DEVELOPMENT OF *DIROFILARIA IMMITIS* IN *AEDES TRISERIATUS* SAY[1]

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ABSTRACT. Dogs with circulating microfilariae were obtained from three geographically distinct areas to determine whether divergent strains of *Dirofilaria immitis* Leidy might develop differently in *Aedes triseriatus*. No significant differences were noted. Results of these experiments were then combined to demonstrate the overall growth of *D. immitis* in *Aedes triseriatus*. An average of twenty microfilariae per mosquito were seen in the midgut and malpighian tubules 24 hours after the blood meal. An average of 6.5 first and second stage larvae per mosquito developed in the tubules over a 15-day period. Infective larvae averaged 2.7 per mosquito and were found in the tubules 11 days after ingestion of microfilariae. Average lengths of infective larvae ranged from 776μ–865 microns. Mortality of *A. triseriatus* during the developmental period of *D. immitis* was not a critical factor.

INTRODUCTION. Canine heartworm research continues to provide impetus in determining the importance of local mosquitoes as vectors, control of these vectors to lessen transmission, and in the utilization of the information as potential models for the study of other filarial diseases. Numerous workers over a period of the last forty years have established efficiency of various mosquito species to serve as suitable invertebrate hosts for *Dirofilaria immitis* Leidy either naturally, experimentally, or both, Ludlam *et al.* (1970). In several laboratory studies, mosquitoes have been shown to differ in their susceptibility to infection from one geographical area to another. Rosario (1936) reported *Aedes aegypti* susceptible to infection to *D. immitis* in the Philippines. Travis (1947) found only 2 percent of *A. aegypti* positive for infective larvae on Guam. The *Culex pipiens* and *C. pipiens quinquefasciatus* complex also demonstrates a variety of results. A Puerto Rican strain of *Psorophora confinnis* was more refractive to *Wuchereria bancrofti* than a United States strain, Newton *et al.* (1945). Kartman (1953) compared the efficiency of several mosquito species as hosts and vectors of *D. immitis* and results varied greatly between genera and species. Therefore, data obtained for one mosquito species in one locality cannot be anticipated to apply elsewhere. Dogs with circulating microfilariae were obtained from three geographically distinct areas to develop conclusions as to how divergent strains of *D. immitis* might influence transmission by the arthropod host. However, because there were no obvious differences in development of parasite strains from Mississippi, Japan, and Southern Texas in *Aedes triseriatus* Say from Jackson, Mississippi, results were combined to reflect the development of *D. immitis* in this indigenous mosquito species.

MATERIALS AND METHODS. *Aedes triseriatus* larvae were collected as needed and reared to adults in the laboratory. Larvae were placed in 8 x 12-inch enamel pans and fed Purina dog chow. No larval mortality occurred. Adults were held in screened cages 24 x 24 x 24 inches and maintained both prior to and after the blood meal by sugar water and sliced apples. Room temperature varied between 70 degrees–80 degrees F and humidity within the cages ranged from 70–80 percent. Mosquitoes obtained a blood meal

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by feeding on the dog's shaven hindquarters over a 3-hour period. Microfilarial density was determined prior to each experiment by examining 20 cm^3 of blood removed from the cephalic vein. Acepromazine (IM) at a dose of .25–.5 mg/lb body weight was administered as a sedative. This drug did not have an apparent effect on either mosquitoes or microfilariae. Mosquito dissection was accomplished in a manner similar to that described by Kartman (1953). For the first 9 days, only the midgut and malpighian tubules were examined for microfilariae and developing larvae. Gut, tubules, haemocoele (abdomen, thorax and head), and proboscis were dissected independently from the 10th–16th day. These structures were dissected in saline tinted with methylene blue. Fast moving infective larvae were killed in 2 percent formalin for observation and measurement. Bausch and Lomb ocular micrometers were employed for all measurements.

RESULTS AND DISCUSSION. Only a few workers have evaluated the receptiveness of *Aedes triseriatus* as a host for *Dirofilaria immitis*: Yen (1938), Phillips (1939) and Keegan *et al.* (1968). This species is discriminate in its breeding habitats utilizing tree holes and automobile tires as primary breeding sites, Womach (1971). For this reason, *Aedes triseriatus* is not generally found in large numbers, yet they will feed on dogs much more readily than many species.

The development of *D. immitis* in *A. triseriatus* was observed over a 16-day period. A total of 1163 mosquitoes fed on six different dogs. Mosquitoes fed readily and 60 percent obtained blood meals. Several factors may influence feeding and subsequent infection of the host. First, the attractiveness of the canine host to the mosquito is of primary importance in the maintenance of *D. immitis*. Keegan *et al.* (1968) obtained a 70 percent rate of engorgement with *A. triseriatus*. This was a greater percentage than he obtained from six other *Aedes* sp., five species of *Culex* sp. and *Culiseta inornata*. Phillips (1939) also noted that *A. triseriatus* readily fed on dogs. Secondly, acceptance of a blood meal and development of the parisite may be influenced by the age of the arthropod host. Duxbury *et al.* (1961) demonstrated that *Anopheles quadrimaculatus* 12–13 days of age before feeding harbored more larvae of *Dirofilaria uniformis* than mosquitoes 4–5 days old prior to obtaining a blood meal. Desowitz and Chellapah (1962) found in their study of *Brugia* sp. transmission by *Culex pipiens fatigans* that older mosquitoes may be more susceptible to infection. Keegan *et al.* (1967) discovered that the ability of *Aedes togoi* to serve as a host for *D. immitis* did not differ in four different age groups ranging from 5–22 days. Jordan (1962) concluded that no significant age difference existed in *Culex fatigans* if all third stage larvae of *Wuchereria bancrofti* were counted and not just those in the proboscis.

Age of the arthropod host does not seem to be critical if within reasonable limits. Therefore, mosquitoes used in each experiment did not differ more than 4 days in age and all mosquitoes ranged between 6–12 days of age prior to feeding. Finally, the development of *D. immitis* may be influenced by the number of microfilariae ingested. The observations of Kershaw *et al.* (1955) demonstrated that *Aedes aegypti* actually take in about half the numbers of microfilariae that would be expected knowing the size of the blood meal and host microfilarial density. However, Wharton (1960) observed that *Culex fatigans* feeding on patients with circulating *Wuchereria bancrofti*, seemed to ingest 3 times more than would normally be expected. In addition, Kershaw *et al.* (1955) theorized that mosquitoes that are potential survivors for a time long enough to support development to the infective form ingest about half as many microfilariae as those that do not survive to harbor infective larvae. The actual uptake, therefore, must be considered to be extremely variable.

Microfilarial density in all dogs ranged from 62–438/cm^3. Microfilariae remained in the midgut a maximum of 4 days. The

majority migrated to the malpighian tubules within 24 hours and characteristic shortening occurred in those microfilariae that remained in the gut more than 48 hours. Generally, microfilariae did not begin their initial development until migration to the malpighian tubules had been completed. This migratory phase proceeded without any apparent histological damage to the gut or tubules. The movement of microfilariae from the midgut to the tubules was slowed after 48 hours. The rate of egress was expressed by Hawking and Worms (1961) and Kartman (1953) as being influenced by the speed in which blood in the midgut coagulated and the clot formed. Encapsulation was observed. Isolated examples of this refractive influence most commonly involved the anterior or posterior one-fourth of 1st and early 2nd stage larvae. A lack of mortal encapsulation of larvae in *Aedes aegypti, Anopheles quadrimaculatus,* and *Culex quinquefasciatus,* was observed by Kartman (1953), although more encapsulation was seen in *Aedes aegypti.* Occasionally, high mortality of stage I and II larvae was evident in mosquitoes which had ingested large numbers of microfilariae. Sporozoites of *Lankesteria* sp. (possibly *L. culicus*) were observed in the tubules of 5–10 percent of the mosquitoes dissected, but no correlation between the presence of this gregarine and development of *D. immitis* in *A. triseriatus* could be demonstrated.

A record of the development of *D. immitis* in *A. triseriatus* is shown in table 1 and the average number of worms for each mosquito dissected and found positive is recorded. Ninety-six percent of the mosquitoes were positive for microfilariae on day 1. The percentage for mosquitoes found positive for microfilariae or any stage of the developing parasite decreased from 96 percent on day 1 to 50 percent over the next 15 days.

An average of 20 microfilariae from the gut and tubules were found on day 1. This is approximately the same intake reported by Kartman (1953) and Travis (1947) in *Aedes* sp. It has been shown that microfilariae intake may vary considerably by species, yet the total number of infective larvae per mosquito may not differ significantly (Intermill, 1970). Of

Table 1.—Development of *Dirofilaria immitis* in *Aedes triseriatus.*

				Average number of parasites per mosquito						
	Mosquitoes		Percent				Infective larvae			
Day *	Dissected	Positive	with parasites	Mff in midgut	Mff in tubules	Larvae in tubules	Tubules	Haemocoele	Labium	All areas
1	28	27	96	24	12.1	..	..	..	..	..
2	16	13	81	19	9.5	2.3	..	..	..	..
3	11	10	90	10	5.2	18.0	..	..	..	..
4	13	10	76	4	1.5	3.3	..	..	..	..
5	5	4	80	..	..	6.5	..	..	..	..
6	13	12	92	..	..	5.9	..	..	..	..
7	33	21	63	..	2.0	24.2	..	..	..	..
8	31	19	61	..	..	6.2	..	..	..	..
9	25	15	60	..	1.0	5.7	..	..	..	..
10	20	11	57	..	..	8.3	..	..	..	..
11	43	26	60	..	..	8.7	2.2	..	..	2.2
12	51	33	64	..	..	5.8	2.5	..	..	2.5
13	72	44	61	..	..	7.2	3.0	1.5	2.0	2.8
14	131	72	54	..	..	6.4	2.9	1.6	1.6	2.3
15	166	88	53	..	..	5.5	2.6	4.8	2.7	3.3
16	126	63	50	..	..	4.5	2.6	3.6	2.8	2.9

* Days following a blood meal.

the total microfilariae observed in the midgut on days 1–4, approximately one-half this number were seen in the tubules. Microfilariae were not seen in the gut after day 4.

The average number of stage II larvae per mosquito remained relatively constant through the 16-day period. The greatest number of stage I and II larvae appeared on the 10th and 11th days just prior to the morphological differentiation of stage II larvae into infective larvae. Yen (1938), in an abbreviated study, reported sausage stage larvae in *A. triseriatus* 5 days after feeding. No other comparable data are available on the larval development of *D. immitis* larvae in *A. triseriatus*.

Infective larvae first appeared in the tubules 11 days after the blood meal. The average number of stage II larvae per mosquito from all areas ranged from 2.2 on day 11 to 3.3 on day 15. Concurrently, the average number of developing larvae per mosquito fell from 8.7 to 5.5. The largest number of infective larvae from a specific body area was seen on day 15 when an average of 4.8 stage III larvae were recovered from the haemocoele. Infective larvae first appeared in the labium 13 days after feeding. There exists a difference in the time of appearance of infected larvae in the labium between species and within species from different areas, Feng Lan-Chou (1930), Keegan (1968), Kartman (1953), Intermill (1970), Travis (1947) and Phillips (1939).

The efficiency of *Aedes triseriatus* as a host and vector for *Dirofilaria immitis* is indicated by the number of parasites that mature to stage III larvae from the original number of microfilariae ingested. An average of 24 microfilariae per mosquito was observed on days 1 and 2. Of these microfilariae, 6.5 stage I and II larvae and 2.7 stage III larvae per mosquito *developed*. In other words, approximately 40 percent of the microfilariae ingested continued development to stage I and II larvae and 11 percent reached the infective stage. Stage I and II larvae were recovered from 47 percent of the mosquitoes dissected from the 2nd to the 16th day. Thirty-nine percent of the mosquitoes dissected from the 11th to the 16th day contained infective stage larvae. Keegan (1968) and Phillips (1939) recovered infective larvae from 26 percent and 75 percent of *A. triseriatus*, respectively. The host and vector efficiency formula, employed by Kartman (1953) to measure the host's ability to support development of microfilariae to infective stage larvae, demonstrates that *A. triseriatus* compares favorably with most *Aedes* sp., is superior to *Culex* sp., but is inferior to *Anopheles* sp.

Mortality of *A. triseriatus* has presented problems in its evaluation as a host for *D. immitis* (Weiner and Bradley, 1970) and Bradley (1953). This difficulty was not evident in these studies. Mosquito mortality was minimal through the first 8 days, never rising above 3 percent each day. However, deaths increased on day 9 to between 3–4 percent and this level of mortality remained until day 14 at which time another slight increase was observed. The rise in deaths observed from days 9–14 corresponds to the movement of late stage II and development of active stage III larvae in the tubules. The increase in daily mortality to 6 percent on day 15 parallels the migration of infective larvae within the haemocoele. Kershaw *et al.* (1955) observed this pattern of mortality with *Aedes aegypti*. Pistey (1959) observed increased mortality in mosquitoes harboring *Dirofilaria tenuis* on the second and eighth day. These peaks were believed to result from larval migration to the tubules and emergence of third stage larvae from the malpighian tubules.

Microfilariae, developing and infective larvae of *Dirofilaria immitis* in *Aedes triseriatus* were measured (Figure 1). The average length of microfilariae examined was 282 microns. The range was 260–312 microns. Developing larvae, however, varied a great deal. This was especially true in the latter days of the experiments when some larvae were still typical sausage-shape stage I larvae not progressing

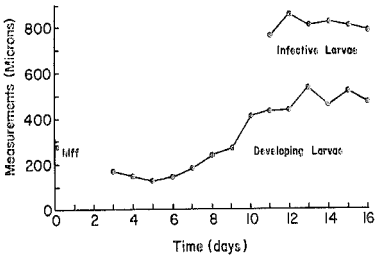

Fig. 1.—Average measurements of microfilariae, developing and infective larvae of *Dirofilaria immitis* in *Aedes triseriatus*.

in development, and other larvae were transforming into stage III. Infective larvae ranged from 776–865 microns.

SUMMARY. The development of three geographically distinct strains of *Dirofilaria immitis* in locally collected *Aedes triseriatus* showed no significant differences. Results of these experiments are combined to demonstrate the overall growth of *D. immitis* in *Aedes triseriatus*. Sixty percent of the experimental mosquitoes took blood meals. Of 784 such mosquitoes dissected, 60 percent harbored at least one stage of the developing parasite. An average of 20 microfilariae per mosquito were seen in the midgut and tubules 24 hours after the blood meal and approximately one-half the number of microfilariae seen in the midgut on days 1–4 migrated to the malpighian tubules. An average number of 6.5 first and second stage larvae per mosquito developed in the tubules over a 15-day period. Infective stage larvae averaged 2.7 per mosquito and were found in the tubules 11 days after ingestion of microfilariae. Using Kartman's (1953) standard for evaluating the host efficiency and vector potential, *Aedes triseriatus* compared favorably with other *Aedes* sp. as a host for *D. immitis*. It was superior to *Culex* sp., but somewhat less efficient than *Anopheles* sp. Mortality of *Aedes triseriatus* during the development period of *D. immitis* was not a critical factor. Peaks of mortality occurred between days 8–14 and days 15–16. These increases in mortality were closely correlated with the movement and migration of the parasite within the arthropod host. During the period of development, stage II larvae varied considerably in their rate of development. Average measurements of stage III larvae ranged from 776–865 microns in length.

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References

Bradley, R. E. 1953. Observations on the development of *Dirofilaria immitis* in mosquitoes. Jour. of the Tenn. Acad. Sci. 28:176.

Desowitz, R. S. and Chellapah, W. T. 1962. The transmission of *Brugia* sp. through *Culex pipiens fatigans;* The effect of age and prior non-infective blood meals in the infection rate. Trans. Roy. Soc. Trop. Med. Hyg. 65:121–125.

Duxbury, R. E., Moon, A. P. and Sudan, E. H. 1961. Susceptibility and resistance of *Anopheles quadrimaculatus* to *Dirofilaria uniformis*. Jour. Parasit. 47:687–692.

Feng Lan-Chou, M. B. 1930. Experiments with *Dirofilaria immitis* and local species of mosquitoes in Peiping, North China. Ann. Trop. Med. Parasit. 24:347–364.

Hawking, F. and Worms, M. 1961. Transmission of filarioid nematodes. Ann. Rev. of Ent. 16:413–432.

Intermill, R. W. and Frederick, R. M. 1970. A study of potential mosquito vectors of *Dirofilaria immitis* Leidy, on Okinawa, Ryukyu Islands. Jour. Med. Ent. 7(4):455–461.

Jordan, P. 1962. Transmission of *Brugia* through *Culex pipiens fatigans* (Correspondance). Trans. Roy. Soc. Trop. Med. Hyg. 56:436.

Kartman, L. 1953. Factors influencing infection of the mosquito with *Dirofilaria immitis*. Exptl. Parasit. 2:27–78.

Keegan, H. L., Betchley, W. W., Haberkorn, T. B., Nakasone, A. Y., Sugiyama, H. and Warne, R. J. 1967. Laboratory and field studies of some entomological aspects of canine dirofilariasis problems in Japan. Jap. Jour. of San. Zoo. 18(1):6–13.

Keegan, H. L., Fitzgerald, C. M., McCrary, T. L. and Doyle, M. D. 1968. Studies on dog heartworm in South Texas. Texas Repts. on Biol. and Med. 26(3):321–330.

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

Ludlam, K. W., Jachowski, L. A. and Otto, G. F. 1970. Potential vectors of *Dirofilaria immitis*. JAVMA 157(10):1354–1359.

Newton, W. L., Wright, W. H. and Pratt, I. 1945. Experiments to determine potential mosquito vectors of *Wuchereria bancrofti* in the Continental United States. Am. Jour. Trop. Med. 25:253–261.

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

Pistey, W. R. 1959. Studies on the mortality of mosquitoes infected with *Dirofilaria tenuis* Chandler. 1942. Exptl. Parasit. 8:596–608.

Rosario, F. del. 1936. *Dirofilaria immitis*, Leidy and its culicine hosts in Manila, P. I. Philipp. Jour. Sci. 60:45–47.

Travis, B. V. 1947. Relative efficiency of six species of mosquitoes from Guam, M. I. as supplemental hosts for *Dirofilaria immitis*. Jour. Parasit. 33:142–145:

Weiner, D. and Bradley, R. E. 1970. Ability of some mosquitoes to transmit *Dirofilaria immitis* in Florida. Mosq. News 30:406–410.

Wharton, R. H. 1960. Studies on filariasis in Malaya: Field and laboratory investigation of the vectors of a rural strain of *Wuchereria bancrofti*. Ann. Trop. Med. Parasit. 54:78–91.

Womack, W. L. 1971. The bionomics of *Aedes triseriatus* (Say) larvae in Jackson, Mississippi area. Thesis. University of Mississippi, School of Medicine, Department of Preventive Medicine, Jackson, Mississippi.

Yen, C. H. 1938. Studies of *Dirofilaria immitis* Leidy with special reference to the susceptibility of some Minnesota species of mosquitoes to the infection. Jour. Parasit. 24:189–205.

COMPARATIVE BEHAVIOUR OF EIGHT SPECIES OF MOSQUITO LARVAE (DIPTERA:CULICIDAE) IN ELECTRIC FIELDS[1]

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ABSTRACT. The behaviour of late-larval instars of three species of *Aedes*, three of *Culex* and one of *Culiseta* in direct-current electric fields was tested and compared to that of *A. aegypti* (L.). All species showed response to electric current but in none was it as pronounced as in *A. aegypti*. As voltage and current were increased the three *Aedes* species showed the negative—positive—negative reversals in reactions found in *A. aegypti* in earlier work. In the *Culex* and *Culiseta* species, only a negative—positive change was evident before the onset of paralysis. Causes of the reactions remain unknown.

INTRODUCTION. The behaviour of larvae and pupae of *Aedes aegypti* (*L.*) in direct-current electric fields was reported by Riordan (1971). Described here is an extension of this work in which third and fourth instar larvae of *A. stimulans*/ complex, *A. canadensis* (Theobald), *A. atropalpus* (Coq.), *Culex pipiens quinquefasciatus* Say, *C. pipiens pipiens* L., *C. tarsalis* Coq. and *Culiseta inornata* (Williston) were similarly tested and their behaviour compared to that of *A. aegypti*. The work was done at the former Research Institute, Canada Agriculture, Belleville, Ontario.

METHODS. The experimental apparatus and procedures were the same as those described previously (Riordan, 1971). Briefly, the plastic trough in which the tests were conducted is 120 cm long, 12.5 cm wide and is filled with water to a depth of 7.5 cm. Carbon electrodes are situated at each end. Tests were of 20 minutes duration and were conducted in complete darkness. At the end of each test partitions dropped to divide the trough into seven compartments, the centre compartment being twice as long as the others. The larvae were released at

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