MALAYAN FILARIASIS STUDIES IN KENDARI REGENCY, SOUTHEAST SULAWESI, INDONESIA II :

Surveillance of mosquitoes with reference to two *Anopheles* vector species

Bahang, Z.[1], L. Saafi[2], N. Bende[3], S. Kirnowardoyo[1], and Lim Boo Liat[4]

ABSTRACT

Studi nyamuk penular filariasis malayi pada empat desa endemis filariasis (Wawolemo, Pondidaha, Lalohao dan Teteona) di Kabupaten Kendari, Sulawesi Tenggara, telah dilakukan dari bulan November 1980 sampai Oktober 1982.

Nyamuk penular Brugia malayi di alam adalah Anopheles barbirostris dan An. nigerrimus sebagai penular yang potensial, serta tiga jenis dari marga Mansonia. Kepadatan bulanan An. barbirostris dan An. nigerrimus mempunyai keeratan hubungan yang positif dengan curah hujan, dengan puncak kepadatan pada bulan Juni. Nisbah nyamuk parous untuk kedua jenis nyamuk ini relatif rendah dan tidak mempunyai keeratan hubungan positif dengan kepadatannya. Kepadatan jentik dari kedua jenis nyamuk ini juga relatif rendah. Daur gonotrofik An. barbirostris di laboratorium berkisar antara 65 sampai 87 jam. An. barbirostris lebih cenderung antropofilik dari An. nigerrimus. Puncak kepadatan waktu menggigit orang dari An. barbirostris dimulai menjelang tengah malam hingga menjelang pagi hari, sedangkan An. nigerrimus aktif menggigit orang antara jam 19.00 sampai 22.00.

Nisbah infeksi alamiah dari larva Brugia pada An. barbirostris lebih tinggi daripada An. nigerrimus. Indeks infeksi buatan rata-rata 0,22 pada An. barbirostris dan 0,83 pada An. nigerrimus. Uji kerentanan DDT terhadap An. barbirostris memperlihatkan bahwa nyamuk ini rentan terhadap DDT.

INTRODUCTION

In our longitudinal studies of mosquito vector of malayan filariasis in the four villages at Kendari Regency, all mosquitoes species collected were identified and recorded. Among the mosquitoes examined, at least three *Mansonia* and two *Anopheles* species were found to be important vectors of the parasite. The present studies report the mosquito fauna distribution in the four villages with emphasis on the biology and seasonal variations of the two *Anopheles* vectors.

1 Sub-directorate Entomology, Communicable Disease Control, Ministry of Health, Jalan Percetakan Negara No. 29, Jakarta, Indonesia.
2 Province Health Office, Southeast Sulawesi, Kendari.
3 Regency Health Office, Southeast Sulawesi, Kendari.
4 WHO/Vector Biology and Control Research Unit-II, P.O. Box 302, Jakarta, Indonesia.

STUDY AREA

The four villages (Teteona, Lalohao, Pondidaha and Wawolemo) surveyed are in the district of Wawotobi, District of Kendari. The topography, ecology and socio-economic status of these villages have been presented by Arbain Joesoef et al.(1984).

MATERIALS AND METHODS

Entomological Studies

Vector density and infection rates were determined each month from November 1980 to October 1982. Six mosquito collectors were employed for each of the villages. Two were assigned for baited net collections, two each for indoor and outdoor. A large mosquito net mea-

suring 4 x 3 x 2 m with a meter side door were used. The two human bait were enclosed with a smaller protective net measuring 2 x 1 x 1 m inside the large net for indoor and outdoor. The large net was opened for 45 minutes each hour and then closed. The collectors, collected the mosquitoes landing inside the large net for 15 minutes, and the large net was opened for the next catch. Catching of net landing mosquitoes for indoors and outdoors started from 19.00 – 23.00 for one night in the 2nd weeks and two nights in the 4th week of each month at 20 man-hour for each of the villages.

Resting mosquitoes collections of indoors at night was made for 15 minutes each hour in a separate house by a collector in each village. Indoor morning resting collection was carried out by visiting 20 houses in each village for four man/hour.

Outdoor morning collection was made with a drop-net in bushes surrounding the villages for an hour. Thus, a total of 5 hours collection was carried in each of these methods for each village each month.

All night collections of mosquito landing on man for both indoors and outdoors at hourly intervals for 12 hours from 18.00 to 06.00 was made in one of the villages for once a month for 12 months.

Anopheles and *Mansonia* mosquitoes collected from these five collecting methods were individually dissected for presence of *Brugia* larvae. The head, thorax, gut and other parts of the abdomen were placed in four separate drops of saline. One ovary from the same mosquito was placed in a drop of distilled water for partial drying and sample age grading, by determining the presence or absence of tracheolar skeins in those specimens which had not developed ovaries beyond Christopher's mid stage II. The various parts of the mosquitoes in saline were each teased apart, covered with a cover-glass, and examined for filarial larvae under the low power compound microscopes. The numbers of stage I, II and III larvae in each part were determined and counted.

The recovered larvae were mounted in glycerine alcohol and taken to Jakarta to be identified. Identification of the *Brugia* larvae was based on keys by Nelson (1960) and Ramachandran (1970).

Anopheles barbirostris and *An. nigerrimus* pupae were collected from disused ponds surrounding the village which were raised to adult in the field laboratory. A total of 80 and 50 of the two species were raised, but only 60 of the former and 30 of the latter species were used for infectivity studies. These mosquitoes were fed to 3 human microfilarial carriers. After the mosquitoes were fed, they were transfered in large paper cups, each covered with fine cloth netting. These were kept in cool places, suspended on trays with water to protect from ant predators. During the holding period the mosquitoes were fed with 10% sugar water soaked in cotton wools placed on top of the cups. They were checked each morning. The weak and dead mosquitoes found were individually dissected, and those survived were killed on day 11. The larvae recovered were preserved and subsequently identified, and their stages determined.

Wild caught *An. barbirostris* and *An. nigerrimus* infested with water mites supposedly to be newly emerged were used for efficacy tests. Before these mosquitoes were used to feed on various Mf carriers. Some of these were individually dissected, and found free from filariasis infection. Three sets of experiments for each species with varying number of mosquitoes were used to feed on Mf carriers with 2, 3. 2 and 7 microfilariae per cu mm blood, 50 *An. barbirostris* and 20 *An. nigerrimus* not infected were used as control. Mosquitoes died during the holding period and those survived at day 11 were individually dissected.

Larval dipping of *An. barbirostris* and *An. nigerrimus* was carried out at monthly intervals from February 1981 to October 1982 in 3 ponds, the sizes varies from 10 x 10 meters to 20 x 50 meters. During November to January these ponds were dried, and no collection was made. The method on larval collection follow WHO Manual on practical Entomology in Malaria, part II (1975). Once a month 250 dips were made. The main vegetations in these ponds are *Pistia sp, Isachne globosa, Panicum*

amplexicaule and *Ipomoea sp.* During the rainy period, the water in these ponds are quite clear, and turbid during lesser rainfall. Dipping of the larvae were confined to the edges of the ponds between 10.00 to 12.00 hours each month.

DDT susceptibility test were carried out for *An. barbirostris.* The mosquitoes were collected between 1900 to 2100 hours while resting on walls of the house. The house has not been sprayed. with any insecticide before. All mosquitoes tested were unfed. Standard exposure to insecticide papers in WHO Manual on practical Entomology in Malaria (1975) was followed. As *An. barbirostris* is a large mosquito, each tube is confined to not more than 15 mosquitoes. During exposure the tubes were held in a wooden box maintained at 26°C to 28°C and 85 – 88% relative humidity. After the 24 hours holding, all dead mosquitoes were removed, counted and identified. Percent mortalities were plotted on logarithmic probability paper and regression lines fitted by eye to determine LC_{50} and LC_{95}.

RESULTS

Mosquito Fauna

A total of 82.771 mosquitoes consisted of 12 genera with 56 species from 5 methods of collection were examined from the four villages. These consisted of 5 *Mansonia spp.*, 14 *Anopheles spp,* 4 *Armigeres spp.*, 4 *Conquillettidia spp.*, 7 *Aedes spp,* and 16 *Culex spp,* and 6 unidentified mosquitoes belonging to 6 genera. Among these, *Mansonia spp,* comparised of 14.9%, *Anopheles* spp. 25.9%, *Armigeres spp.* 0.4%, *Coquillettidia* spp. 0.3%, *Aedes* spp. 8.0 %, *Culex spp.* 48.3% and 2.2% of 6 other genera.

The frequency distribution of these mosquito species are remarkedly similar in each of the villages. *Culex spp.* were shown to have the highest prevalence rate. Chi-square test at 5% level showed that there was no significant different on the density of mosquitoes between each of the villages, although Teteona village showed a slightly lower catch than the other 3 villages (Table 1).

Among the 5 *Mansonia spp.* examined, *Ma. uniformis* and *Ma. indiana* were more prevalent in all the villages with higher mean number of mosquito per man hour in Lalohao and Pondidaha villages than the other two villages. The lowest index were *Ma. annulata* and *Ma. annulifera,* while *Ma. bonneae/dives* were relatively abundant.

Of the *Anopheles spp.* examined, *An. barbirostris* was the most abundant in all the villages with equally high index in 3 villages, and lower in Teteona village. *An. nigerrimus* comes next after *An. barbirostris,* while relatively low index were shown in the other 12 species. Among the *Armigeres spp.* and *Coquillettidia spp.* examined, *Ar. malayi* and *Cq. crassipes* have higher indices in all the villages than the other species of these two genera. Three of the *Aedes spp. Ae. vexans, Ae. lineatopennis* and *Ae. albopictus* were found to have higher indices than the four other *Aedes spp.* examined, however, the highest mean mosquitoes per man per hour was *Ae. vexans. Culex* mosquitoes, on the other hand, are the most prevalent of the mosquito fauna sampled in all the four villages. Among 16 *Culex spp.* examined, the highest indices were *Cx. tritaeniorhynchus, Cx. gelidus, Cx. quinquefasciatus, Cx. vishnui* and *Cx. pseudovishnui.* The prevalence of the unidentified species of six genera were low, except *Uranotaenia spp.* which showed a higher index than the others (Table 1).

Evaluation of Trapping Methods with Two *Anopheles* sp.

The mosquito fauna survey revealed that two *Anopheles spp., An. barbirostris* and *An. nigerrimus,* and most of the *Mansonia spp.* are vectors of brugian filariasis in the study areas. The current report includes detail studies of *Anopheles* vectors only.

A total of 20,502 *An. barbirostris* and 615 *An. nigerrimus* were caught by five catching methods during 24 months survey. Among the five catching methods, very few of these two mosquito species were caught in the outdoor drop net catches, thus they were not statistically analysed. Analysis were made between the other four catching methods at monthly period for 24

Table 1. Relative abundance of mosquito species in four villages of Kendari Regency, Southeast Sulawesi Province, Indonesia.

MOSQUITO SPECIES	TETEONA Number m/h	LALOHAO Number m/h	PONDIDAHA Number m/h	WAWOLEMO Number m/h
MANSONIA				
Ma. uniformis	0.95	1.70	1.65	0.80
Ma. indiana	0.25	1.24	1.05	0.72
Ma. bonneae/dives	0.28	0.69	0.09	0.08
Ma. annulifera	0.001	0.004	0.006	0.008
Ma. annulata	0.003	0.003	–	0.005
TOTAL	1.48 (1867)	3.64 (3844)	2.79 (4101)	1.61 (2496)
ANOPHELES				
An. barbirostris	1.61	4.56	3.68	4.25
An. nigerrimus	0.13	0.19	0.07	0.02
An. albotaeniatus	0.003	0.01	0.01	0.001
An. barbumbrosus	0.005	0.001	0.003	0.004
An. crawfordi	0.01	0.08	0.04	0.01
An. nitidus	–	0.001	–	0.001
An. parangensis	–	0.002	0.001	–
An. peditaeniatus	0.01	0.03	0.01	0.001
An. pseudobarbirostris	0.001	0.002	0.005	0.001
An. sulawesi	0.001	0.001	–	0.001
An. tesselatus	0.004	0.04	0.03	0.03
An. vagus	0.03	0.01	0.006	0.006
An. flavirostris	0.001	–	–	0.001
An. kochi	0.001	–	–	–
TOTAL	1.81 (2816)	4.93 (6284)	3.86 (5688)	4.33 (6667)
ARMIGERES				
Ar. denbesteni	0.001	0.001	0.006	0.001
Ar. malayi	0.03	0.12	0.03	0.003
Ar. moultoni	0.005	0.002	0.002	0.003
Ar. subalbatus	0.003	0.008	0.001	0.005
TOTAL	0.04 (63)	0.13 (187)	0.04 (59)	0.01 (19)
COQUILLETTIDIA				
Cq. crassipes	0.04	0.04	0.06	0.03
Cq. hodgkini	0.001	–	–	-
Cq. nigrosignata	–	–	0.001	–
Cq. ochracea	0.001	0.001	0.001	–
TOTAL	0.04 (68)	0.04 (67)	0.06 (83)	0.03 (39)
AEDES				
Ae. albopictus	0.02	0.10	0.04	0.05
Ae. kochi group	0.03	0.002	0.003	0.001
Ae. lineatopennis	0.25	1.46	0.17	0.27
Ae. panayensis	0.05	0.17	0.16	0.06
Ae. quadripunctis	0.001	0.001	0.001	0.001
Ae. vexans	0.40	0.47	0.28	0.65
Aedes sp.	0.03	0.03	0.06	0.02
TOTAL	0.76 (1152)	2.23 (2843)	0.71 (1044)	1.05 (1612)

Continued .....

MOSQUITO SPECIES	TETEONA Number m/h	LALOHAO Number m/h	PONDIDAHA Number m/h	WAWOLEMO Number m/h
CULEX				
Cx. tritaeniorhynchus	1.82	2.00	1.12	1.22
Cx. gelidus	1.71	1.44	1.65	1.63
Cx. quinquefasciatus	1.66	1.55	0.83	0.69
Cx. vishnui	1.51	1.40	1.18	0.86
Cx. bitaeniorhynchus	0.25	0.21	0.16	0.08
Cx. cinctellus	0.21	0.19	0.29	0.11
Cx. fuscanus	0.01	0.001	0.02	0.006
Cx. halifaxii	0.001	0.02	0.001	0.003
Cx. mimulus	0.001	0.001	0.001	0.001
Cx. nigropunctatus	0.05	0.07	0.04	0.03
Cx. pseudovishnui	1.40	0.61	0.85	0.52
Cx. sinensis	0.002	–	0.006	–
Cx. sitiens	0.03	0.02	0.04	0.02
Cx. whitmorei	0.07	0.02	0.005	0.02
Cx. pallidothorax	0.002	0.005	–	0.001
Culex sp.	0.01	0.03	0.03	0.01
TOTAL	8.74 (13165)	7.58 (9664)	6.22 (9159)	5.20 (8010)
Ficalbia sp.	0.04 (65)	0.05 (69)	0.03 (40)	0.02 (36)
Heizmannia sp.	0.02 (28)	0.03 (41)	0.05 (84)	0.02 (33)
Mimomyia sp.	0.04 (59)	0.02 (33)	0.08 (126)	0.05 (75)
Orthopodomyia sp.	0.001 (1)	– –	–	–
Uranotaenia sp.	0.21 (327)	0.21 (276)	0.22 (329)	0.09 (151)
Tripteroides sp.	–	–	0.001 (1)	–
Total examined	19611	23308	20714	19138

months. These two mosquito species were analysed separately, and the following statements apply equally to both species.

The paired 't' test shows no significant difference between indoor and outdoor bait net catches. However, significantly more mosquitoes were caught resting indoors at night than in the morning ($p < 0.01$). An analysis of variance shows no significant difference between the indoor bait net, outdoor bait net, and morning indoor resting monthly catches. However, when the night indoor resting catches were added to this analysis, there was a difference ($p < 0.01$). There were significantly more mosquitoes caught resting indoors at night than at any other catching methods.

Seasonal Variations on Density and Parous Rate

Attempts were made to find out the seasonal variations and parous rates of *An. barbirostris* and *An. nigerrimus* from outdoor and indoor net bait collections of the two *Anopheles spp.* from the four villages were combined.

The density of *An. barbirostris* is shown to be positively correlated with rainfall, the correlation coefficient is 0.47. The 1981's peak was in June and it was considerably higher than that of the 1982's (Figure 1).

Although the density of *An. nigerrimus* was considerably lower than *An. barbirostris* in all the study areas, it was also positively correlated with rainfall ($r = 0.50$). The peaks of this mosquito is identical to that of *An. barbirostris,* but the highest was in June 1982 with a slightly decrease in 1981 (Figure 1).

The monthly parous rate of *An. barbirostris* ranged from 28.3% to 67.6% with a monthly

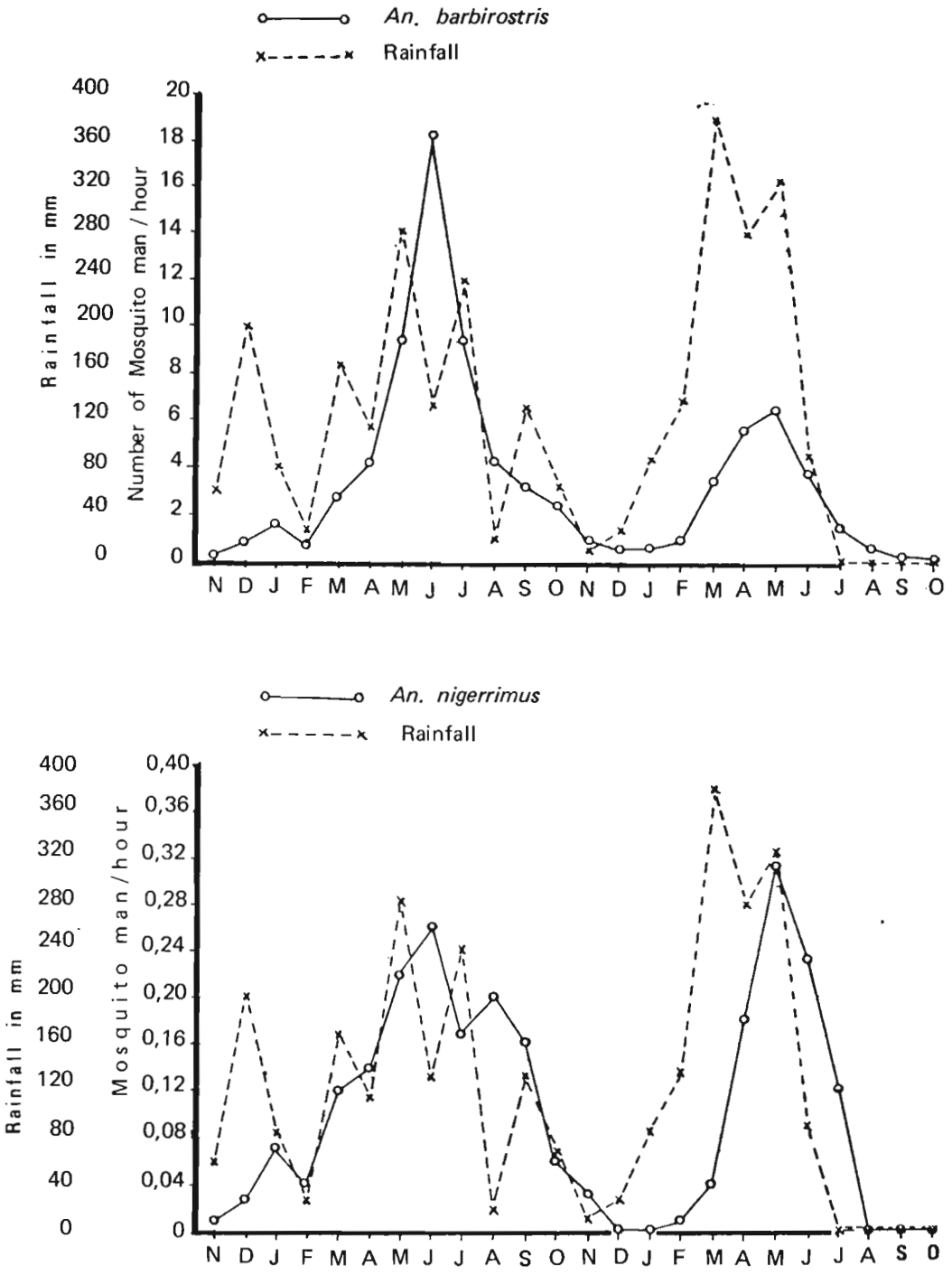

Fig. 1. Seasonal variation of *Anopheles barbirostris* and *An. nigerrimus*.

average of 45.0% compared to *An. nigerrimus* which ranged from 27.8% to 100% and monthly average of 39.9%. The monthly parous rate of *An. barbirostris* was shown to be negatively correlated with the densities, the correlation coefficient was – 0.74, but no correlation (r = – 0.18) was observed for *An. nigerrimus* (Figure 2).

Seasonal Larva Density

The monthly larva density of *An. barbirostris* was very low throughout the 21 month dipping collections. The density per man hour each month ranged from 0.1 to 1.7 larvae. There was no significant correlation between the larva density with the adult populations as well as

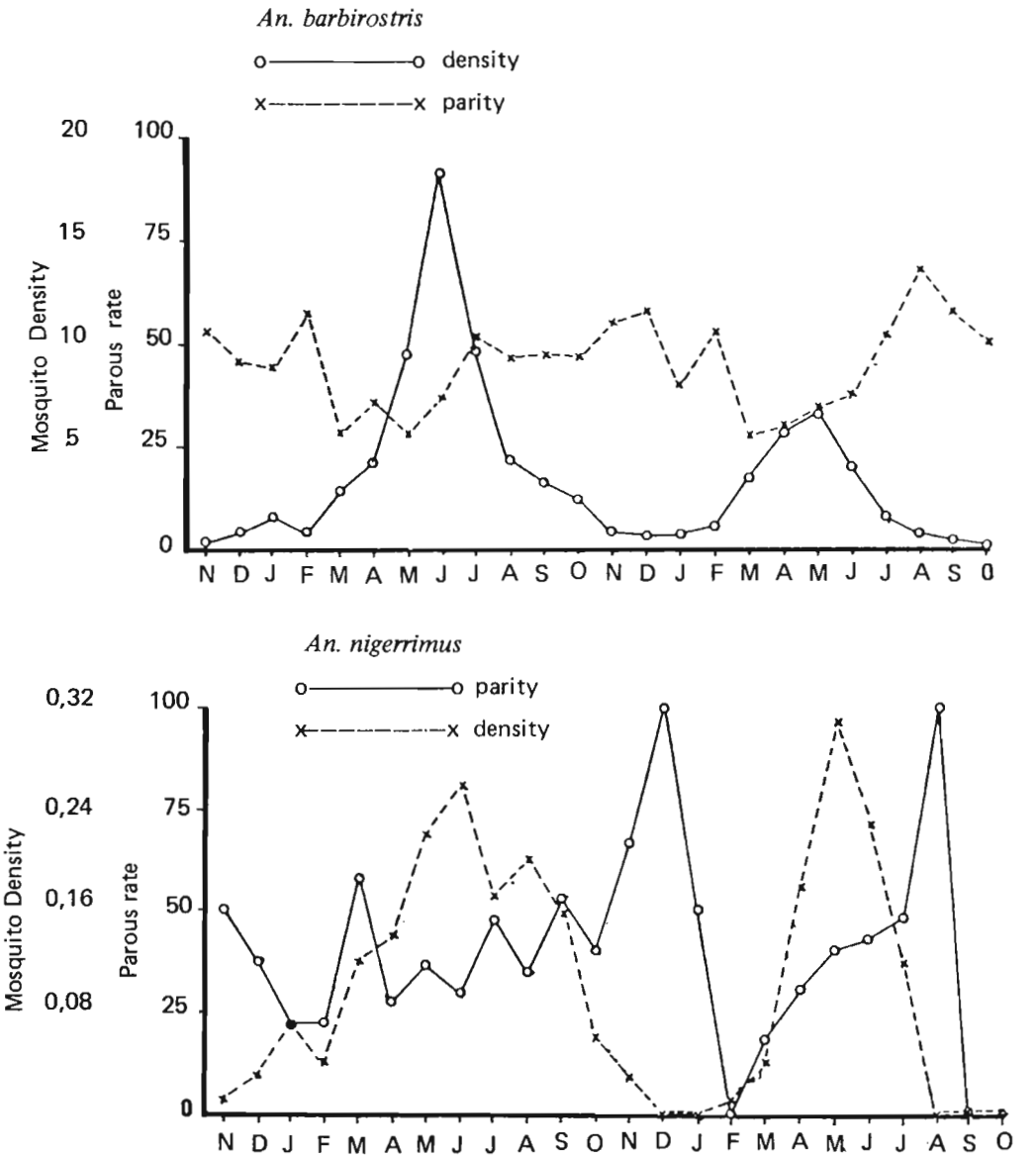

Fig. 2. Seasonal variation of parous rates on *Anopheles barbirostris* and *An. nigerrimus*

with rainfall ($p > 0.05$). There was a trend of decreasing density from October to January 1981/1982 during very low rainfall when the ponds were dried. Similarly, there was no significant correlation between density of *An. nigerrimus* with adult and as well as with rainfall ($p > 0.05$). The lower density of this mosquito was similar to that of *An. barbirostris* during months of low rainfall.

Gonotrophic Cycle

A series of experiments were made with 100 newly blood fed *An. barbirostris* collected resting inside the houses. Specimens were individually held in plastic tube and allowed to oviposit on wet filter paper. During the holding period, they were fed with 10% sugar water. Eggs laying from these mosquitoes, began from 65 to 87 hours after blood meals.

Information was also obtained by examining 50 wild females from landing collections after blood meal had been completed. About 76% of them oviposited after 73 hours and 15% after 98 hours in the laboratory.

Biting Peak

All night collection of landing mosquitoes were made for 12 months with one night for each month. More than 50% of *An. barbirostris* from both indoor and outdoor were caught from 2300 to 0500 hours, and for *An. nigerrimus* it was from 1900 to 2200 hours. The biting peaks for *An. barbirostris* were from 2400 to 0300 hours compared to *An. nigerrimus* were from 1900 to 2200 hours.

Host Preference

Precipitin tests on 75 females *An. barbirostris* and 36 *An. nigerrimus* from outdoor resting collections showed the following results: 90.7% *An. barbirostris* fed on man, 2.7% on cats, 4.2% on bovids and 2.4% on unknown, and for *An. nigerrimus*, it was 66.7%, 8.3%, 21.4% and 3.6% respectively.

Natural Infection of Filarial Worm of Mosquitoes

The natural filarial infection of *An. barbirostris* between the 5 collecting methods was presented in Table 2. The infection rate of *An.*

Table 2. Natural filarial infections in *Anopheles barbirostris* in different methods of catches from the four vilages combined at Wawotobi district, Kendari Regency, Southeast Sulawesi Province, Indonesia.

Remarks	Indoor net bait	Outdoor net bait	Indoor resting (night)	Indoor resting (morning)	Outdoor drop-net	Total
Number of mosquito dissected	4,553	5,232	4,439	2,747	499	17,470
Infection rates	0.1% (5/4,553)	0.15% (8/5,232)	0.1% (5/4,439)	0.25% (7/2,747)	2.6% (12/499)	0.21% (37/17,470)
Infective rates	0.02% (1/4,553)	0.04% (2/5,232)	0.02% (1/4,439)	0.11% (3/2, 747)	1.00% (5/499)	0.07% (12/17,470)
Mean number of infected larvae	4.9	6.7	4.8	4.6	5.2	5.24
Mean number of infective larvae	3.7	5.1	3.00	2.5	3.8	3.62

barbirostris between the five different collecting methods vary, however infective mosquitoes were found to be significantly higher in outdoor drop net collections than the other four collecting methods ($p < 0.05$). The mean number of *Brugia* larvae was found to be the highest in moquitoes from the outdoor net bait collections. There was no significant difference on infection rates for *An. nigerrimus* collected between the 5 collecting methods. Only one mosquito was found with 4 infective larvae (Table 3).

Experimental Infections

In the present experimental infection studies, comparison of infectivity was made between laboratory raised and wild caught mosquitoes fed to human Mf carriers (Table 4). Mosquitoes died during the experimental periods were dissected, and all live ones were sacrificed at day 10.5 or 11. The infection rates between laboratory raised and wild caught *An barbirostris* showed no significant difference: the rates being 81.7% and 71.7% respectively. Infective *Brugia malayi* larvae developed in 7.5 days onwards, although developing infective stage was detected on mosquitoes killed at 6.5 days. The mean worm-load of 1st and 2nd stages larvae was 5.8 larvae per host, compared to 4.7 infective larvae in mosquitoes killed from day 7.5 onwards. There was also no marked difference on the infectivity of *An. nigerrimus* between laboratory raised and wild caught ones. The mean worm-load of 1st and 2nd stages was 4.2 compared to 2.6 of the infective stage.

Efficacy of Mosquitoes as Experimental Vectors

Comparison of the host efficiency of laboratory raised *An. barbirostris* and *An. nigerrimus* over three different microfilarial densities was conducted (Table 5). Indices of experimental infection for *An. barbirostris* show the effect on microfilarial densities on the production of stage III (infective) larvae on Mf carriers with 2, 3.2 and 7 Mf per mm^3 blood were 0.16, 0.18 and 0.32 with an average of 0.22. The indices for *An. nigerrimus* were 1.1, 1.25 and 0.14 respectively with an average of 0.83.

Insecticide Susceptibility Test

The insecticide susceptibility tests with DDT impregnated papers on *An. barbirostris* revealed the LC_{50} and LC_{95} determined were 0.58% and 1.70% respectively. The result showed that this mosquito was susceptible to DDT (Table 6).

Table 3. Natural filarial infections in *Anopheles nigerrimus* in different methods of catches from the four villages combined at Wawotobi district, Kendari Regency, Southeast Sulawesi Province, Indonesia.

Remarks	Indoor net bait	Outdoor net bait	Indoor resting (night)	Indoor resting (morning)	Outdoor drop-net	Total
Number of mosquito dissected	146	204	165	85	15	615
Infection rates	0	0.5% (1/204)	0	0	0	0.16% (1/615)
Infective rates	0	0.5% (1/204) *	0	0	0	0.16% (1/615)*

*** Single infection with 4 infective larvae.**

Table 4. Number of infected mosquitoes after feeding on microfilariae carriers in experimental infection studies.

Species	Number of infected mosquitoes/number of mosquito dissected on days after feeding																					
	Laboratory raised mosquitoes											No. of mosq	Field caught mosquitoes										No. of mosq
	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5		0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	
Anopheles barbirostris	1/2	–	–	–	3/4	2/4	1/1	3/4*	8/9*	4/4*	27/32*	49/60	0/1	0/1	1/2	2/3	–	–	2/5	1/1*	4/6*	7/9*	26/32*	43/60
Anopheles nigerrimus	0/1	0/1	–	–	0/1	1/2	1/5	1/2*	–	–	3/18*	6/30	–	0/2	0/1	0/3	–	0/3	1/2	0/1	1/3*	–	3/15*	5/30
	Control												Control											
Anopheles barbirostris	–	–	–	–	0/3	–	–	0/4	0/1	0/3	0/49	0/60	–	0/3	0/2	0/3	0/1	–	–	0/8	0/1	0/2	1/40**	1/60
Anopheles nigerrimus	–	0/1	–	–	0/2	0/1	–	0/2	–	–	0/24	0/30	0/1	–	–	0/5	–	–	–	0/2	–	–	0/28	0/30

* Infective larvae found
** Other mematode

Table 5. Observation of the eficacy of *Anopheles barbirostris* and *An. nigerrimus* as laboratory vector of *Brugia malayi* (mosquitoes dissected 10.5 – 11.0 days after feeding on human carriers with different densities of microfilariae in the peripheral blood)

Experimental number	Microf. per Cu. mm x	Mosq. dissected	% of mosquitoes			No. of larvae found			% Stage III	Larvae Larvae mosquito Mean (rage)	Stage III Larvae per infective mosquito (c)	Index of experimental infection a x b x c / x
			Infected	Infective a x 100	Survived b x 100	Stage I	Stage II	Stage III				
Anopheles barbirostris												
1	2	254	16.5	15.7	72	0	13	118	95.2	3.1 (1–25)	3.0	0.16
2	3.2	31	22.5	22.5	66	0	1	26	100	3.9 (1–13)	3.7	0.18
3	7	162	32.0	32.0	84	0	3	440	100	8.5 (1–31)	8.5	0.32
		447	23.7	23.4	74	0	5.6	194.7	98.4	5.2 (1–31)	5.0	0.22
Anopheles nigerrimus												
1	2	32	46.9	43.8	91	0	4	77	93.3	5.4 (1–24)	5.5	1.1
2	3.2	5	40	40	100	0	0	20	100	10 (9–11)	10.0	1.25
3	7	1	100	100	100	0	0	1	100	1.00	1.00	0.14
		38	62.3	61.3	97	0	1.3	32.7	97.8	5.5 (1–24)	5.5	0.83

Table 6. 24 hours mortality of *Anopheles barbirostris* exposed to DDT for one hour. In the study area, district of Wawotobi, Kendari Regendy, Southeast Sulawesi Province.

% DDT	Replicate I		Replicate 2		Relicate 3		Replicate 4		T o t a l		
	number tested	number dead	number tested	number dead	number tested	number dead	number tested	number dead	number tested	number dead	% mortality
0.25	12	4	11	0	12	1	12	1	47	6	12.76
0.50	11	4	12	3	12	6	13	5	48	18	37.50
1.00	12	9	12	7	12	11	13	9	49	36	73.46
2.00	14	14	13	13	11	11	12	10	50	48	96.00
4.00	15	15	11	11	12	12	13	13	51	51	100.00
Control	12	0	13	0	11	0	13	1	49	1	2.04

Eye fitted regression line about : LC_{50} = 0.58%

LC_{95} = 1.70%

DISCUSSION

O'Connor and Tine Sopa (1981) listed 111 mosquito species comprised of 15 genera in Sulawesi island. The present study examined 56 species of 12 genera. Six genera *Ficalbia, Heizmannia, Mimomya, Orthopodomyia, Tripteroides* and *Uranotaenia* were listed by them have yet to be found in the study areas. About 60.0% among the listed mosquito species, were found in the study areas.

Among the *Anopheles* and *Mansonia* species listed, 14 of 34 *Anopheles* spp. and all 5 *Mansonia* spp. listed were present in the study areas.

Of the *Anopheles* spp. examined *An. barbirostris* and *An. nigerrimus* were the most abundant, the density of the former was significantly higher than the latter ($p < 0.05$). The seasonal density of both these mosquitoes were shown to be correlated with rainfall with distinct peak density in June. The generally low parous rates of these two *Anopheles* spp., suggest that the monthly sampling consisted of more young mosquitoes than older adults. This could be due to a consistant migration of these species from and to surrounding habitats.

Gonotrophic cycle studies carried out on *An. barbirostris*, although crudely done, however, does indicate that the feeding cycle of this mosquito was between 2.7 and 3.6 days. *An. barbirostris* was shown to be highly anthropophilic, and its biting peaks was observed during midnight and morning hours. *An. nigerrimus*, although anthropophilic, but fairly high percentage fed on bovids as well, and its biting peaks were immediately after dusk to before midnight. Both species were found to be more indoors than outdoors feeders. In Flores island, *An. barbirostris* biting peak was between 2100 to 0300 hours and it had also been shown to feed mostly indoors (Soeroto Atmosoedjono, 1977), and in South Sulawesi, the biting activities were observed from 2100 with a slight peak between 0100–0300 hours (Partono et al., 1972).

An. barbirostris has been establish as a good natural vector for Malayan filariasis in South Sulawesi (Partono et al., 1972), Central Sulawesi (Soeroto Atmosoedjono et al., 1976) and for Timor filariasis in Flores island (Soeroto Atmosoedjono et al., 1977). In South Sulawesi, 11.7% of 111 *An. barbirostris* from indoor resting were positive for all stages of larvae with 3.5% for infective larvae, while in Flores 27% of 35 resting indoor mosquitoes were infected with larvae of *Brugia timori*. In the present study the overall infection rate was 0.21%, and 0.07% of the infected mosquitoes harboured infective stage larvae. For *An. nigerrimus*, only 1 of 615 mosquitoes examined were infected with infective larvae.

Experimental infections on *An. barbirostris* and *An. nigerrimus* revealed that 81.7% of 60 and 20% of 30 were susceptible, and the micro-

filariae developed to infected stage larvae from day 7.5 onwards. Partono et al., (1972) showed 43.4% of 145 *An. barbirostris* took the infection, and infective mosquitoes were detected from six days onwards, while Soeroto Atmosoedjono (1977) showed 57.7% of 26 mosquitoes were experimentally infected, and 50% of the infected ones harboured infective larvae in day 12.

Following the method of host efficiency by Wharton (1962) comparison was made between *An. barbirostris* and *An. nigerrimus* on three ranges microfilarial densities, showed the overall index of the former species was 0.22 and that of the latter species 0.83. Based on this study and that of previous workers, it was apparent that *An. barbirostris* is not only a good natural host, but is also a good laboratory susceptible and efficient host. The short developmental period to infective mosquitoes undoubtedly contributes to the high endemicity of Malayan filariasis in the study areas. There were too few *An. nigerrimus* available for the experiments, however they were found to be good experimental host. Its potential as one of the important vectors should not be over-looked.

Comparison with the various methodology of trapping mosquitoes excluding the conventional method of landing mosquitoes directly on man, it was found among the five methods used, bait net collections were equally efficient for indoors and outdoors. However, resting indoor at night collections were found with more mosquitoes than the rest of the other four methods. In view of the efficiency of bait net collections shown in this study, it is recommended that mosquitoes studies in highly endemic filariasis areas, consideration should be given to use net-bait methods.

Based on various collecting techniques of adult and larval mosquitoes in the study areas, it showed that the breeding sites of *An. barbirostris* and *An. nigerrimus* are in ponds, grass-field and riverside channals within the village surroundings. In Flores, *An. barbirostris* breeeds in irrigated ricefields and river-side channals 0.5 Km from the village (Soeroto Atmosoedjono et al., 1977).

The overall picture of adult behaviours in *An. barbirostris* derived from this study is fairly clear-cut. However, there are still informations, e.g. flight range, establishing of laboratory colonies from larval stage, etc. are needed to further understand its biology. Reid et al., (1979) stated that the vector form of *An. barbirostris* in Flores and Sulawesi and that of the non vector form in Java are morphologically difficult to warrant sub-specific status, but differing more in biology. In view of this fact, further biological studies of this species is needed to ilucidate between the two conspecific mosquitoes.

Insecticide susceptibility tests on *An. barbirostris* found this species was susceptible to DDT impregnated papers.

SUMMARY

Application of 5 collecting methods, a total of 82,771 mosquitoes comprised of 12 genera with 56 species were sampled. No significant difference in the monthly catches were found between the indoor bait net, outdoor bait net and morning indoor resting, but significantly more mosquitoes were caught resting indoors at night, and least of all was from the outdoor resting collection. The most predominant mosquito is *Culex* spp. followed by *Anopheles* and *Mansonia* spp., with few of each of the other species. The malayan filariasis vectors established are *An. barbirostris* and possibly *An. nigerrimus,* and at least 3 of five *Mansonia* spp. The densities of *An. barbirostris* and *An. nigerrimus* were shown to be correlated with rainfall, with peak densities in June.

Parous rate for both moquito species were relatively low. Larval densities of both species were low, and were neither correlated with adult population nor rainfall, and with lowest numbers during period of very low rainfalls. *An. barbirostris* was more anthropophilic than *An. nigerrimus.* The biting peaks for the former species began from midnight to early morning while

that of the latter started immediately after dusk to nearly midnight.

The natural filariasis infection rate of *An. barbirostris* was significantly higher than *An. nigerrimus.* Experimental infections of these mosquito species to human microfilarial carriers showed the Mf developed to infective stage larvae in them at 7.5 days post infections. Indices of experimental infection on *An. barbirostris* show the index of this species was 0.22 per mosquito compared to 0.83 in *An. nigerrimus.*

DDT susceptibility test on *An. barbirostris* found the mosquito was susceptible to the insecticide with LC_{50} and LC_{95} determined being 0.58% and 1.70% respectively.

ACKNOWLEDGEMENTS

We wish to express our appreciation to: Dr. Arwati Soepanto, Director of the Directorate of Vector-borne Diseases Control, Communicable Diseases Control for her administrative support, Dr. H. Aman Nasution MPH, former head of Provincial health Services, Southeast Sulawesi, Dr. Fuad Imanudin, head of Provincial Communicable Disease Control, Southeast Sulawesi, Dr. Bagus Asiadi S. MSc, head of Regency Health Services, Kendari, for their very helpful assistance in the Province. Our thanks are also due to Mr. Soeroto Atmosoedjono, Medical Entomologist, US NAMRU–2 Jakarta, for his guidance during this study.

REFERENCES

Annonymous (1975) Manual on practical entomology in malaria. WHO. Publication, Geneva, 13 : part 2, p. 55.

Arbain Joesoef, Lifwarni, Wardiyo, L. Maneoba, Z. Bahang, S. Kirnowardoyo, S. Arwati, and Lim Boo Liat (1983) Malayan filariasis in Kendari Regency, Southeast Sulawesi I: Parasitological survey. *Health Studies in Indonesia.*, 12 (1) 1–7.

Atmosoedjono, S., P.F.D. Van Peenen, and J. Putrali (1976) *Anopheles barbirostris* (van der Wulp) still an efficient vector of *Brugia malayi* in Central Sulawesi (Celebes), Indonesia. *Trans. Roy. Soc.* Trop. Med. Hyg. vol. 70, 3, 259.

Atmoseodjono, S., F. Partono, D.T. Dennis, and Purnomo (1977) *Anopheles barbirostris (Diptera: Culicidae)* as a vector of the Timor Filaria on Flores Island: Preliminary observations. *J. Med. Ent.*, 13, 611 – 13.

Nelson, G.A. (1960) The identification of filarial larvae in their vectors. *Indian J. Malariology,* 14, 555 – 92.

O'Connor, C.T. and Tine Sopa (1981) A checklist of the mosquitoes of Indonesia. A special publication, *US. Naval Medical Research Unit-2.* Jakarta, Indonesia, 26 p.

Partono, F., Hudojo, Sri Oemijati, M. Noor, Borahima, J. H., Cross, M. D. Clarke, G. S. Irving and C. F. Duncan (1972) Malayan filariasis in Margolembo, South Sulawesi, Indonesia. *Southeast Asian J. Trop.* Med. Publ. Hlth., 3, 537 – 47.

Ramachandran, C.P. (1970) A guide to methods and techniques in filariasis investigation. *Institute for Medical Research Bull.,* Kuala Lumpur, 15, 39 pp.

Reid, J. A., B.A., Harrison, and S. Atmosoedjono (1979) Variation and vector status in *Anopheles barbirostris. Mosquito Systematics,* II, 235 – 51.

Wharton, R.H. (1962) The biology of *Mansonia* mosquitoes in relation to the transmission of filariasis in Malaya. *Bulletin Institute for Medical Research,* Federation of Malaya, 11, 114 pp.