RESEARCH NOTES

COMPARATIVE SUSCEPTIBILITY OF TWO STRAINS OF *ANOPHELES BARBIROSTRIS* VAN DER WULP 1884 (DIPTERA: CULICIDAE) TO INFECTION WITH *BRUGIA MALAYI*

Anopheles (Anopheles) barbirostris species group has been long time incriminated as the natural vectors of malaria and filariasis in Southeast Asia. At least three species of this group, i.e., *An. barbirostris*, *An. campestris* and *An. donaldi* are of medical importance (Reid, 1968. *Stud. Inst. Med. Res. Malaya. 31*: 117). These three species have also been recorded as the suspected vectors of malaria and Brugian filariasis in southern Thailand (Reid, 1968, *Stud. Inst. Med. Res. Malaya., 31*: 117 and Harinasuta *et al.*, 1976. *Southeast Asian J. Trop. Med. Pub. Hlth., 7* : 645).

Recently, evidence from morphologic, cross-mating, and cytogenetic studies have revealed that at least two strains of *An. barbirostris* exist and exhibited possible presence of a species complex. These are *An.barbirostris* Chumphon strain which is *campestris*-like in adult characters and Chon Buri strain which is typical adult *barbirostris* in all characters (Choochote *et al.*, 1983. *Southeast Asian J. Trop. Med. Pub. Hlth. 14*: 204). The studies reported herein described the experiments on the susceptibility of two strains of *An. barbirostris* to subperiodic *Brugia malayi*.

Adult engorged females of two strains of *An.barbirostris*, the same strains as studied by Choochote *et al.*, 1983. (*Southeast Asian. J. Trop. Med. Pub. Hlth., 14*: 204) were collected from baited water buffaloes in Bang Luke Canton, Chumphon Province and Kao Mai Kaew, Chon Buri Province of Southern and Central Thailand respectively. These two strains were successfully reared in the laboratory, Chumphon strain for 56 generations and Chon Buri strain for 43 generations, by induced mating technique (Ow Yang *et al.*, 1963. *Mosquito News, 23*: 24). Seven-day-old mosquitoes of both strains of *An. barbirostris* and *Aedes togoi* Taiwan strain, vector of periodic and subperiodic strains of *B. malayi* (Ramachandran *et al.*, 1963. *Ann. Trop. Med. Parasit., 57*: 443) were used. All three populations of mosquitoes were allowed to feed on cat infected with nocturnally subperiodic *B.malayi*. The origin of the parasite was from a 20-year old woman, a resident of Bang Paw District, Narathiwat Province and experimentally infected to domestic cats and are now kept in the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University Bangkok. Fourteen days after the infected blood meal, the mosquitoes were dissected in normal saline solution and examined under a dissecting microscope. The number of mosquitoes with one or more infective larvae in any part of the body (head, thorax and abdomen) were recorded. The infective rate of *Ae.togoi*(Taiwan strain) and *An. barbirostris* (Chumphon and Chon Buri strains) to subperiodic *B.malayi* were 30.9%, 27.7% and 18.9% respectively. The results suggested that *Ae.togoi* (Taiwan strain) and *An. barbirostris* (Chumphon and Chon Buri strains) have similar competency as laboratory vector for subperiodic *B.malayi* (Table 1, $X_2^2 = 1.56$, $p > 0.01$). It was also found that more than 50% of the infective larvae could migrate out from the thoracic muscle to the proboscis, head and abdomen and behaved similarly in these three mosquito populations (Table 2).

Table 1

The infection rate and density load in *Ae. togoi* and *An.barbirostris* (2 strains) 14 days after feeding on cat infected with subperiodic *B. malayi*.

Species and strains of mosquitoes	Mf. density per c.mm.	No. dissected	No.* infected (%)	Average No. larvae per mosq.	Range of larve per. mosq.
Ae. togoi, Taiwan strain	1.8	42	13 (30.9)	3.69	1-7
An .barbirostris, Chumphon strain	1.8	47	13 (27.7)	2.15	1-9
An. barbirostris, Chon Buri strain	1.8	37	7 (18.9)	2.29	1-6

* $X_2^2 = 1.56$, $p > 0.01$.

Table 2

The distribution of infective larvae of *B. malayi* in head, thorax and abdomen of *Ae. togoi* and *An.barbirostris*.

Species and strains of mosquitoes	No. of infective larvae found in			
	Head (%)	Thorax (%)	Abdomen (%)	Total
Ae. togoi, Taiwan strain	38 (79.2)	4 (8.3)	6 (12.5)	48
An. barbirostris, Chumphon strain	12 (42.9)	9 (32.1)	7 (25.0)	28
An. barbirostris, Chon Buri strain	9 (56.2)	1 (6.2)	6 (37.5)	16

It is interesting to note that *An. barbirostris* in Pahang, Malaysia was refractory to infection with subperiodic *B. malayi* (Poynton and Hodgkin, 1938. *Bull. Inst. Med. Res. F.M.S.*, *1*:1 and Wharton, 1960. *Ann. Trop. Med. Parasit.*, *54*:78) whereas *An. barbirostris* strain from Thailand was highly susceptible. The reason may be host-parasite specificity. Even though there is evidence of these two strains of *An. barbirostris* are genetically isolated there is similarity in the susceptibility to infection. The *An. barbirostris* Chumphon strain which is *campestris*-like in adult characters tend to be more susceptible to subperiodic *B. malayi* than *An. barbirostris* which is typical adult *barbirostris* in all characters. Another interesting point is the incorrect field identification betweeen *An. barbirostris* which is *campestris*-like and *An. campestris*, because of the difficulty to confirm the developmental stages (Harrison and Scanlon, 1975. *Contrib. Am. Entomol. Inst.*, *12*:78). The latter is the major natural vector of malaria and periodic form of Brugian filariasis in Malaysia and it is probably also a minor vector of both diseases in Thailand (Reid, 1968 : *Stud. Inst. Med. Res. Malaya.*, *31*:117). Therefore, further studies on natural vector competence and behavioral traits of these two types of mosquitoes are needed in order to elucidate the type (s) responsible for the transmission of the diseases in nature.

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LABORATORY STUDIES ON THE CONTROL OF *MANSONIA* LARVAE BY SOME BIO-AGENTS

Mansonia mosquitoes, the main vector of *Brugia malayi* filariasis are also vectors of *B. pahangi* and *Dirofilaria* in both wild and domestic animals. A recent control trial with Abate (Gass *et al.*, in press) showed that the larvicide quickly losses its activity in the natural breeding habitats of this vector and that repeated applications were required to suppress the mosquito population making it an expensive measure. To find cheaper alternative control measures, some bioagents were tested against *Mansonia* larvae in the laboratory.

A mermithid nematode, *Romanomermis culicivorax* was tested against larvae of *Mansonia uniformis* and *M. annulifera*. Various instars of mosquito larvae, laboratory bred, were exposed to preparasitic stage of *R. culicivorax* at the host/parasite ratio of 1:20 in *Mansonia* larval media (infusion of 1.25 gm of guinea pig dung in 1 litre of tap water). *Pistia stratiotes* roots were used as the air source for *Mansonia* larvae. Twenty four hours later, larvae samples were dissected to examine whether they were infected by the parasite.

From the infection rate in *Mansonia* larvae (Table 1) it was found that *M. annulifera* larvae were more susceptible to *R. culicivorax* than *M. uniformis*. The results also show that the infection rate depended on the stage of development of the mosquito larvae. The older stages became less susceptible (Petersen and Willis, 1970. *J. Econ. Entomol.*, *63*: 175), and was probably due to the thickness of the larval cuticle which retarded the penetrations of the preparasitic stage of the parasite. However, in nature with mixed larval stages, it was reported that infection levels of first instar were significantly lower than second or third instar (Petersen, 1981. *J. Nematol.*, *13*: 228).

Table 1

Susceptibility of larval stages of *Mansonia* exposed to *Romanomermis culicivorax* at the host/parasite ratio of 1: 20.

Mosquito species Larval stage	Infection rate
M. annulifera	
1	80.0
2	58.1
3	0
4	0
M. uniformis	
1	66.7
2	49.7
3	29.0
4	0

The aquatic stage of some insects were also tested as bio-agents for the control of *M. uniformis* larvae in the laboratory. These were larvae of *Toxorhynchitis splendens*, naiads of dragonflies *(Diplacodes trivialis)* and larvae of water scavenger beetles *(Hydrophilus* sp.*)*.

The predators were confined with a number of third or fourth instar larvae of *M. uniformis* in tap water, with *Pistia stratiotes* as the air source for the larvae. The number of larvae that were taken up by the predators were recorded daily. The predator efficiency (number of mosquito larvae that were taken up/predator/day) is shown in Table 2.

The results show that under experimental conditions, the larvae of *Hydrophilus* sp. were the most effective predators of *M. uniformis* larvae. This predator however is rarely found

Table 2

Predator efficiency of insect predators on third or fourth instar larvae of *Mansonia uniformis*.

Insect predator	Predator efficiency
Toxorhynchitis splendens (larva)	2.9
Diplacodes trivialis (naiad)	11.3
Hydrophilus sp. (larva)	19.0

in natural breeding places of *Mansonia* mosquitoes. Therefore, naiads of dragonflies would be more appropriate as bio-agent for the control of *Mansonia*, as they share the same larval habitats and are often found resting on the roots of aquatic vegetation.

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DINOBDELLA FEROX (ANNELIDA: HIRUDINEA) INFESTATION OF THE NASO-PHARYNX

Leeches constitute a constant threat to outdoor life. Infestations on the boby surface have been reported frequently but infection on other sites of the body are relatively rare. Previous reports relate to infestations of the rectum (Smythies, 1953, *J. Bombay Nat. Hist.*, *51*:957), respiratory channel (Chauduri, 1971, *J. Indian Med. Assoc.*, *6*: 234; Gerlach and Gerlach, 1975, *Laryng., Rhino., Oto.*, *54*:123), vagina (Malik, 1970, *J. Pakistan Med. Assoc.*, *20*:227; Tur'Ani, 1974, *Jordan Med. J.*, *9*:52), excretory passage (Mukerjee, 1974, *J. Indian Med. Assoc.*, *63*:284).

More common are apparently infestations of the naso-pharyngeal region as demonstrated by Keegan *et al.*, (1970, *Amer. J. Trop. Med. Hyg.*, *19*:1029) and Rehman (1972, *J. Pakistan Med. Assoc.*, *22*:237). The present report relates to similar infestations from Indonesia whereby two specimens of *Dinobdella ferox* were recovered from two patients at two separate occasions. One specimen, in a state of full engorgement, 5 cm long and 1 cm wide, was secured through forcefully pulling the parasite from the nasal wall. The patient (female, 4 years) suffered from a week-long epistaxis. The second specimen (Fig. 1) also fully engorged, was reported to be secured from the naso-pharyngeal region of a boy (age unknown). No information was received as to the exact site of attachment, the clinical picture of the infestation as well as how the specimen was recovered. The patient was reported to have played in a river 4-5 months prior to seeking medical assistance.

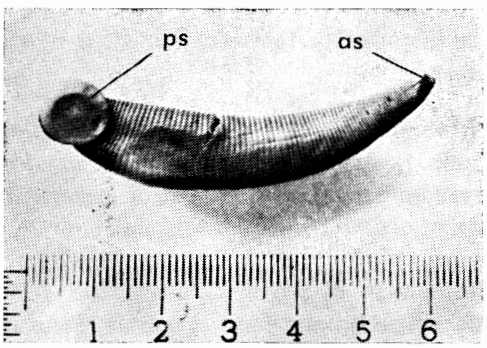

Fig. 1—Ventral view of *D. ferox*.
a s = anterior sucker and mouth
p s = posterior sucker

D. ferox is not a sole parasite of human beings. The same species was recovered from the nasal cavity of an Orang Utan, *Pongo*

pygmeus (Rijksen, 1978, *Med. Landb. Hoogesch Wageningen, 78*:420), a rhesus monkey, *Macaca mulatta* (Kagei, 1973, *Nittai, 8*:1) and a Taiwan monkey, *Macaca cyclopsis* (Pryor *et al.*, 1970, *J. Amer. Vet. Med. Assoc., 157*: 1926). Harding & More (1927, *Fauna Brit. Ind. Hirudinea* : 175) has long noticed the association of this species with cattle.

This species is a slow feeder and for that matter needs to be associated for long periods with its host, lasting for several days up to several weeks. Though clearly a parasite of terrestrial animals, circumstantial evidence indicate that the parasite must have entered the body of its host during its aquatic stage and when its size was still unnoticeable. In addition to blood loss, no other reports are available as to the harmful effect of its infestation. In monkey, *D. ferox* may produce chronic light inflammation with excessive mucous discharge (Pryor *et al.*, *op. cit.*).

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