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Summary

1) *Ae.* aegypti was the most susceptible to *D. immiis* among 5 species of mosquitoes used in this study. Complete development of *D. immitis* was not achieved in one species tested - *Armi geres subalbatus.*

2) Older *Ae.* aegypti were more susceptible to D. *immitis* than the younger mosquitoes, while older *An. stephensi* were less susceptible than yonnger mosquitoes.

3) The most optimum temperature of larvae in mosquitoes was 27°C, but at 31°C mosquitoes developed longer larvae in a shorter period of time than they did at two low temperatures. Beyond that, larvae developed very well in *An. stephensi* at the lowest temperature (23°C) while in *Ae. aegypti* larvae failed to reach as great a stage of development as in *An. stephensi.*

4) The percentage of mosquitoes surviving following infection decreased as the intensity of microfilaremia in the ingested blood increased. *An. stephensi* was less able than *Ae. aegypti* to tolerate the ingestion of high number of microfilaria.

INTRODUCTION

Dirofilaria immitis (Leidy 1856) is the common heart–worm of dogs, and this parasite also occurs in cats, foxes, and wolves. The worms live mainly in the right ventricle and the pulmonary artery, and have also been found in other parts of the body. The males, measuring 120 to 250 mm. in length by 1 mm. in diameter, possess blunt tails which are armed with caudal alae and are spirally coiled. The females are larger and measure 250 to 310 mm. by 1 mm.; the vulva is situated near the posterior extremity of the oesophagus.

The microfilariae, measure 218 to 330 μ by 5 to 6 μ, are unsheathed and are found in the peripheral circulation at all times, but there is a tendency towards periodicity. This appears to vary in different countries. In Thailand, Manee Apichatbut (1969) observed that in four infected dogs the microfilaria seemed to be nocturally subperiodic the microfilariae intensity was highest during the night, with a maximum near 12 p.m. followed by a drop which reached the lowest point between 6 a.m. and 10 a.m.

Life–cycle. The insect vector for *D. immitis* is a mosquito. Again there appears to be little host specificity, for many species of mosquitoes can serve as vectors (Bemrick and Sandholm, 1966). Other arthropods, such as lice, flies, and ticks, have been suspected as possible vectors.

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Taylor (1960) has described the development in the mosquito. Within 24 to 36 hours after ingestion by the mosquito, the larvae are found primarily in the cells of the malpighian tubules. After 3–4 days the infective larvae escape from the malpighian tubules and migrate to the coelom. Eleven to 12 days after their escape most of the larvae are found in and among the fat bodies in the lower half of the thorax. From there they migrate to the mouth parts and are in position to be introduced into another vertebrate when the mosquito next partakes of a blood meal. The adults appear in the vertebrate's heart, particularly the right ventricle and pulmonary arteries, 8 to 9 months after infection. According to Kume and Itagaki (1955), the adults may live more than two years.

Medical Importance. Heartworm infection in domestic dogs is prevalent throughout Southern Europe, China, Japan, Fiji, New Caledonia, Australia, Hawaii, and the warmer regions of the North and South America. In a survey of stray dogs in Bangkok conducted by Manee Apichatbut during 1968, 28 out of 32 animals were found to be infected with *Dirofilaria immitis*. It is of interest to note that the mosquitoes which are able to transmit this worm are common house mosquitoes in Bangkok and elsewhere in Thailand. It seems to be possible that humans in this area which are bitten many times by these species of mosquitoes, may be infected with this worm. Jung (1960) reported a human case of superficial dirofilarial infection and it is possible that this infection was due to *D. immitis*, since this worm, even in its normal host, resides for a time in the subcutaneous tissue before moving to the heart (Kume and Itagaki, 1955). In Florida Sams and Beck (1959) reported 39 cases of human infection with filarial parasites which have been indentified as *D. conjunctiva*. It has been suggested by Faust (1952) that *D. conjunctiva* may be *D. immitis* in an abnormal host. Dashiell (1961) reported the finding of a worm in a pulmonary artery of a patient and Abadis (1965) also reported the discovery of a worm in the right side of the heart of a forty year old woman in New Orleans. Both of the parasites were identified as the heart worm of dog, *Dirofilaria immitis*.

Objectives. According to Manee Apichatbut (1969), *C. pipiens quinquefasciatus*, *Ae. aegypti*, and *Ae. albopictus* were highly susceptible to *D. immitis* and only one species of mosquito, *An. stephensi* was examined in which the larvae did not develop to the infective stage. This study was designed to determine whether there is any factor which influences the susceptibility of *Ae. aegypti* (susceptible species) and *An. stephensi* (resitant species) to infection with *D. immitis*. The objectives of this study were as follows:–

a. A preliminary experiment to determine the susceptibility of several of colonized mosquitoes to infection with *D. immitis*.

b. To study the effect of mosquitoes'age on development of larvae.

c. To determine effect of tempera-

ture on development of *D. immitis* larvae in mosquito host.

d. To determine relationship between intensity of microfilaremia and development of infective larvae.

MATERIALS AND METHODS

a. Source of microfialariae

The source of microfilaria of *D. immitis* was a natural infection in two dogs (designated T. and M.) reared in screened quarters in the animal house of the Department of Veterinary Medicine, SEATO Medical Research Laboratory for the last 2 years. Microfilaremia was measured by drawing 20 cu. mm. of blood routinely at the initiation of an experiment. Then the blood was expelled in a thick film about 30 X 15 mm. on a clean slide. The smear was allowed to dry, then laked in distilled water and stained with Giemsa stain. The entire a area of each blood film was examined with a compound microscope. If microfilariae were present, they were counted with the aid of a hand tabulator.

b. Infection of mosquitoes.

The mosquitoes used in these studies were: *An. stephensi* (Calcutta strain), *Ae. aegypti* (Koh Samui strain), *Armigeres subalbatus* (Malayan strain), *An. maculatus* (Malayan strain), *Anopheles balabacensis* (Khao Mae Kaew strain). These colonized mosquitoes were maintained in the insectary at 27°C and 80–90% relative humidity. They were provided with a 10% multivitamin-syrup solution as a source of fluids between blood meals. Adult mosquitoes ot be used in experiments were deprived of food for half a day to stimulate their appetite for the infective blood meal. They were allowed to feed on an infected dog which was anesthetized with Pentobarbital Sodium by intravenous injection, by placing the mesh-covered surface of a small box with a given number of mosquitoes against the shaved abdomen of the infected dog for 30–50 minutes. Several small containers were used when simultaneously feeding mosquitoes of different ages and different species.

After feeding, the fully engorged females were removed with an aspirator and maintained in small rearing cages approximately 12X12X12 inches in size. The infected mosquitoes were etherized and dissected at various intervals after engorgement.

c. Dissection techniques.

A slit was made on either side of the eighth abdominal segment of the mosquito and the intestinal tract was drawn out posteriorly in a drop of saline solution on slide under a streoscopic microscope. In the earlier stages of infection, the intestinal tract and the malpighian tubules were placed in a drop of saline salution and examined under a compound microscope for filarial larvae. With mosquitoes that were in the later stages of infection, the head, thorax and abdomen of the specimen were cut apart and placed in drops of saline solution and examined for filarial larvae.

When measurements were required, larvae were placed in 2 percent formalin and the length determined with the aid of a calibrated ocular micrometer under compound microscope. In some studies, the mosquitoes were kept in an incubator where constant temperatures and a relative humidity of 80–90% were maintained.

RESULTS

1. A preliminary experiment was designed to determine the susceptibility of several mosquito species to *D, immitis* (Tables I-V). Five species of mosquito *Ae. aegypti*, *An. stephensi*, *An. maculatus*, *An. balabacensis* and *Arm. subalbotus*, aged 5–8 days, were allowed to feed on dog T. between 2.30–3.30 p.m. when there were 100–200 microfilariae in 20 cu. mm. of blood. All of these species became infected with *D. immitis*, although the number of positive mosquitoes seemed to be small. It is important to note that among these species *Ae. aegypti* was most susceptible while *Arm. subalbatus* never developed infective larvae at the head and also seemed to be less susceptible than other species (Table VI).

2. In order to determine the mosquito's age on the number and growth of developing larvae. *Ae. aegypti* and *An, stephensi.* in several groups of varying age, were exposed to infection of dog T. between 2.30–3.30 p.m. when counted microfilaremia were 100–200 microfilariae per 20 cu.mm. These mosquitoes were dissected 4,8 and 12 days later. The number and length of larvae which were recoverd from 15 positive mosquitoes is given in Tables VII, VIII, Older *Ae. aegypti* developed more and longer larvae than the younger mosquitoes, on the other hand, the older *An. stephensi* seemed to less susceptible than the younger mosquitoes.

3. In order to determine the role of temperature in the development of *D. immitis* larvae, *Ac. aegypti* any *An. stephensi*, aged 5–8 days, were maintained at 31, 27 and 23°C after feeding on infected dog T. when there were 100–200 mf. in 20 cu.mm. of blood. Thirty mosquitoes from each of the three groups were dissected 4, 7, 10 and 13 days after infection. At 27°C both species developed higher number than the group maintained at 23 and 31°C, however, the number of larvae recovered from *Ae. aegypti* was considerably higher than from *An. stephensi*, especially at 23°C

The larvae developing in *Ae. aegypti* and in *An. stephensi* maintained at 31°C grew more rapidly than those maintained at the two lower temperatures. There was a slightly difference between these 2 species of mosquito in this respect, for in *Ae. aegypti* larvae developed rapidly at 31, 27 and 23°C, respectively, from 1st stage to later stages, while in *An. stephensi*, they grew very rapidly at 31°C during the first 10 days, but by the 13th day, the larvae at 27 and 23°C had grown to greater lengths

4. The last experiment was attempted to study the role of infections of varying intensity on the survival of mosquitoes and on the numbers of

developing larvae. *Ae. aegypti* and *Anopheles stephensi*, aged 5–8 days, were fed on dog T. at 2.30–3.30 p.m. when there were 123 mf. in 20 cu.mm. of blood; on dog M., at 6.30 7.30 p.m. when microfilaremia was 558 mf./20 cu.mm. and also during 11.30–12.30 p.m. when there were 1030 mf./20 cu.mm. (Tables IX,X).

The percentage of mosquitoes surviving decreased as the intensity of microfilaremia during feeding increased. The few *Ae. aegypti* which survived until the 12th day after exposure to high intensity infections developed significantly higher numbers of larvae than those which fed during the two lower intensities. *An. stephensi* was less able to sustain high numbers of microfilaria, for few survived until the 12th day. Moreover, it appeared that the few surviving *An. stephensi* were all negative, and they never developed larvae at all.

TABLE I

Experimental infection of *Ae. aegypti* with *D. immitis**

Days after feeding	No. mosq. dissected	No. mosq. pos.	Organ Abd.	Positive Thor.	Head	% mosq. Pos.	% mosq. with larvae at head
4 – 5	27	18	18	0	0	66.6	0
7 – 9	25	6	6	0	0	24	0
14 – 16	42	9	5	2	8	21.4	19

* Fed on dog with 100 – 200 microfilariae/20 cu.mm.

TABLE II

Experimental infection of *An. stephensi* with *D. immitis**

Days after feeding	No. mosq. dissected	No. mosq. pos.	Organ Abd.	Positive Thor.	Head	% mosq. pos.	% mosq. with larvae at head
4 – 5	7	4	4	0	0	57.1	0
7 – 9	9	3	3	0	0	33.3	0
14 – 16	36	4	2	0	2	11.1	5.7

* Fed on dog with 100 – 200 microfilariae/20 cu.mm.

TABLE III

Experimental infection of *An. maculatus* with *D. immitis**

Days after feeding	No. mosg. dissected	No. mosq. pos.	Organ positive Abd.	Thor.	Head	% mosq. pos.	% mosq. with larvae at head
4 – 5	12	5	5	0	0	41.7	0
7 – 9	14	3	3	0	0	21.4	0
14 – 16	28	2	1	1	2	7.1	7.1

* Fed on dog with 100 – 200 microfilariae/20 cu.mm.

TABLE IV

Experimental infection of *An. balabacensis* with *D. immitis**

Days after feeding	No. mosq. dissected	No. mosq. pos.	Organ positive Abd.	Thor.	Head	% mosq. pos.	% mosq. with larvae at head
4 – 5	15	4	4	0	0	26.6	0
7 – 9	12	2	2	0	0	16.6	0
14 – 16	18	4	1	1	4	22.2	22.2

* Fed on dog with 100 – 200 microfilariae/20 cumm.

TABLE V

Experimental infection of *Armigeres subalbatus* with *D. immitis*

Days after feeding	No. mosq. dissected	No. mosq. pos.	Organ positive Abd.	Thor.	Head	% mosq. pos.	% mosq. with larvae at head
4 – 5	33	11	11	0	0	33.3	0
7 – 9	35	7	7	0	0	20.0	0
14 – 16	42	2	2	2	0	4.8	0

* Fed on dog with 100 – 200 microfilariae/28 cu.mm.

TABLE VI

Infection of Different Mosquito Species with *D. immitis**

Species	No. mosq. dissected	% mosq. infected	% mosq. with larvae at head
Ae. aegypti	94	35.1	19
An. stephensi	52	21.2	5.7
An. maculatus	54	18.5	7.1
An. balabacensis	45	22.2	22.2
Arm. subalbatus	110	18.2	0

* Fed on dog with 100 – 200 microfilariae/20 cu.mm.

TABLE VII

Effect of Mosquitoes' Age on Development of Larvae*

(*Ae. aegspti*)

Age of Mosq. when infected (days)	Larvae recovered from 15 positive mosq. 4 days Total larvae	No.larvae/mosq.	Mean length (Micron)	8 days Total larvae	No.larvae/mosq.	Mean length (Micron)	12 days Total larvae	No.larvae/mosq.	Mean length (Micron)
4–5	168	11	275	48	3	357	24	2	186
9–10	92	6	263	18	1	368	48	3	892
14–15	201	13	287	56	4	408	107	7	1020

* Fed on dog with 100–200 microfilariac/20 cu. mm.

TABLE VIII

Effect of Mosquitoes'age on Development of Larvae*

(*An. stephensi*)

Age of Mosq. when infected (days)	Larvae recovered from 15 positive mosq. 4 days Total larvae	No.larvae/mosq.	Mean length (Micron)	8 days Total larvae	No.larvae/mosq.	Mean length (Micron)	12 days Total larvae	No.larvae/mosq.	Mean length (Micron)
4–5	93	6	212	19	1	342	23	2	867
9–10	60	4	230	17	1	365	20	1	520
14-15	40	3	133	17	1	289	18	1	561

* Fed on dog with 100–200 microfilariae/20 cu. mm.

TABLE IX

Relationship between intensity of microfilaremia and development of infective larvae in *Ae. aegypti.*

Microfilaremia (Mf./20 cu. mm.)	Number of Mosq. fed	Mosq. surviving 12 day Number	Percent	Mean No. of Larvae
123	198	108	54.6	3
558	225	90	40	2
1030	268	19	7.1	8

TABLE X

Relationship between intensity of microfilaremia and development of infective larvae in *An. aegypti*

Microfilaremia (Mf./20 cu. mm.)	Number of Mosq. fed	Mosq. surviving 12 days Number	Percent	Mean No. of Larvae
123	77	12	15.5	4
558	64	6	9.4	0
1030	89	2	2.2	0

Discussion

The principal objective of this study was to define some of the factors which influence the infection of local species of mosquitoes by *D. immitis*. However, before the importance of some of these factors could be measured it was necessary first to study the course of development of this parasite within these species. It was found necessary to feed large numbers of mosquitoes on infected dogs because of the high mortality which occurred in these mosquitoes following the infective blood meal. It is possible that some of this mortality was due the injurious effect of the developing parasites on the mosquito hosts. In addition, the sodium pentobarbitol used to anaesthetize the dogs prior to mosquito feedings may have affected the mosquitoes which engorged on the dogs. The number of *An. stephensi* used in these studies was smaller because of difficulties encountered in rearing the larval stages of that mosquito through to maturity.

In the preliminary experiments, all of the mosquito species used became infected with *D. immitis*, but with varying degrees of intensity. *Armigeres subalbatus* was the only species which failed to develop infective stage larvae in the head and mouthparts. These different reponses are probably determined, in part, by the different genetic constitutions of the mosquito hosts (Kartman, 1951). A given mosquito species may differ in susceptibility to infection with this parasite from one area to another, also due, presumably, to differences in the genetic makeup of different geogaphic populations (Yen, 1938).

A direct relationship was observed between the age of mosquitoes at infection and length of larvae developing in them. Attention should be called to the fact that in these experiments increased susceptibility in *Ae. aegypti* was apparently related to senility. In contrast to *Ae. aegypti*, *An. stephensi* became more resistant to infection with increasing age. The relation of the age of insects to their resistance to infection is influenced by many factors, among which are the intrinsic qualities of the insects' tissues and/or metabolism on the physiology of the parasites (Steinhaus, 1949).

It was found (Fig. 1–4) that 27°C was an optimum temperature for parasite development. It is also important to note that, at 23°C, mortality of mosquitoes was high so that the experiment had to be repeated many times in order to obtain enough mosquitoes for dissection at different intervals of time. At 31°C *D. immitis* larvae grew more rapidly especially during the first few days. Although the number of larvae recovered from *An. stephensi* was small, larvae did develop in that species even at the lowest temperature (23°C), and were longer than larvae in *Ae. aegypti* (Fig. 3–4).

In the last experiment it was observed that the greater the intensity of microfilaremia at time of feeding the greater was the mortality in the infected mosquitoes. It is possible that heavy infections overwhelm the defense mechanism of the mosquito host and that tissue demage produced by the activities of the

parasites result in death of the mosquitoes. Large numbers of mosquitoes died within periods of from one hour to two days after feeding on dogs with a high concentration of circulating microfilaria.

Of considerable interest was the fact that the number of larvae recovered from mosquitoes of both *Ae. aegypti* and *An. stephensi* decreased as the interval between the infective blood meal and time of dissection increased (Table. VII, VIII and Fig. 1,2). This might be due in part to the encapsulation of parasite larvae within host tissues. Kartman (1956) suggested that encapsulation of worms increased in direct proportion to the length of time spent by the parasites in the mosquito. In these studies it was observed that, encapsulation was usually found more often in *An. stephensi* than in *Ae. aegypti*. Brug (1932) suggested that filarial encapsulation was not a successful means of defense by the host but he also noted that encapsulation occurred more consistenly in some mosquito hosts than in others.

Other factors could also result in the destruction of larvae prior to their complete development in the mosquito host. Kartman (1953) suggested that there might be some factors in the midgut or salivary glands which are capable of killing microfilariae at the time of their ingestion. The activity of enzymes in the digestive process, the trapping of microfilariae by the clotted blood in the midgut, and the excretory processes might play a role in the loss of larvae before they reach maturity. The mosquitoes were not allowed to feed on blood again after the infectious blood meal, but they were given multivitamin syrup and it is possible that some infective stage larvae were lost during that feeding process. Differences in activity of digestive enzymes, rate of peritrophic membrane and blood clot formation in the midgut, speed of the excretory process, which may vary in different mosquito species might result in the different degrees of susceptibility observed in the mosquitoes in these studies. In view of these findings *Ae. aegypti* seemed to be more highly susceptible than the anophelines because of its ability to sustain much larger numbers of larvae.

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