.	Ae. hendersoni	Ae. triseriatus
1	1	6
2	1	3
3	6	7
4	3	2
5	1	2
6	1	0
7	0	2
8	0	1
9	3	1
10	3	1
11	10	6
12	15	1

Table 9 (cont'd).

Replicate 6		
Mosquito No.	Ae. hendersoni	Ae. triseriatus
1	1	8
2	2	4
3	2	7
4	11	10
5	4	0
6	7	0
7	17	9
8	34	4
9	8	0
10	4	6
11	5	5
12	3	6

Replicate 7		
Mosquito No.	Ae. hendersoni	Ae. triseriatus
1	8	0
2	11	7
3	14	1
4	5	5
5	5	7
6	3	5
7	23	6
8	7	7
9	11	7
10	12	1
11	14	1
12	9	4

Replicate 8		
Mosquito No.	Ae. hendersoni	Ae. triseriatus
1	14	3
2	13	20
3	10	3
4	7	2
5	4	6
6	13	4
7	3	7
8	12	7
9	8	8
10	8	0
11	5	7
12	5	6

Table 9 (cont'd).

Replicate 9		
Mosquito No.	Ae. hendersoni	Ae. triseriatus
1	18	0
2	7	0
3	5	1
4	1	15
5	12	8
6	17	1
7	16	3
8	9	4
9	17	2
10	23	10
11	24	2
12	7	11

Replicate 10		
Mosquito No.	Ae. hendersoni	Ae. triseriatus
1	8	0
2	1	3
3	0	0
4	10	5
5	7	2
6	17	3
7	4	0
8	1	0
9	5	0
10	7	1
11	3	1
12	2	6

APPENDIX C

Table 10. Survival of *D. immitis*-infected and uninfected *Ae. hendersoni*.

	No. infected survivors						No. uninfected survivors						
Day after blood meal	cage number						Day after blood meal	cage number					
	1	2	3	4	5	6		1	2	3	4	5	6
1	60	59	62	51	47	53	1	69	70	68	69	70	69
2	53	53	55	40	36	37	2	69	70	67	67	69	67
3	50	46	51	37	32	34	3	69	68	65	66	67	67
4	46	41	49	28	29	32	4	68	68	64	65	66	66
5	43	38	47	26	24	30	5	68	68	64	65	65	66
6	37	33	43	21	18	21	6	68	66	64	65	64	66
7	36	31	39	21	15	18	7	68	64	63	65	64	63
8	35	29	35	20	12	15	8	68	64	63	65	64	63
9	34	29	32	18	12	13	9	68	64	62	65	64	61
10	32	29	30	14	12	13	10	68	64	62	65	64	60
11	28	24	28	13	12	11	11	67	64	62	65	63	60
12	27	24	28	12	12	10	12	67	64	62	65	63	60
13	22	22	26	12	12	10	13	67	64	62	64	63	58
14	20	18	25	10	11	9	14	66	63	60	63	63	56
15	15	16	23	9	11	9	15	66	60	60	63	63	56
16	15	15	22	5	10	9	16	64	59	59	62	63	56
17	14	15	21	4	10	8	17	64	59	59	60	60	55

*each cage originally had 70 mosquitoes

APPENDIX D

Table 11. Ovitrapping results.

Trap no. & height (meters)		June 20		June 27		July 4		July 11		July 18	
		h*	t	h	t	h	t	h	t	h	t
1	0	0	0	5	0	0	69	0	108	0	17
	6	0	0	18	0	66	30	62	92	69	0
2	0	0	0	0	0	39	15	0	74	0	89
	6	0	0	0	0	15	0	74	0	0	33
3	0	0	0	0	0	0	0	0	0	0	105
	6	0	0	24	0	80	0	0	0	51	80
4	0	0	0	0	0	0	0	0	0	0	6
	6	0	0	26	0	12	13	24	64	13	39
5	0	0	0	0	0	0	0	0	0	11	99
	6	0	0	0	0	35	0	47	59	53	0
6	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	15	0	46	0	0	0	0	94
7	0	0	0	0	0	0	0	80	18	0	154
	6	19	0	26	0	20	9	54	0	11	70
8	0	0	0	0	0	0	0	0	19	0	45
	6	0	0	32	0	0	9	65	17	0	0
9	0	0	0	0	0	0	0	0	5	0	0
	6	46	0	44	0	7	0	0	96	25	0

*h=Ae. hendersoni, t=Ae. triseriatus

Table 11. (cont'd).

Trap no. & height (meters)		June 20		June 27		July 4		July 11		July 18	
		h	t	h	t	h	t	h	t	h	t
10	0	0	0	0	0	0	0	0	178	0	107
	6	0	0	0	0	43	0	0	175	0	54
11	0	0	0	0	0	0	0	0	10	0	0
	6	0	0	0	0	23	0	63	0	48	0
12	0	0	0	0	0	0	0	40	0	76	0
	6	94	0	0	0	0	0	81	0	0	0
13	0	0	0	0	0	0	0	0	0	17	17
	6	22	0	0	0	88	0	0	135	5	0
14	0	0	0	0	0	0	22	0	46	0	82
	6	0	0	0	0	0	0	0	135	98	37
15	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	32	0	173	105	0	33	30	0
17	0	0	0	55	0	0	0	0	0	49	0
	6	0	0	0	0	80	0	7	30	24	87
18	0	0	0	0	0	0	0	0	28	0	0
	6	0	0	105	33	82	0	27	49	0	0
19	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	35	0	80	0	0	40	72	31
20	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	24	0	83	0	0	0	85	0
21	0	0	0	0	0	25	0	0	21	0	0
	6	0	0	65	0	31	0	0	50	0	0

Table 11 (cont'd).

Trap no. & height (meters)		End of trapping week									
		July 25		Aug. 1		Aug. 8		Aug. 15		Aug. 22	
		h	t	h	t	h	t	h	t	h	t
1	0	0	48	0	2	0	10	0	0	0	0
	6	11	55	116	0	82	0	0	0	0	0
2	0	0	98	0	3	22	0	0	0	0	0
	6	55	15	0	0	71	19	0	0	0	0
3	0	0	67	0	0	0	2	0	0	0	0
	6	30	55	0	51	76	0	132	0	159	0
4	0	0	85	0	46	0	50	0	0	0	0
	6	34	210	76	0	83	0	126	41	0	0
5	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	29	0	48	0	0	0	0	0
6	0	0	0	0	0	0	4	0	0	0	49
	6	0	0	0	139	19	0	15	0	0	0
7	0	0	0	99	0	29	14	0	0	20	9
	6	0	0	0	0	0	0	0	0	0	0
8	0	0	111	0	6	0	0	0	0	0	0
	6	27	0	100	0	40	0	52	0	0	0
9	0	0	0	43	0	0	0	0	37	0	0
	6	0	0	33	0	0	0	0	43	0	0
10	0	0	0	0	0	0	0	0	0	28	0
	6	0	0	0	0	0	0	0	0	0	0

Table 11 (cont'd).

Trap no. & height (meters)		End of trapping week									
		July 25		Aug. 1		Aug. 8		Aug. 15		Aug. 22	
		h	t	h	t	h	t	h	t	h	t
11	0	0	0	0	0	0	0	0	0	0	0
	6	0	10	0	0	0	0	0	0	0	0
12	0	0	0	0	23	30	61	0	0	0	0
	6	0	0	0	0	89	0	0	0	0	0
13	0	0	33	0	6	0	0	0	0	0	0
	6	0	52	0	25	0	0	0	0	0	0
14	0	0	58	3	5	0	0	0	30	0	10
	6	15	0	30	0	59	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	126	30	0	0	0	0	0	0
16	0	105	69	0	0	0	0	0	0	0	0
	6	93	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0
18	0	0	22	50	15	0	0	0	0	0	0
	6	0	94	0	0	9	0	0	0	0	0
19	0	0	34	0	0	0	0	0	0	0	0
	6	99	30	0	0	0	15	0	0	0	0
20	0	0	44	0	0	0	0	0	0	0	0
	6	179	28	18	0	35	0	0	21	0	0
21	0	0	4	0	0	0	0	0	10	0	0
	6	0	0	0	0	123	0	32	0	0	0

APPENDIX E

Table 12. Rat-baited mosquito trapping

Species	Date:	July 13		July 14		July 15		July 16		July 17		July 18		July 19		July 25	
	Height (meters):	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9
Ae. hendersoni		0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ae. triseriatus		0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0
Ae. vexans		1	0	5	0	2	1	4	0	0	0	0	0	0	0	0	0
Ae. trivitattus		1	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0
C. restuans-pipiens		0	0	0	0	1	3	0	0	0	4	0	0	0	0	1	0

Table 12 (cont'd).

Species	Date:	July 26		July 27		July 28		July 29		July 30		July 31		Aug. 1		Aug. 2	
	Height (meters):	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9
Ae. hendersoni		0	1	0	0	0	0	1	2	0	0	4	0	3	1	0	0
Ae. triseriatus		0	0	0	0	0	0	0	0	0	0	9	1	2	0	0	0
Ae. vexans		1	0	0	0	3	0	1	0	0	0	0	0	0	0	0	0
Ae. trivitattus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. restuans-pipiens		0	3	0	1	0	0	0	0	0	0	1	0	0	0	0	0

APPENDIX F

Table 13. Dog-baited mosquito trapping.

Species	Date in June*																
	19d	20n	20d	21n	21d	22n	22d	23n	25d	26n	26d	27n	27d	28n	28d	29n	29d
Ae. triseriatus	0	0	3	0	4	0	2	0	1	0	0	0	1	1	4	1	1
Ae. hendersoni	2	0	1	1	2	0	1	0	0	0	1	0	4	0	1	0	0
Ae. trivitattus	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0
Ae. stimulans	12	12	6	12	13	6	5	6	6	2	4	5	4	3	3	3	8
Ae. cinereus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Ae. vexans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ae. sticticus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. restuans-pipiens	1	1	0	1	0	1	0	0	0	2	0	3	0	4	4	4	1
C. tarsalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
An. quadrimaculatus	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

*d=7:00 p.m. collection, n=10:00 a.m. collection

Table 13 (cont'd).

Species	Date in July															
	2d	3n	3d	4n	4d	5n	5d	6n	6d	9d	10n	10d	11n	11d	12n	12d
Ae. triseriatus	0	0	2	0	3	0	2	0	3	4	2	9	1	0	0	0
Ae. hendersoni	1	0	2	0	2	0	1	0	0	0	0	0	0	1	0	2
Ae. trivitattus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ae. stimulans	8	3	3	0	6	1	5	3	7	4	4	4	1	6	2	0
Ae. cinereus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ae. vexans	0	0	0	0	0	0	0	0	0	0	0	0	0	15	42	12
Ae. sticticus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. restuans-pipiens	0	8	1	9	10	10	2	11	0	1	12	5	14	5	0	3
C. tarsalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
An. quadrimaculatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 13 (cont'd).

Species	Date in July															
	13n	13d	14n	14d	15n	15d	16n	16d	17n	17d	18n	18d	19n	19d	20n	20d
Ae. triseriatus	0	0	0	2	0	1	0	1	0	0	0	1	0	0	1	0
Ae. hendersoni	2	0	0	2	0	2	0	0	1	0	1	2	0	0	0	1
Ae. trivitattus	0	4	0	3	4	1	3	3	3	2	1	0	1	1	1	1
Ae. stimulans	6	0	0	1	1	0	0	3	0	2	1	0	0	1	0	0
Ae. cinereus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ae. vexans	42	16	120	20	75	17	42	8	0	4	0	1	1	0	0	0
Ae. sticticus	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0
C. restuans-pipiens	10	4	2	2	21	0	27	1	44	4	11	2	25	12	31	6
C. tarsalis	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	0
An. quadrimaculatus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0

Table 13 (cont'd).

Species	Date in July														August		
	25n	25d	26n	26d	27n	29d	28n	28d	29n	29d	30n	30d	31n	31d	1n	1d	2n
Ae. triseriatus	3	12	3	2	2	9	3	1	1	0	1	0	10	6	2	4	1
Ae. hendersoni	3	15	1	4	0	3	0	9	2	7	2	15	6	9	4	3	0
Ae. trivitattus	1	0	0	1	0	1	0	1	0	0	0	11	0	1	1	0	0
Ae. stimulans	1	0	1	0	0	1	1	0	2	0	0	0	0	0	0	0	2
Ae. cinereus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ae. vexans	13	3	0	0	1	1	3	11	3	8	3	1	12	10	17	12	0
Ae. sticticus	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
C. restuans-pipiens	0	11	52	13	19	8	24	12	4	5	20	1	6	3	43	2	10
C. tarsalis	0	0	0	0	0	0	0	0	2	0	1	1	0	1	1	0	1
An. quadrimaculatus	2	1	0	0	2	0	1	0	2	2	0	0	0	0	4	1	0

REFERENCES

Ansari, J. A. 1970. A review of the mechanisms of microfilarial periodicity. Zool. Anz. 185:387-392.

Barnett, H. C. 1960. The incrimination of arthropods as vectors of disease. Proc. 11th Inter. Congr. Entomol. 2:341-345.

Bemrick, W. J., and D. M. Bemrick. 1969. Sugar solution feeding and its effect on the retention of infective Dirofilaria immitis larvae in Anopheles quadrimaculatus. J. Med. Entomol. 6:278-279.

Benach, J. L., L. G. Cuiston, and J. Azzone. 1971. Preliminary studies on the blood meal source and olfactory responses of Aedes triseriatus with notes on the biology of the species. Proc. N. J. Mosq. Exter. Soc. 58:126-138.

Bickley, W. E., R. S. Lawrence, G. M. Ward, and R. B. Shillinger. 1977. Dog-to-dog transmission of heartworm by Aedes canadensis. Mosq. News 37:137-138.

Blecka, L. J. 1978. Human subcutaneous dirofilariasis in Illinois. J. Amer. Med. Assn. 240:245-246.

Breland, O. P. 1960. Restoration of the name, Aedes hendersoni Cockerell, and its elevation to full specific rank (Diptera: Culicidae). Ann. Entomol. Soc. Amer. 53:600-606.

Christensen, B. M. 1977. Laboratory studies on the development and transmission of Dirofilaria immitis by Aedes trivittatus. Mosq. News 37:367-371.

Church, E. M., J. R. Georgi, and D. S. Robson. 1976. Analysis of the microfilarial periodicity of Dirofilaria immitis. Cornell Vet. 66:333-345.

Cockerell, T. D. A. 1918. The mosquitoes of Colorado. J. Econ. Entomol. 11:195-200.

Dyar, G. G. 1919. Westward expansion of the Canadian mosquito fauna (Diptera: Culicidae). Ins. Ins. Mens. 7:11-39.

Eyles, D. E., C. L. Gibson, F. E. Jones, and M. E. G. Cunningham. 1954. Prevalence of Dirofilaria immitis in Memphis, Tennessee. J. Parasitol. 40:216-227.

Fowler, J. L., J. L. Young, R. T. Sterner, and R. C. Fernau. 1973. Dirofilaria immitis: lack of correlation between numbers of microfilariae in peripheral blood and mature heartworms. Amer. Anim. Hosp. Assn. J. 9:391-394.

Furlow, B. M., and W. W. Young. 1970. Larval surveys compared to ovitrap surveys for detecting Aedes aegypti and Aedes triseriatus. Mosq. News 30:468-470.

Garlick, N. L. 1975. The management of canine dirofilariasis. Canine Practice 2:22-27

Goble, F. C. 1942. Dog heartworm in the muskrat in New York. J. Mammal. 23:346.

Gorton, R. J. 1973. An epidemiological study of mosquito-borne California encephalitis in a southern Michigan subdivision. M. S. thesis, Michigan State University.

Grimstad, P. R., C. E. Garry, and G. R. DeFoliart. 1974. Aedes hendersoni and Aedes triseriatus (Diptera: Culicidae) in Wisconsin: characterization of larvae, larval hybrids, and comparisons of adult and hybrid mesoscutal patterns. Ann. Entomol. Soc. Amer. 67:795-804.

Groves, H. F., and F. R. Kutz. 1964. Survey of microfilariae in Ohio dogs. J. Amer. Vet. Med. Assn. 144:600-602.

Hamilton, D. R., and R. E. Bradley, Sr. 1979. Observations on early death by Dirofilaria immitis-infected mosquitoes. J. Med. Entomol. 15:305-306.

Hanson, R. P., and M. G. Hanson. 1970. The effect of land use practices on the vector of California encephalitis (La Crosse) in north central United States. Mosq. News 30:215-221.

Harmston, F. C. 1969. Separation of the females of Aedes hendersoni Cockerell and Aedes triseriatus (Say) Diptera: Culicidae by the tarsal claw. Mosq. News 29:490-491.

Hawking, F. 1967. The 24 hour periodicity of microfilariae: biological mechanisms responsible for its production and control. Roy. Soc. (London) Proc., Ser. B. Biol. Sci. 169:59-76.

Ho. B. C., M. Singh, and E. H. Yap. 1974. The fate and migratory patterns of the infective larvae of Brugia malayi, Dirofilaria immitis, and Breinli sergenti in Aedes togoi denied access to a host. J. Med. Entomol. 11:622-628.

Hu, S. M. K. 1931. Studies on host-parasite relationships of Dirofilaria immitis Leidy and its culicine intermediate hosts. Amer. J. Hyg. 14:614-629.

Intermill, R. W. 1973. Development of Dirofilaria immitis in Aedes triseriatus. Mosq. News 33:176-181.

James, M. T., and R. F. Harwood. 1969. Herm's Medical Entomology. Macmillan Co., N. Y. 484 pp.

Johnson, C. A. 1975. Ursus americanus (black bear) a new host for Dirofilaria immitis. J. Parasitol. 61:940.

Jones, J. C. 1967. Methods for dissection of mosquitoes. Mosq. News 27:76-82.

Kartman, L. 1953a. Factors influencing infection of the mosquito with Dirofilaria immitis (Leidy). Expt. Parasitol. 2:27-78.

Kartman, L. 1953b. An observation on the loss of microfilariae from the mosquito host during its infective blood meal. J. Parasitol. 39:571-572.

Kazacos, K. R. 1979. The prevalence of heartworms (Dirofilaria immitis) in dogs from Indiana. J. Parasitol. 64:959-960.

Keegan, H. L., C. M. Fitzgerald, T. L. McCrary, and M. D. Doyle. 1968. Studies on dog heartworm in South Texas. Tex. Rep. Biol. Med. 26:321-330.

Kershaw, W. E., M. M. F. Lavoipierre, and W. N. Beesley. 1955. Studies on the intake of microfilariae by their insect vectors. VII. Further observations of the intake of microfilariae of Dirofilaria immitis by Aedes aegypti in laboratory conditions: the pattern of the uptake of a group of flies. Ann. Trop. Med. and Parasitol. 49:203-211.

Kocan, A. A., and H. E. Laubach. 1976. Dirofilaria immitis and Dipetalonema reconditum infections in Oklahoma dogs. J. Amer. Vet. Med. Assn. 168:419-420.

Kume, S. 1974. Experimental observations of seasonal periodicity of microfilariae. In: Proceedings of the heartworm symposium '74, pp. 26-31. H. C. Morgan, G. Otto, R. F. Jackson, and W. F. Jackson, eds. V. M. Publishing, Inc., Bonner Springs, Kansas.

Kume, S., and S. Itagaki. 1955. On the life cycle of Dirofilaria immitis in the dog as the final host. Brit. Vet. J. 111:16-24.

Kutz, F. R., and R. C. Dobson. 1974. Effects of temperature on the development of Dirofilaria immitis (Leidy) in Anopheles quadrimaculatus Say and on vector mortality resulting from this development. Ann. Entomol. Soc. Amer. 67:325-331.

Leash, A. M., and N. Hanson. 1961. The incidence of heartworm reported at the Michigan State University Clinic. Mich. State Univ. Vet. 21: 70.

Lewandowski, H. B., Jr. 1977. Determination of the important potential vectors of dog heartworm in Michigan. Ph. D. dissertation, Michigan State University.

Loor, K. A., and G. R. DeFoliart. 1969. An oviposition trap for detecting the presence of Aedes triseriatus (Say). Mosq. News 29: 487-488.

Ludlam, K. W., L. A. Jachowski, and G. F. Otto. 1970. Potential vectors of Dirofilaria immitis. J. Amer. Vet. Med. Assn. 157:1354-1359.

Lunt, S. R., and G. E. Peters. 1976. Distribution and ecology of tree-hole mosquitoes along the Missouri and Platte rivers in Iowa, Nebraska, Colorado, and Wyoming. Mosq. News 36:80-84.

Marquardt, W. D., and W. E. Fabian. 1966. The distribution of Illinois filariids of dogs. J. Parasitol. 52:319-322.

Mayr, E. 1963. Animal species and evolution. Harvard Univ. Press. 781 pp.

McDaniel, I. N., and W. R. Horsfall. 1957. Induced copulation of Aedine mosquitoes. Science 125:745.

McGreevy, P. B., J. H. Theis, M. M. J. Lavoipierre, and J. Clark. 1974. Studies on filariasis. III. Dirofilaria immitis: emergence of infective larvae from the mouthparts of Aedes aegypti. J. Helminth. 48:221-228.

Morris, C. D., and G. R. DeFoliart. 1971. Parous rates in Wisconsin mosquito populations. J. Med. Entomol. 8:209-212.

Newton, W. L. 1957. Experimental transmission of dog heartworm, Dirofilaria immitis by Anopheles quadrimaculatus. J. Parasitol. 43: 589.

Newton, W. L., and W. H. Wright. 1956. The occurrence of a dog filariid other than Dirofilaria immitis in the United States. J. Parasitol. 42:246-258.

Otto, G. F. 1969. Geographical distribution, vectors, and life cycle of Dirofilaria immitis. J. Amer. Vet. Med. Assn. 154:373.

Otto, G. F. 1972. Epizootiology of canine heartworm disease. In Canine heartworm disease: the current knowledge, pp. 1-14. R. E. Bradley, ed. Univ. Fla. Press, Gainesville.

Phillips, J. H. 1939. Studies on the transmission of Dirofilaria immitis in Massachusetts. Amer. J. Hyg. 29:121-129.

Prouty, D. L. 1972. Canine heartworm disease in southeastern Michigan. J. Amer. Vet. 161:1675-1676.

Rogers, J. S. 1978. Unpublished data.

Sawyer, T. K. 1974. Seasonal fluctuations of microfilariae in two dogs naturally infected with Dirofilaria immitis. In: Proceedings of the heartworm symposium '74, pp. 23-25. H. C. Morgan, G. F. Otto, R. F. Jackson, and W. F. Jackson, eds. V. M. Publishing, Inc., Bonner Springs, Kansas.

Schlotthauer, J. C., E. G. Harrison, and J. H. Thompson. 1969. Dirofilariasis-an emerging zoonosis? Arch. Environ. Hlth. 19:887-890.

Scholl, M. J., and G. R. DeFoliart. 1977. Aedes triseriatus and Aedes hendersoni: vertical and temporal distribution as measured by oviposition. J. Environ. Entomol. 6:355-358.

Shaw, D., Jr. 1976. The determination of the potential vectors of eastern equine encephalitis in Michigan. M. S. thesis, Michigan State University.

Sinsko, M. J., and P. R. Grimstad. 1977. Habitat separation by differential vertical oviposition of two tree-hole Aedes in Indiana. J. Environ. Entomol. 6:485-487.

Slocombe, J. O. D. 1978. Heartworm in dogs in Canada. Can. Vet. J. 19:244.

Soulsby, E. J. L. 1968. Helminths, arthropods, and protozoa of domesticated animals. London: Bailliere, Tindall, and Cassel. 824 pp.

Streitel, R. H., P. C. Stromberg, and J. P. Dubey. 1977. Prevalence of Dirofilaria immitis infections in dogs from a humane shelter in Ohio. J. Amer. Vet. Med. Assn. 170:720-721.

Taylor, A. E. R. 1960. The development of Dirofilaria immitis in the mosquito Aedes aegypti. J. Helminth. 34:27-38.

Truman, J. W., and G. B. Craig. 1968. Hybridization between Aedes hendersoni and Aedes triseriatus. Ann. Entomol. Soc. Amer. 61: 1020-1025

Watts, D. M., P. R. Grimstad, G. R. DeFoliart, and T. M. Yuill. 1975. Aedes hendersoni: Failure of laboratory-infected mosquitoes to transmit La Crosse virus (California encephalitis group). J. Med. Entomol. 12:451-453.

Weinmann, C. J., and R. Garcia. 1974. Canine heartworm in California, with observations on Aedes sierrensis as a potential vector. Calif. Vector Views 21:45-50.

Williams, J. F., and A. W. Dade. 1976. Dirofilaria immitis infection in a wolverine. J. Parasitol. 62:174-175.

Worley, D. E. 1964. Helminth parasites of dogs in southeastern Michigan. J. Amer. Vet. Med. Assn. 144:42-46.

Wright, R. E., and G. R. DeFoliart. 1970. Association of Wisconsin mosquitoes and vertebrate hosts. Ann. Entomol. Soc. Amer. 63:777-786.

Zaim, M. 1978. Impact of wastewater irrigation on the population of the tree hole breeding mosquito, Aedes triseriatus (Diptera: Culicidae). Ph. D. dissertation, Michigan State University.

Zaim, M., H. B. Lewandowski, H. D. Newson, and G. R. Hooper. 1977. Differentiation of Aedes triseriatus and Ae. hendersoni (Diptera: Culicidae) based on the surface of the egg shell. J. Med. Entomol. 14:489-490.

Zavortink, T. J. 1972. Mosquito studies (Diptera: Culicidae) XXVII. The New World species formerly placed in Aedes (Finlaya). Contrib. Amer. Entomol. Inst. 8:1-206.

Zydeck, F. A., I. Chowdkowski, and R. R. Bennett. 1970. Incidence of microfilariasis in dogs in Detroit, Michigan. J. Amer. Vet. Med. Assn. 156:890-891.