RELATIVE SUITABILITY OF *AEDES ALBOPICTUS* AND *AEDES AEGYPTI* IN NORTH CAROLINA TO SUPPORT DEVELOPMENT OF *DIROFILARIA IMMITIS*[1]

CHARLES S. APPERSON,[2] BARRY ENGBER[3] AND JAY F. LEVINE[4]

ABSTRACT. The relative suitability of two colonies established from local strains of *Aedes albopictus* (Wilmington and Rockingham) and a local (Raleigh) and laboratory (Liverpool) strain of *Ae. aegypti* to support development of *Dirofilaria immitis* was investigated. High levels of mortality occurred 1–2 days after mosquitoes fed on a heavily microfilaremic (28,617 microfilariae/ml) dog but not when mosquitoes were fed on a dog exhibiting a moderate microfilaremia (3,300 microfilariae/ml). At 15–16 days postfeeding, development of *D. immitis* to the third larval stage occurred to some extent in mosquitoes of all four strains. Microfilariae were only found in the Raleigh strain of *Ae. aegypti. Aedes albopictus* most frequently contained first and second stage larvae that were deteriorated which suggested that their development had been arrested. Third stage larvae were found most often in the Liverpool strain of *Ae. aegypti.* Colonies established from local strains of *Ae. albopictus* and *Ae. aegypti* do not appear to be suitable hosts of *D. immitis.*

INTRODUCTION

The discovery (Sprenger and Wuithiranyagool 1986) and probable widespread establishment of *Aedes albopictus* (Skuse) in the United States (Nawrocki and Hawley 1987) has prompted concern over the potential impact of this species on the public's health (Knudsen 1986, Moore 1986). *Aedes albopictus* has also been listed as a potential vector of canine heartworm (Ludlam et al. 1970).

Aedes albopictus has been collected from four sites in North Carolina in 1987 (Apperson and Engber, unpublished data) and three additional sites in 1988 (M. Slaff, personal communication); thus, it is highly likely that this species will become widely established in North Carolina. *Aedes aegypti* (Linn.) is already established throughout the Coastal Plain and Piedmont regions of North Carolina, and in some of these areas of North Carolina, canine infection with *Dirofilaria immitis* (Leidy) is widespread (Rowley 1977,[5] Butts 1979, Falls and Platt 1982). Although these mosquito species are not known to be natural hosts for *D. immitis*, they have been found to support the development of infective stage larvae in the laboratory (Kartman 1953, Keegan et al. 1967, Webber and Hawking 1955). Therefore, laboratory experiments were conducted to determine whether local populations of *Ae. albopictus* and *Ae. aegypti* from North Carolina were suitable hosts for *D. immitis.*

MATERIALS AND METHODS

Mosquito rearing and feeding procedures: Laboratory colonies of *Ae. albopictus* were established from several hundred larvae collected in June 1987 from tires in Rockingham County and in July 1987 from rain barrels in Carolina Beach near Wilmington, New Hanover County, North Carolina. These populations were in generation F_4 when tested. *Aedes aegypti* was collected in Raleigh, Wake County, in 1984 and has since been continuously maintained in the laboratory. The Liverpool strain of *Ae. aegypti* was obtained from Karen Snowden, College of Veterinary Medicine, North Carolina State University. This strain is a competent vector of *D. immitis* (Hendrix et al. 1986) and is commonly used in laboratory experiments involving transmission of filarial nematodes.

Details of rearing procedures for mosquito larvae have been described previously (Benzon and Apperson 1988). After pupation, mosquitoes were removed and placed in screened cages. A 10% sucrose solution was available to adults until 24 h prior to feeding when ca. 150 females of each species and strain were transferred to individual cardboard cartons.

Dogs of mixed breeding obtained from the Laboratory Animal Resources Facility at the College of Veterinary Medicine, North Carolina

---

[1] Paper No. 12090 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.

[2] Department of Entomology, Box 7613, North Carolina State University, Raleigh, NC 27695-7613.

[3] Vector Control Branch, North Carolina Division of Health Services, Raleigh, NC 27602.

[4] Department of Microbiology, Pathology and Parasitology, Box 8401, North Carolina State University, Raleigh, NC 27695-8401.

[5] Rowley, B. J. 1977. The prevalence of heartworm, *Dirofilaria immitis* (Leidy, 1856), infection in privately owned and free-ranging dogs in Wake, Durham, and Orange counties, North Carolina. M. S. thesis, North Carolina State University, Raleigh. 22 p.

State University were used.[6] A Knott's test (Altman and Yarbrough 1973) had been previously conducted on anticoagulated blood taken from each dog to determine its approximate microfilaremia. *Dirofilaria immitis* microfilariae were differentiated from microfilariae of other species based on their size and morphology (Falls and Platt 1982). Dogs with heavy and moderate microfilaremias were selected for our investigation. Before mosquitoes were allowed to feed, each dog was first anesthesized with Acepromazine and Numorphan (oxymorphone hydrochloride) administered intramuscularly, and then each carton was held against the dog's shaved midsection for 30–45 min. When mosquitoes were feeding, three 20-μl samples of blood were withdrawn from each dog and thick films were spread on a glass slide; subsequently, the microfilariae were counted after staining with Geimsa (10%). Microfilaremias averaged 28,617 ± 4,436 and 3,300 ± 522 microfilariae per ml for the first and second feeding trials, respectively. After feeding, approximately 75 engorged females of each species and strain were transferred to another cardboard carton that contained 10% sucrose. The cartons were examined twice daily and, dead females were removed and counted.

Dissection procedures: Females alive 15–16 days after feeding were dissected in saline solution (0.85% NaCl). The head, thorax and abdomen of each mosquito were separated, teased apart and examined at 150× for the various stages of *D. immitis*. A drop of acetic acid:saline solution (1:1, v:v) was added to the Malpighian tubules to solubilize uric acid crystals and clarify the tubules. Developmental stages of *D. immitis* were identified using descriptions of their size, shape and location given in Taylor (1960). The occurrence and location of juvenile *D. immitis* in each mosquito were recorded.

Statistical analysis: The daily percent mortalities and percent of females surviving to the end of the 15–16 day observation periods for the strains of each species were calculated, subjected to arcsine transformation and analyzed for independence by analysis of variance using the General Linear Models (GLM) procedure of SAS (1985). Percent mortality or survival of females was the dependent variable and, the feeding trial, species strain, strain replicate, and observation day were used as independent variables in the model statements. Significantly different mean values were separated using the Student-Newman-Keuhls option of GLM (SAS 1985). Unless stated otherwise, statistical tests were carried out at $P \leq 0.05$.

[6] All dogs used in this study were given humane care in accordance with recommendations set forth in the *Guide for the care and use of laboratory animals* (NIH Publ. No. 78-23) and the regulations of the USDA Animal Welfare Act. The College of Veterinary Medicine at North Carolina State University is AALAC certified.

RESULTS

Mosquito mortality and survival: Daily mortality of *Ae. albopictus* and *Ae. aegypti* after feeding on a dog heavily infected with *D. immitis* is presented in Fig. 1. High levels of mortality were observed for all strains on the first and/or second day following the feedings. For *Ae. albopictus*, highest mortalities recorded occurred on the first and second day after feeding. These mortality levels varied from 30 to 54% and 34 to 50% for the replicates of the Wilmington and Rockingham strains, respectively. In contrast, the highest mortality of *Ae. aegypti* was observed on the second day after feeding. It was 53% for the Liverpool strain and it varied between 28 and 29% for both replicates of the Raleigh strain. Mortality levels for both strains of *Ae. albopictus* and the Raleigh strain of *Ae. aegypti* on day 1 postfeeding were significantly different ($P < 0.005$), but a comparison of the mean daily percent mortalities over the 15 day period did not reveal any significant difference among the four strains.

At the end of the 15 day period, low but comparable levels of survival were observed for mosquitoes that fed on the heavily infected dog relative to mosquitoes that fed on an uninfected dog (Table 1). Survival of females varied between a low of 8.2% for the Liverpool strain of *Ae. aegypti* to a high of 19.2% for the Raleigh strain of *Ae. aegypti*. Differences in the percent survival of females of all four strains that fed on the infected dog were found to be insignificant ($P > 0.4$).

For the second feeding trial, highest mortalities were observed during the first four days postfeeding for all strains except the Liverpool strain of *Ae. aegypti* (Fig. 2). For these days, levels of mortality averaged 20.7, 27.6 and 22.1% for the Wilmington and Rockingham strains of *Ae. albopictus* and the Raleigh strain of *Ae. aegypti*, respectively, but only 9.3% for the Liverpool strain of *Ae. aegypti*. Differences between the Liverpool strain of *Ae. aegypti* and other *Aedes* mosquitoes for the total percent mortality that occurred on days 1–4 postfeeding were significant ($P < 0.001$). In contrast, mortality for the last four days of the 16 day observation period averaged 6.6, 8.3 and 10% for the Wilmington and Rockingham strains of *Ae. albopictus* and Raleigh strain of *Ae. aegypti*, respectively, but 19.9% for the Liverpool strain of *Ae.*

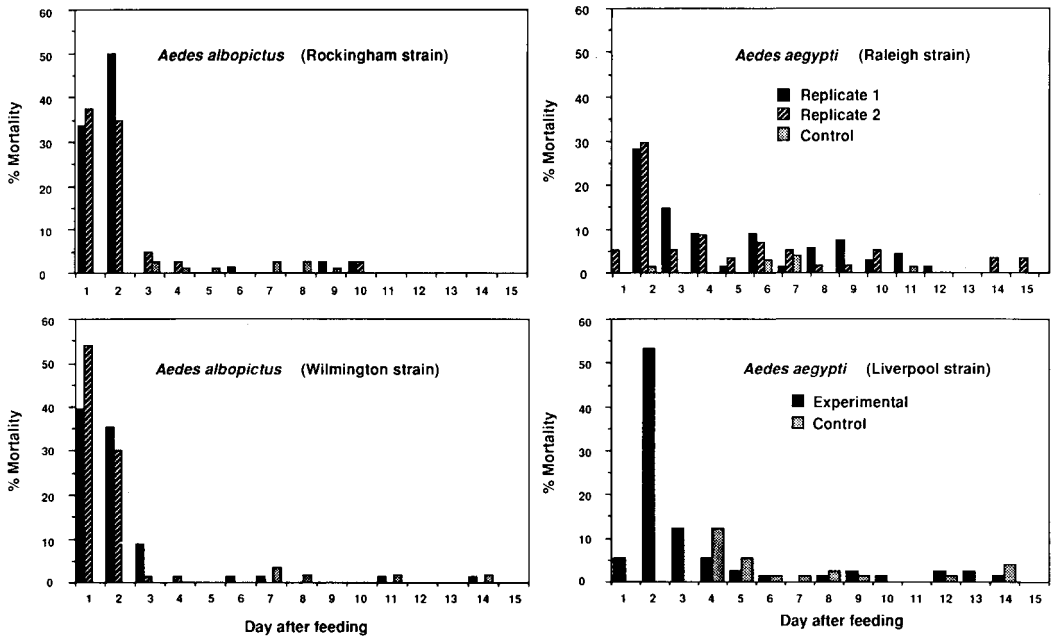

Fig. 1. Daily mortality of *Aedes albopictus* and *Aedes aegypti* after feeding on a dog that was heavily infected (28,617 microfilariae/ml) with *Dirofilaria immitis*.

Table 1. Survival of *Aedes albopictus* and *Aedes aegypti*, 15–16 days after feeding on dogs infected with *Dirofilaria immitis*.

Mosquito species and strain		1st feeding trial (28,617 microfilariae/ml)		2nd feeding trial (3,300 microfilariae/ml)	
		No. surviving/ no. fed	Percent surviving[a]	No. surviving/ no. fed	Percent surviving[a]
Aedes albopictus					
Wilmington strain					
Replicate	1	8/68	11.7	35/64	54.7
Replicate	2	10/76	13.2	49/73	67.1
Control[b]	—[c]	53/58	91.4	52/67	77.6
Rockingham strain					
Replicate	1	8/74	10.8	29/63	46.0
Replicate	2	7/40	17.5	38/64	59.4
Control	—	67/82	81.7	58/70	82.9
Aedes aegypti					
Raleigh strain					
Replicate	1	9/67	13.4	36/68	52.9
Replicate	2	11/57	19.3	36/72	50.0
Control	—	65/72	90.3	65/74	87.8
Liverpool strain					
Replicate	1	6/73	8.2	34/76	44.7
Replicate	2	—	—	38/75	50.7
Control	—	52/74	70.3	64/70	91.4

[a] Within each trial, differences in percent survival for mosquitoes fed on an infected dog were not significant ($P > 0.25$). Between the trials, differences in the percent survival for mosquitoes fed on infected dogs were significant ($P < 0.001$).

[b] Mosquitoes in the control groups fed on dogs that were not infected with *D. immitis*.

[c] Not replicated.

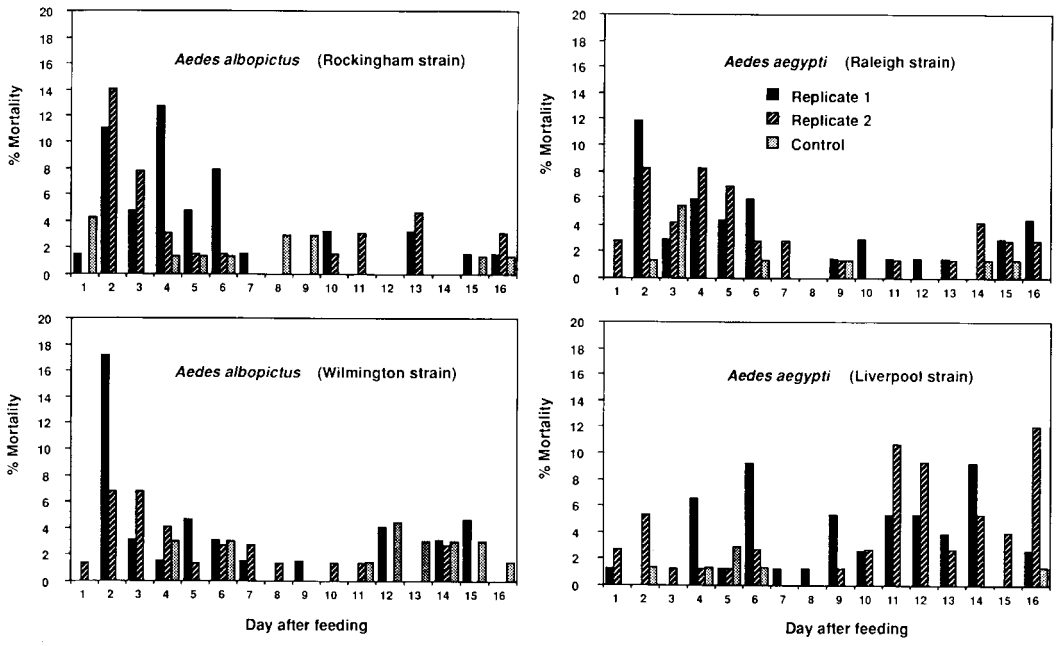

Fig. 2. Daily mortality of *Aedes albopictus* and *Aedes aegypti* after feeding on a dog that was moderately infected (3,300 microfilariae/ml) with *Dirofilaria immitis*.

aegypti. These differences in total percent mortality for days 13–16 were significant ($P < 0.001$). Generally, the daily percent mortality of mosquitoes was low to moderate throughout the 16 day observation period. For *Ae. albopictus*, daily mortality ranged from 7 to 17% for the Wilmington strain and from 11 to 14% for the Rockingham strain. Similarly, the death rate varied from 8 to 11% for the Raleigh strain and from 9 to 12% for the Liverpool strain of *Ae. aegypti*. Mean daily levels of mortality were not significantly different among the four mosquito strains for the 16 day period. In the second feeding trial, mean daily percent mortalities were significantly lower ($P < 0.001$) than in the first feeding trial.

Percent survival of females of the four strains that fed on the moderately filaremic dog varied from a low of 44.7% for the Liverpool strain of *Ae. aegypti* to a high of 67.1% for the Wilmington strain of *Ae. albopictus* (Table 1). In the second feeding trial, differences in the percent survival of females among the four strains were not significant ($P > 0.25$), but survival of females was significantly higher ($P < 0.001$) than in the first trial.

Development of Dirofilaria immitis: Infection rates and, the location and developmental stages of *D. immitis* in *Ae. albopictus* and *Ae. aegypti* at 15–16 days after feeding are presented in Table 2. After entry into the Malpighian tubules, some development of microfilariae occurred in all mosquito strains in both feeding trials. In *Ae. albopictus*, development of *D. immitis* was usually arrested at the L_1-L_2 stage; however, some L_3 were found in the Malpighian tubules. From their appearance, these larvae were dead. Only the Liverpool strain of *Ae. aegypti* consistently developed L_3 *D. immitis*. At the end of both trials, microfilariae were only found in the Raleigh strain of *Ae. aegypti*. For most mosquitoes of this strain dissected at the end of both feeding trials, *D. immitis* did not develop to L_3, but a L_3 was found in the head of one mosquito in each replicate of the Raleigh strain at the end of the second feeding trial. Encapsulated microfilariae and L_1 were occasionally found in mosquitoes of all strains except the Liverpool strain of *Ae. aegypti*.

DISCUSSION

High levels of mortality occurred 24–48 h after mosquitoes fed on the dog with high filaremia. The mortality was probably caused by the movement of large numbers of microfilariae from the midgut into the Malpighian tubules. Kartman (1953) and Buxton and Mullen (1981) found that 24 h after *Ae. aegypti* and *Ae. albopictus* had fed on a heartworm-infected dog, a large percentage of the specimens dissected contained microfilariae in the Malpighian tubules. Weiner and Bradley (1970) and Buxton and Mullen (1981) found that mortality of several strains of *Ae. aegypti* was highest 1–3 days after feeding on heartworm-infected dogs. Encapsulation in

melanin and arrestment of larval development are mechanisms of refractoriness to *D. immitis* that occur to varying degrees in mosquitoes (Buxton and Mullen 1981, Christensen et al. 1984, Kartman 1953, Nayar and Sauerman 1975). Arrested development of larvae in the microfilariae stage has been found (Buxton and Mullen 1981, Kartman 1953, Nayar and Sauerman 1975) to be the more important mechanism of refractoriness for *Ae. aegypti*. Our results confirm these findings with the Raleigh strain of *Ae. aegypti* but not with *Ae. albopictus*.

Differences in the suitability of various populations of *Ae. aegypti* and *Ae. albopictus* to support development of L_3 *D. immitis* have been previously reported. In some investigations (Webber and Hawking 1955, Keegan et al. 1967, Chellappah and Chellappah 1968) a greater percentage of *Ae. aegypti* were found to have developed infective stage larvae, but in another laboratory study (Kartman 1953), *Ae. albopictus* was found to be a more suitable host of *D. immitis*. Buxton and Mullen (1981) investigated the comparative susceptibility of four strains of *Ae. aegypti* to infection by *D. immitis* and found substantial differences among mosquito strains in the proportion of ingested microfilariae that developed to the third larval stage. With strains of *Ae. aegypti* at 20 days postinfection, mosquitoes either contained microfilariae or mature larvae. Similar results were obtained in our investigation for the Raleigh strain but not for the Liverpool strain of *Ae. aegypti* or either strain of *Ae. albopictus*. Microfilariae were only found in the Raleigh strain of *Ae. aegypti* while mosquitoes of other strains most frequently contained L_1-L_2 that were deteriorated suggesting that these colonies are largely refractory to infection by *D. immitis*. Like Buxton and Mullen (1981), we found that mosquitoes of the Liverpool strain of *Ae. aegypti* developed L_3 more frequently than mosquitoes of other strains. It was noteworthy that in the second feeding trial the highest mortality of the Liverpool strain of *Ae. aegypti* occurred toward the end of the 16 day observation period when L_3 were found migrating from the Malpighian tubules to the head. It is ironic that production of L_3 was greater in the second feeding trial when mosquitoes ingested lower numbers of microfilariae. However, it is well established that entry into the Malpighian tubules of large numbers of microfilariae results in high mortality of mosquitoes (Weiner and Bradley 1970, Hamilton and Bradley 1979). Ingestion of low numbers of microfilariae is more conducive to mosquito survival and L_3 production.

The extent to which these mosquitoes would use dogs as hosts in North Carolina is unknown, but Tempelis et al. (1970) found both species of

Table 2. Infection rates and number of *Aedes albopictus* and *Aedes aegypti* with developmental stages of *Dirofilaria immitis*, 15–16 days after feeding on heartworm-infected dogs.

Mosquito species and strain	Replicate	No. infected/ no. dissected	Malpighian tubules			L_3 larva				
			Microfilariae	L_1	L_2	No. of mosquitoes	Malpighian tubules	Abdomen	Thorax	Head
1st feeding trial (28,617 microfilariae/ml)										
Aedes albopictus										
Wilmington	1	7/8	0	6	2	0	0	0	0	0
Wilmington	2	8/10	0	7	2	1	1	0	0	0
Rockingham	1	5/8	0	3	1	1	1	0	0	0
Rockingham	2	7/7	0	4	3	0	0	0	0	0
Aedes aegypti										
Raleigh	1	7/9	7	7	0	0	0	0	0	0
Raleigh	2	8/11	7	8	0	0	0	0	0	0
Liverpool	—	6/6	0	0	5	5	4	3	0	3
2nd feeding trial (3,300 microfilariae/ml)										
Aedes albopictus										
Wilmington	1	22/35	0	18	4	1	1	0	1	1
Wilmington	2	36/49	0	30	2	4	1	1	2	2
Rockingham	1	26/27	0	23	5	2	1	0	0	1
Rockingham	2	30/38	0	30	1	0	0	0	0	0
Aedes aegypti										
Raleigh	1	21/36	20	0	0	1	0	0	0	1
Raleigh	2	18/36	17	0	0	1	0	0	0	1
Liverpool	1	29/34	0	6	8	21	4	7	11	19
Liverpool	2	31/38	0	2	8	27	6	9	14	22

Aedes to feed on a broad range of mammals including dogs. In our investigation, the finding of L_3 in some females of both strains of *Ae. albopictus* and the Raleigh strain of *Ae. aegypti* indicated they can support development of *D. immitis*. However, L_3 were found in only a few mosquitoes (Table 2) which suggests that the suitability of these local strains of *Aedes* as hosts, and their consequent vector potential, is low.

ACKNOWLEDGMENTS

Technical assistance provided by Gene Powell is appreciated. Grover Miller, Bea Parker and Mike Fletcher read the manuscript and made some suggestions that improved it.

REFERENCES CITED

Altman, N. H. and B. Yarbrough. 1973. A rapid screening program for the diagnosis of *Dirofilaria immitis* infections in dogs. Lab. Anim. Sci. 23:701–703.

Benzon, G. L. and C. S. Apperson. 1988. Reexamination of chemically mediated oviposition behavior in *Aedes aegypti* (L.) (Diptera:Culicidae). J. Med. Entomol. 25:158–164.

Butts, J. A. 1979. Survey of *Dirofilaria immitis* in Mecklenburg County, North Carolina. J. Am. Vet. Med. Assoc. 174:1088–1089.

Buxton, B. A. and G. R. Mullen. 1981. Comparative susceptibility of four strains of *Aedes aegypti* (Diptera: Culicidae) to infection with *Dirofilaria immitis*. J. Med. Entomol. 18:434–440.

Chellappah, W. T. and G. R. Chellappah, Jr. 1968. Susceptibility of four common Singapore mosquitoes to *Dirofilaria immitis* Leidy. J. Med. Entomol. 5;358–361.

Christensen, B. M., D. R. Sutherland and L. N. Gleason. 1984. Defense reactions of mosquitoes to filarial worms: comparative studies on the response of three different mosquitoes to inoculated *Brugia pahangi* and *Dirofilaria immitis* microfilariae. J. Invertebr. Pathol. 44:267–274.

Falls, R. K. and R. R. Platt. 1982. Survey for heartworm. *Dirofilaria immitis* and *Dipetalonema reconditum* (Nematoda: Filarioidea) in dogs from Virginia and North Carolina. Am. J. Vet. Res. 43:738–739.

Hamilton, D. R. and R. E. Bradley. 1979. Observations on the early death experienced by *Dirofilaria immitis*-infected mosquitoes. J. Med. Entomol. 3:305–306.

Hendrix, C. M., C. J. Brunner and L. K. Bellamy. 1986. Natural transmission of *Dirofilaria immitis* by *Aedes aegypti*. J. Am. Mosq. Control Assoc. 2:48–51.

Kartman, L. 1953. Factors influencing the infection of the mosquito with *Dirofilaria immitis* (Leidy 1856). Exp. Parasitol. 2:276–280.

Keegan, H. I., W. W. Betchley, T. B. Haberkorn, A. Y. Nakasone, H. Sugiyama and R. J. Warne. 1967. Laboratory and field studies of some entomological aspects of the canine dirofilariasis problem in Japan. Jap. J. Sanit. Zool. 18:6–13.

Knudsen, A. B. 1986. The significance of the introduction of *Aedes albopictus* into the southeastern United States with implications for the Caribbean, and perspectives of the Pan American Health Organization. J. Am. Mosq. Control Assoc. 2:420–423.

Ludlam, K. W., L. A. Jachowski and G. F. Otto. 1970. Potential vectors of *Dirofilaria immitis*. J. Am. Vet. Assoc. 157:1354–1359.

Moore, C. G. 1986. The Centers for Disease Control's perspective of the introductions of *Aedes albopictus* into the United States. J. Am. Mosq. Control Assoc. 2:416–417.

Nawrocki, S. J. and W. A. Hawley. 1987. Estimation of the northern limits of distribution of *Aedes albopictus* in North America. J. Am. Mosq. Control Assoc. 3:314–317.

Nayar, J. K. and D. M. Sauerman, Jr. 1975. Physiological basis of host susceptibility of Florida mosquitoes to *Dirofilaria immitis*. J. Insect Physiol. 21:1965–1975.

SAS Institute Inc. 1985. SAS user's guide: Statistics, version 5 edition. Cary, NC.

Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. J. Am. Mosq. Control Assoc. 2:217–219.

Taylor, A. E. R. 1960. The development of *Dirofilaria immitis* in the mosquito *Aedes aegypti*. J. Helminthol. 34:27–39.

Tempelis, C. H., R. O. Hayes, A. D. Hess and W. C. Reeves. 1970. Blood-feeding habits of four species of mosquito found in Hawaii. Am. J. Trop. Med. Hyg. 19:335–341.

Webber, W. A. and F. Hawking. 1955. Experimental maintenance of *Dirofilaria repens* and *D. immitis* in dog. Exp. Parasitol. 4:143–164.

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