Susceptibility of Aedes albopictus mosquitoes (Oahu strain) to infection with Dirofilaria immitis.

Zytoon, EM
el-Belbasi, HI
Konishi, E
Matsumura, T

(Citation)
The Kobe journal of the medical sciences,38(5):289-305

(Issue Date)
1992

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/E0034074

Kobe J. Med. Sci. 38, 289/305, October 1992

SUSCEPTIBILITY OF *AEDES ALBOPICTUS* MOSQUITOES (OAHU STRAIN) TO INFECTION WITH *DIROFILARIA IMMITIS*

Eiman M. ZYTOON, Hussein I. El-BELBASI, Eiji KONISHI, and Takeo MATSUMURA

Department of Medical Zoology, Kobe University School of Medicine

INDEXING WORDS

Dirofilaria immitis; *Aedes albopictus*; susceptibility; intermediate host

SYNOPSIS

The susceptibility of the Oahu strain of *Aedes albopictus* mosquitoes to infection with *Dirofilaria immitis* was examined to study the development of larvae in detail. Most of the developing larvae were found in the Malpighian tubules and infective larvae in the labium, indicating that *Aedes albopictus* Oahu strain is susceptible to the infection with *D. immitis*. New different patterns of development from those reported by earlier studies were observed: (i) the second-stage larvae in the thorax muscles of the mosquitoes and (ii) a small projection from the head of the sausage-form first-stage larvae.

INTRODUCTION

Dirofilaria immitis (Leidy) is a parasitic nematode called canine heartworm that is transmitted by an intermediate host, mosquitoes (7, 13, 15). Since Grassi and Noe (1900) first detected *D. immitis* larvae developing in mosquitoes (5), many workers have observed a wide variation among mosquitoes in susceptibility to infection with *D. immitis* (14). In species of susceptible mosquitoes, the ingested microfilariae (Mf) of *D. immitis* migrate

Received for publication : October 1, 1992

Authors'names in Japanese : イーマン・M・ザイトン, フセイン・I・エルベルベシ
小西英二, 松村武男

from the midgut to the primary cells of the Malpighian tubules, where they develop to the infective form (13).

Aedes albopictus is a mosquito species susceptible to infection with *D. immitis* (5). Zytoon (22, 23, 25) found that the concurrent ingestion of chikungunya virus and Mf of *D. immitis* in *Aedes albopictus* mosquitoes enhanced the viral multiplication and dissemination. In experiments to confirm the development of *D. immitis* larvae in this strain, we found new different developing patterns (24) from those reported by earlier studies (13, 15, 19). This paper reports on detail of the development pattern of *D. immitis* Mf to the third-stage larvae in *Aedes albopictus* Oahu strain.

MATERIALS AND METHODS

Mosquitoes

Laboratory colonies of the Oahu strain of *Aedes albopictus* (21) were used. Mosquito eggs were hatched, larvae were reared, and pupae were allowed to emerge as adults in the standard procedure. Adults were maintained with 10% sugar water under the constant insectary condition at 28±1 C and 70±10% relative humidity with a cycle of 16 h of light and 8 h of dark.

Microfilariae

A 4-year-old male beagle dog naturally infected with D. immitis was used as the Mf donor. Blood used for artificial feeding was taken from the radial vein of a foreleg. The dog was cared for at the Institute for Experimental Animal Facilities of this school. The density of circulating Mf range from 35,000 to 60,000 per ml of blood as measured by a direct count in 10µl of infected blood.

Infection of mosquitoes

A group of mosquitoes was infected with *D. immitis* by using an artificial feeding apparatus as previously described (21). Briefly, 5- to 7-day-old females starved for 4 to 6 h were allowed to feed on a mixture of Mf and sheep defibrinated blood. Another mosquito group was allowed to feed directly on the infected dog. A partly shaved distal half hind leg was extended gently into the mosquito cage to allow the mosquitoes to bite the shaved area. After feeding, the engorged females were maintained under the insectary condition, and dead individuals were counted daily.

Dissection of mosquitoes

Ten mosquitoes were examined daily for the number of developing larvae in the midgut, Malpighian tubules, thorax, and head. The larvae in the individual organs were counted, and measured in their lengths and widths. If the larvae moved actively, their movement was stopped by gentle heating of the slide glass to facilitate measurement of their body size.

Periodical changes in number of Mf ingested by mosquitoes

Six groups of female mosquitoes, 50 in each group were allowed to feed on the infected dog blood by an artificial feeding apparatus with each group fed every four hours during a 24-hour period. After each group was infected, the fully engorged mosquitoes were separated, dissected to count the number of Mf.

Degree of Mf penetrating midgut

We compared the possibility of differential delay of Mf in the midgut by comparing the number of Mf remaining in the midgut within 1, 2, 4, and 6 h after infection using different method of feeding. Midguts were excised to count Mf.

Histopathology

Mosquitoes were used for histological studies 9 to 12 days after feeding on Mf. After being fixed at room temperature for 3 days with 10 % formalin, mosquito samples were embedded in wax that melts at 56 C, sectioned at 1μm thickness, and then stained with haematoxylin and eosin (HE). To facilitate the sectioning, blocks were immersed in distilled water for 3 days (14).

RESULTS

The periodical change in the number of Mf ingested by mosquitoes from the infected dog within one day at 4h intervals was examined in two repeated experiments on different days (Fig. 1). A relatively high mean number of Mf obtained from 10 mosquitoes (49-64 Mf per mosquito), was observed at 6 p.m. and 10 p.m. and a lower mean number (17-24 Mf per mosquito) at 6 a.m. and 10 a.m. Based on this result, subsequent feeding experiments were done between 6 p.m. and 10 p.m.

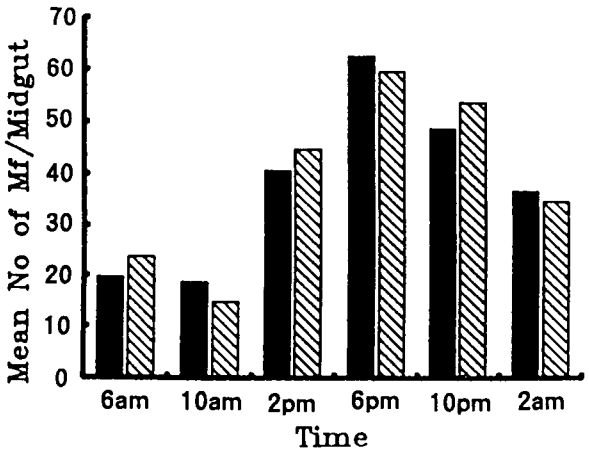

Fig. 1. Periodical changes in the number of Mf ingested by mosquito.

The mortality of the mosquitoes until 14 days after infection was related to the Mf density in the infected blood used for the mosquito feeding (Fig. 2). The lowest mortality was found in mosquitoes that ingested 5 Mf (supposing the blood meal size of 2 μl), which was similar to those ingested uninfected blood. However, the mortality in mosquitoes infected with more than 10 Mf increased gradually from day 5 through day 14. The mortality rate increased more rapidly in mosquitoes fed on blood that had a higher microfilarial density.

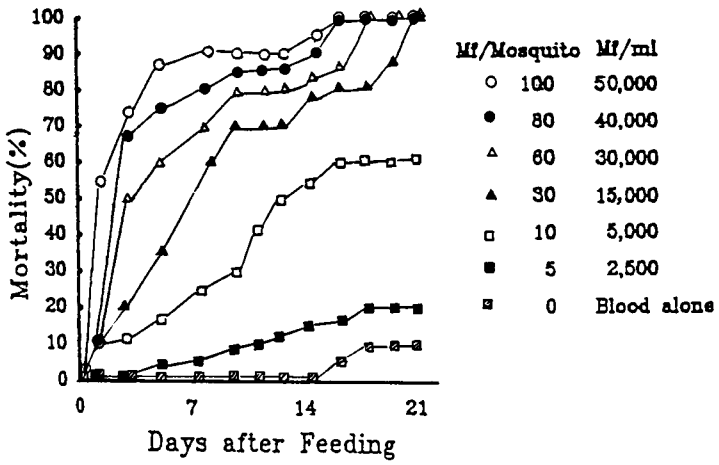

Fig. 2. Mortality rate of female *Aedes albopictus* (Oahu strain) after feeding with different concentration of Mf.

The number of Mf retained from the midgut was examined using mosquitoes fed either on blood from the infected dog directly or infected blood from the feeding apparatus. A 35 % proportion of Mf ingested by mosquitoes from a dog was retained in the midgut after 1 hr which decreased to 10 % 6h after infection, whereas 22 % of Mf ingested from the feeding apparatus were still present in the midgut 6 h after (Table 1). This suggested that Mf ingested from an apparatus may be released from the midgut more slowly than Mf ingested from the infected blood of a dog.

Table 1. Time course of larvae of Mf *D. immitis* present in the midgut of *Aedes albopictus* Oahu strain after feeding on microfilarial blood.

Feeding source	Hour post feeding				
	Ø	1	2	4	6
Apparatus	68.6±3.3*	3Ø.6±2.9	23.8±2.8 (45%)**	2Ø.4±1.4 (35%)	14.9±1.4 (39%)
Infected dog	52.8±3.2	18.2±2.5	11.4±1.9 (35%)	1Ø.4±1.3 (22%)	2.5±1.1 (2Ø%)

* Mean number ± standard deviation of Mf per mosquito obtained from 1Ø mosquitoes.

** Percentage of the Mf number obtained immediately after feeding (Ø h).

Table 2. Development of *D. immitis* Mf to the third-stage larvae in *Aedes albopictus* Oahu strain under different feeding conditions*.

Feeding Source	No. of Mf Ingested			No. of L3/Mosq.		
	Mean	Max.	Min.	Mean	Max.	Min.
Apparatus	58.3	8Ø	2Ø	36.6	45	12
Infected dog	51.5	65	15	25.8	3Ø	8

*Mf density in the donor dog was 35,ØØØ Mf/ml in both feeding methods.

Table 2 shows the development of *D. immitis* larvae in the *Aedes albopictus* Oahu strain by the two different feeding methods. Mosquitoes ingested similar number of Mf that developed to the third-stage larvae in a similar ratio, indicating no significant difference between these two feeding methods.

Figure 3 shows the total number of developing larvae in 10 infected mosquitoes that fed on infected blood at a density of 35,000 Mf/ml. The mean number of the first-stage larvae on day 1 was 70, which decreased by day 5. Ten second-stage larvae were detected on day 8 in the Malpighian tubules, which increased to 25 by day 10 and then decreased to zero by day 12. Some second-stage larvae were detected in the thorax muscles from day 9 to 12 with a mean number of 5-8, which decreased to zero by day 12. Third-stage larvae were observed on day 9, with the mean number of 11 per mosquito, which increased to a maximum of 31.2 on day 14. In both methods, mosquitoes ingested similar number of Mf that developed to the third stage in a similar ratio, indicating no significant differences between these two feeding methods.

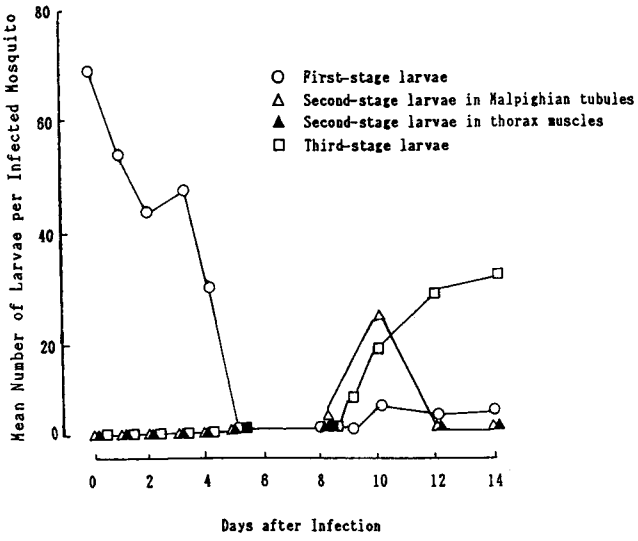

Fig. 3. Time course of the mean number of larvae per infected mosquito.

After the Mf migrated from the midgut to the Malpighian tubules, they developed into the first stage larvae: type I, type II, type III and then the type IV (sausage-form). In the sausage-form, the larvae became shorter and broader than type III. In addition to these known characteristics, a small projection from the head of approximately 6-8 % of the sausage form was found on day 3 in type IV (Fig. 4A). The length of the projection observed on day 4 (Fig. 4B) was more than that observed on day 3. This projection switched back and forth during observation. This was not observed for any

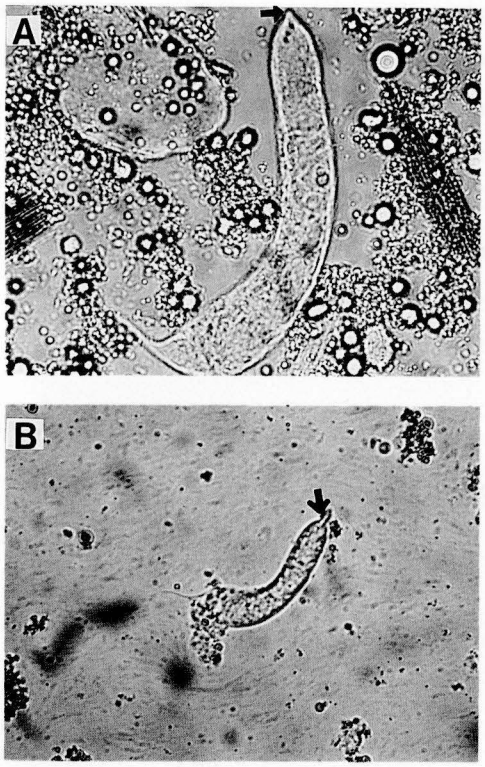

Fig. 4. Photomicrographs of (A) the first-stage larvae (sausage-form) 3 days after infection showing a small projection from the head (x 70 Mag.) and (B) the late form of the first-stage larvae (sausage form) 4 days after infection showing the increase of the projection part (x 300 Mag.).

stage of larvae in earlier studies (10). The mean length of the sausage-form larvae was 146 μm and the mean width was 19 μm (Table 3).

From days 9 to 12, the larvae grew longer and more narrow than the sausage form, and the elastic movement increased in this 2nd stage. Some larvae were observed in the Malpighian tubules (Fig. 5A), the usual site of development, and some others were observed in the crushed thorax muscles of the mosquitoes (Fig. 5B).

The sheath of the larvae was observed in the haemocoel of the abdominal part. We also observed second-stage larvae coming out of the anterior end of the sheath. Furthermore, microscopical observation of sections of the thorax tissues stained with HE revealed second-stage larvae (Fig. 5C), indicating that these second-stage larvae did not migrate from the Malpighian tubules on the dissection of mosquitoes. As this stage, the larvae in the Malpighian tubules were 21.6 μm long and 10 μm wide (Table 3).

Table 3. Measurement of the length and width of *D. immitis* Mf and larvae in *Aedes albopictus* mosquitoes.

	Microfilaria	First stage larvae				Second stage larvae	Third stage larvae
		Type I	Type II	Type III	Type IV		
Length	254.3±4.2* (304-313)**	244.3±3.2	215.7±1.1 (150-300)	172.6±1.2	146.2±1.5	834.1±4.6 (460-1100)	1255.3±4.1 (915-1097)
Width	4.8±0.2 (6.3-6.9)	6.5±0.1	9.7±0.9 (5-60)	11.1±1.9	18.8±8.4	35.0±1.4 (60-70)	19.8±0.8 (26)

*Mean±standard deviation (μm).

**Data from the review of Ohishi (15).

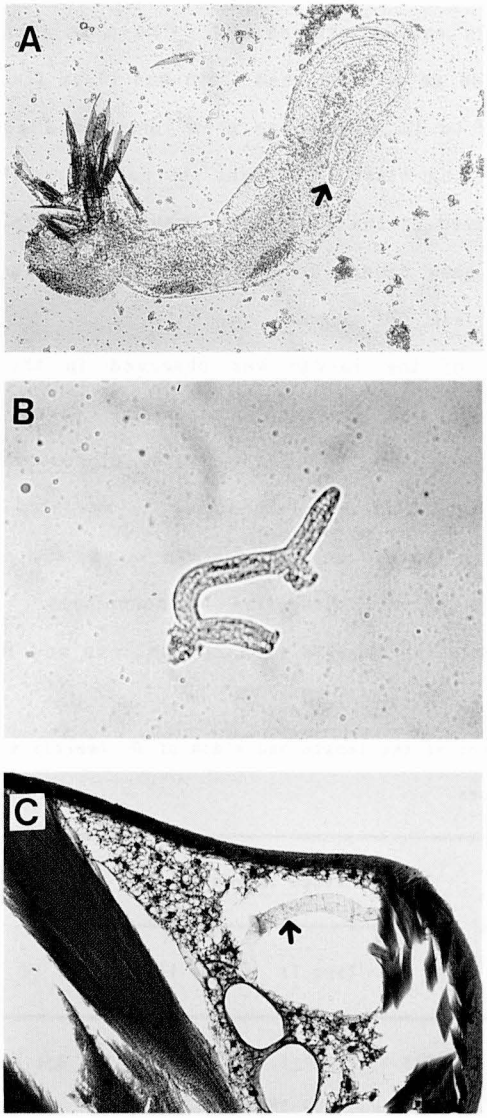

Fig. 5. Photomicrographs of (A) the second-stage larva of *D. immitis* in Malpighian tubule of *Aedes albopictus* Oahu strain 9 days after infection (x 300 Mag), (B) the second-stage larva in the crushed thorax muscle of *Aedes albopictus* Oahu strain 9 days after infection (x 300 Mag.), and (C) the second-stage larva in the thorax tissues in 10th day after infection (HE) (x 300 Mag.).

Third-stage larvae detected from day 9 were observed mainly in the labium (Fig. 6A), occasionally in the crushed thorax muscles (Fig. 6B), and in the Malpighian tubules. At this stage, the larvae were 1255 μm long and 19.8 μm wide (Table 3).

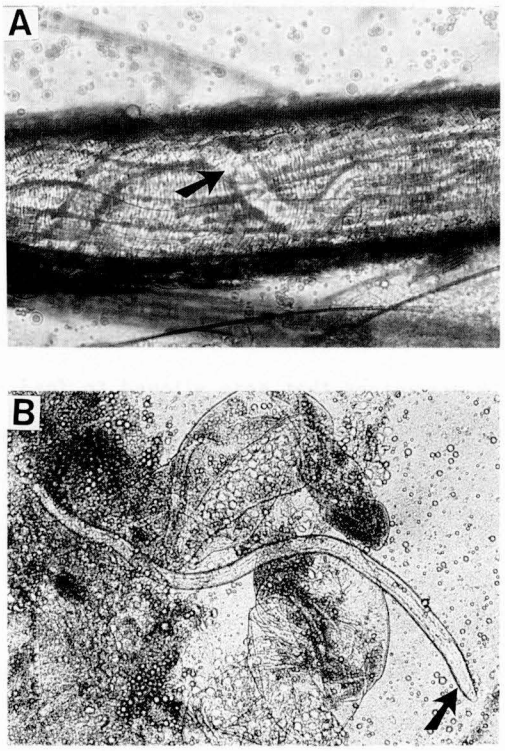

Fig. 6. Photomicrographs of (A) the third-stage larva in the labium of *Aedes albopictus* 14 days after infection and (B) the third-stage larva in the crushed thorax muscle of mosquito 10 days after infection.

DISCUSSION

Many researchers have checked different arthropods for the vector competency of *D. immitis*, and mosquitoes are the major intermediate host identified so far (9). A total of 86 mosquito species including *Aedes albopictus* have been reported as potential vectors of *D. immitis* as determined by observation of development into third-stage larvae under experimental and natural conditions (10). In this study, we confirmed that the Oahu strain of *Aedes albopictus* is susceptible to infection with *D. immitis*: Mf can develop in this host to the infective stage (26).

The density of the circulating *D. immitis* Mf varies over a 24 h period and are generally higher during the day (between 2 p.m and 10 p.m.) than during the night (3, 6). We examined the periodical changes by counting the Mf ingested by 20-30 mosquitoes that were allowed to feed on a dog every 4 h. Although the number of Mf appears to be affected by the blood meal size of each mosquito under this experimental condition, our result showed a consistent periodical pattern. It is generally accepted that the optimum number of Mf in the peripheral blood coincides with the peak time for feeding activity of the vector.

The number of Mf taken in by individual mosquitoes varied greatly, but appeared to depend on the density of Mf in the dog blood. After ingestion, the Mf migrated from the midgut to the Malpighian tubules, which generally occurs within an hour of ingestion. This represents one of the earliest signposts of parasite infectivity (10). Kershaw et al. (11) reviewed early literature indicating that mosquito vector of filarial worms ingested more Mf than those expected or that occasionally mosquitoes took in surprisingly large numbers of microfilariae. Results obtained in this study suggest that mosquitoes ingested a mean of 70 Mf in both feeding methods from a blood containing 35,000 Mf/ml, indicating that an expected number of Mf was ingested by mosquitoes.

In general, the degree of Mf penetration through the midgut depends on the number of Mf ingested. However, some other studies have shown that many viable Mf are retained in the midgut of several *Aedes* mosquito species (2, 9, 18), and the number of Mf ingested may be quite different from the number of Mf migrating into the Malpighian tubules. In our experiment, most of Mf escaped from the midgut within 6 h after feeding. Comparisons between the two feeding methods suggests that Mf ingested from dog blood may penetrate the midgut faster than Mf ingested from feeding apparatus.

Some of the developing pattern of *D. immitis* larvae observed in this study differed from those observed in previous studies (8, 13, 15, 17) in terms of (i) the presence of the second-stage larvae in thorax muscles and (ii) the presence of a small projection on the head part of the sausage form. Our study did not show that all second-stage larvae were present in the Malpighian tubules: some second-stage larvae were found in the crushed thorax muscles. Histological examination of sections of 30-40 infected mosquitoes from days 9 to 12, confirmed the presence of second-stage larvae in the thorax tissues. Although successful migration of some second- and third-stage larvae into the thorax muscles of mosquitoes seems to be accompanied by damage of the flight muscles, the mosquito activities were not affected, suggesting that the range of tissues invaded by these larvae might be limited.

The function of the small projection is not known. Since this projection was observed only in the sausage form and since this projection was very active in contrast to the poor activity of the whole body at this stage, the projection may have a role in its development to the second-stage. However, it should be noted that the projection was observed only in 6-8 % of the first-stage larvae.

The measurement of length and width of Mf and larvae at each stage (Table 3) showed a smaller size than those reported before (15). However,

examination of the adult worm after we sacrificed the dog used for all the present experiments indicated typical *D. immitis* morphology. It seems important to solve the reason why the *D. immitis* larvae in this mosquito strain showed different developing patterns from previous reports. Further studies will be needed to determine whether the development of *D. immitis* larvae depends upon a specific factor derived from the host mosquito.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Prof. Dr. H. Itoh for his support during the histopathological study and Dr. S. Uga (Kobe University School of Medicine) for his collaboration of sacrificing the dog to identify the filaria species. We also would like to express our sincere gratitude to Dr. I. Ohishi (Tokyo University of Agriculture and Technology) and Dr. O. Suenaga (Institute of Tropical Medicine, Nagasaki University) for their valuable advice and comments, Dr. H. Yamanishi (Kobe Gakuin Women's Junior College) for supplying the Oahu strain of *Aedes albopictus*.

REFERENCES

1. Bradley,T.J., and Nayar, J.K.:J. Parasitol. 1987.73. 1Ø35/1Ø43. An ultrastructural study of *Dirofilaria immitis*. infection in the Malpighian tubules of *Anopheles quadrimaculatus*.
2. Christensen,B.M.:Mosquito News. 1977. 37. 367/372. Laboratory studies on the development and transmission of *Dirofilaria immitis* by *Aedes trivittatus*.
3. Church, E.M., George, J.R. and Robson, D.S.: Cornell Vet. 1976. 66. 333/346. Analysis of the microfilarial periodicity of *Dirofilaria immitis* microfilariae.

4. Crans, W.J., and Fedaufer, M.F.: Pap. Annu.Conf. Mosq. Control Assoc. 1974. 42. 168. The likely mosquito vectors of dog heartworm in a coastal area of Southern New Jersey.

5. Grassi, B., and Noe,G.: Brit. Med. J. 1900. 2079. 1306/1307. The propagation of the filariae of the blood exclusively by means of the puncture of peculiar mosquitoes.

6. Grieve,R.B. and Lauria,S.: Acta Tropic. (Basel) 1983. 40. 121/127. Periodicity of *Dirofilaria immitis* microfilariae in canine and murine hosts.

7. Grieve,R.B.,Lok, J.B., and Glickman,L.T.: Epidem. Rev. 1983. Epidemiology of canine heartworm infection.

8. Kartman,L: Exp. Parasit. 1953. 27/78. Factors influencing infection of mosquitoes with *Dirofilaria immitis*(Leidy, 1856).

9. Kartman, L: J. Parasit.1953.39. 296/299. On the problem of non-infective microfilariae.

10. Kartman, L: J. Parasit.1953. 39.572/ 577. Effect of feeding mosquitoes upon dogs with differential microfilaremias.

11. Kershaw, W.E., Lavoipierre, M.M.J., and Chalmers, T.A. : Ann. of Trop. Med. and Parasitol. 1953. 47. 207/221. Studies on the intake of microfilaria by their insect vectors, their survival and their effect on the survival of their vectors. I. *Dirofilaria immitis* and *Aedes aegypti.*

12. Konishi,E: J. Med. Entomol. 1989. 26. 420/424. Susceptibility of *Aedes albopictus* and *Culex tritaeniorhynchus* (Diptera : Culicidae) collected in Miki City, Japan, to *Dirofilaria immitis* (Spirurida : Filariidae).

13. Ludlam, K.W., Jachowsk,L.A., and Otto,G.F: J. Am. Vet. Med. Assoc. 1970. 157.1354/1359. Potential vectors of *Dirofilaria immitis.*

14. Mayar, J.K. and Sauerman, D.M.: J.Insect Physiology 1975. 21.

1965/1975. Physiol. basis of host susceptibility of Florida mosquitoes to *Dirofilaria immitis*.

15. Ohishi, I.: Bun-eido, Tokyo, Japan (in Japanese). 1986. *Dirofilaria immitis*.

16. Rogers,J.S., and Newson,H.D.: Mosq. News. 1979. 39. 463/466. Comparisons of *Aedes hendersoni* and *Aedes triseriatus* as potential vectors of *Dirofilaria immitis*.

17. Suenaga, O.: Trop.Med. (in Japanese with English summary)1972. 14. 144/159. Studies on the filarial prevalence among dogs and the Mosquito vectors in Nagasaki City, Western Japan. 3. On the susceptibility of *Aedes albopictus* and *Culex pipiens molestus* to the larvae of *Dirofilaria immitis* in Nagasaki City.

18. Travis,B.V.: J. Parasitol. 1947. 33. 142/145. Relative efficiency of six species of mosquitoes from Guam, M.I., as developmental host for *Dirofilaria immitis*.

19. Wada,Y., Tsuda,Y., and Suenaga,O: Trop. Med. 1989. 31. 35/47. Transmission dynamics of *Dirofilaria immitis* in southwestern Japan.

2Ø. Wright, J.C., Hendrix, C.M., and Brown,R.G : J. Am. Vet. Med. Assoc. 1989. 194. 644/648. Dirofilariasis.,

21. Yamanishi, H., Konishi, E., and Matsumura, T.: Jpn.J. Sanit.Zool. 1984. 4. 361/365. Comparison of the susceptibility to Chikungunya virus on 2 strains of *Aedes albopictus*.

22. Zytoon, E., El-Belbasi, H., and Matsumura, T. : The 5th International Congress for Infectious Diseases. (Nairobi, Kenya) 1992. Mechanism of enhancement of arboviral multiplication in mosquitoes ingesting microfilaria of *Dirofilaria immitis* using histopathology and electron microscopy.

23. Zytoon, E., El-Belbasi, H., and Matsumura, T. : The 5th International Congress for Infectious Diseases. (Nairobi, Kenya) 1992. Transovarial transmission of chikungunya virus in *Aedes albopictus* mosquito of

concurrently ingesting *Dirofilaria* spp. microfilariae.

24. Zytoon, E., Konishi, E., and Matsumura, T. : The 13th International Congress for Tropical Medicine and Malaria. (Pattaya, Thailand) 1992. New development pattern of *Dirofilaria immitis* larvae in mosquitoes.

25. Zytoon, E., Itoh, H., Homma, M., El-Belbasi, H., and Matsumura, T. : The 13th International Congress for Tropical Medicine and Malaria. (Pattaya, Thailand) 1992. Mechanism of enhancement of arboviral multiplication in mosquitoes with artificial punctures in midgut.

26. Zytoon, E., El-Belbasi, H., Yamanishi H., and Matsumura, T : The 13th International Congress for Tropical Medicine and Malaria. (Pattaya,Thailand) 1992. Susceptibility of *Aedes albopictus* (Oahu Strain) to *Dirofilaria immitis*.