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Entomological Survey for New Vector Incrimination and Re-Confirmation of Established Malaria Vectors in Endemic Areas of Bangladesh (4 Sentinel Sites)

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Abstract **Original Research Article**

In Bangladesh malaria is one of the major public health problems and there are 3 malaria endemic hill tract districts which are the major areas among the red zones of malaria enedemicity. This study was aimed at assessment of the new vector incrimination as well as re-confirmation of the established vectors in Naikhangchari, Thanchi, Laxmichari and Baghachari. The anopheline population were collected and tested to incriminate the vector of malaria and their role in transmission, to find out the sporozoite rate, determination of oocyst rate of parasite and entomological innocultaion rate was diagnosed. Anopheline mosquito samples were directly collected from the sites using human bait, cattle landing and CDC light traps. The collected mosquitoes were dissected to find out the sporozoite in the salivary gland, oocyst in the midgut and to see the ovary for determination of the mosquito's status of parous or nail-parous to determine the length of gonotropic cycle. The highest collection of a primary vector *An. philippinensis* was in Laxmichari (186 samples) and the highest collection of a secondary vector species *An. vagus* was in Thanchi (392). After the dissection no sporozoite or oocyst were detected among the collected anopheles' samples. The vectoral capacity of *An. philippinesis* should be re-evaluated in the further studies. The pick biting hours of *An. philippinensis* and *An. vagus* varied in the 3 study sites which is an important finding of this study.

Keywords: Entomological survey, vector incrimination, malaria, endemic areas.

INTRODUCTION

Malaria is a major public health problem in Bangladesh. The endemicity of this disease is high in north-eastern border districts of Bangladesh [1]. Specifically the hill tract districts Bandarban, Khagrachari and Rangamati are the red zone for malaria prevalence also there is another district namely Cox's Bazar which is still considered as malaria red zone. Those 4 districts are geographically connected and adjacent to each other [2].

There are 34 anopheline species recorded, out of these, seven species (spp.) are proven vector in Bangladesh [3]. Among them, 4 spp. are primary vector namely An. *baimaii(dirus), An. philippinensis, An. minimus* and *An. sundaicus* and other three are *An. annularis, An. aconitus* and *An. vagus* are known as secondary vector (epidemic vector) [3]. During Malaria Eradication Era, DDT was sprayed all over the country to control the vector mosquitoes and eradicate malaria [4]. Case was dropped sharply in many places except bordering districts of the eastern part of the country.

Surveys showed that though the recognized malaria vector of the area, *Anopheles minimus* disappeared but still malaria persist. Though the cases dropped but reappeared quickly after sometime [5]. It was later found that another mosquito, *An. baimaii* (=dirus) is transmitting malaria the eastern part of the country mainly in the Chittagong Hill Tracts and Sylhet district [6]. Since 1977 Malaria Eradication Programme was switched over to Malaria Control Programme and continued till 1994 [7]. In 1994 under the revised control strategy (Role Back Malaria) of WHO, the programme started to implement insecticide (deltamethrin) treated mosquito net as a measure in limited scale for transmission reduction in addition to other disease control activities. Initially it was started in a piece meal manner and was difficult to retreat one regularly [8].

There was information of increasing number of malaria cases, increasing number of chloroquine drug resistance malaria cases and increasing number of *falciparum* cases, this situation remained so still 2007 [9]. Pyrethroid treated mosquito nets have shown to be effective in reduction of malaria transmission and subsequently morbidity. After introducing the LLINs and ITNS, malaria transmission was reduced 40 to 50% or more in some Upazila in northern border belt areas [10]. There are some sporadic entomological surveillance, but no in depth longitudinal entomological study to determine vectors behavior in changed ecological i.e.; deforestation, land utilization, Insecticidal Treated Nets (ITN) & introducing Long Lasting Insecticidal Nets (LLINs), and global warming whether any other mosquitoes are playing any role along with the old vectors or independently acting as vector [11]. If so, we need to incriminate the new vectors if any, we are to know whether the existing vectors are behaving in the same manner or changed their habits in the changed ecological situation and global warming. There is no update information regarding the present anopheline fauna as well as vector status. It is not adequately known how the vector species are transmitting the disease. So, we have to know the real vector status and control it properly. We shall try to evaluate whether any anophelines with high density, early and outdoor biting habit those who are playing role in malaria transmission in malaria areas in Bangladesh.

Statement of the problem

The vectoral capacity of the primary vectors and the emergence of new vectors from the secondary as well as suspected vectors is a basic need for a successful vector incrimination program which is a pre-requisite for the elimination of malaria from Bangladesh. Vector incrimination is one of the routine entomological activity carried out by the National Malaria Elimination and *Aedes* Transmitted Diseases Control Program, CDC, DGHS but the surveillance results have not been disseminated among the stakeholders associated to the malaria elimination program. The results of this study imply that mosquitoes may have developed a resistance to the pesticides employed in the sentinel sites.

METHODOLOGY

Dissection has been performed on mosquito recently dead or killed by chloroform. Study sites: Four sites have been developed as per the previous entomological survey report, malaria incidence, and situation of ongoing malaria transmission. Four sites have been selected from four Upazila under 3 hyper endemic hill tract districts. Nakhangchari and Thanchi Upazila in Bandarban district, Baghaichari Upazila in Rangamati district and Laxmichari upazila in Khagrachari district were selected as our study site. These sites are highly endemic to malaria cases and the primary vector mosquitoes of malaria are abundant there as well. Mosquito collection, carrying and preservation for dissection: Mosquito has been collected through human bait and CDC light trap. The mosquitoes transferred into paper cup and killed the mosquitoes by chloroform. The presence of an infection in Anopheles confirmed the importance of a given species as a vector of malaria. In relation to the cycle of development of Plasmodium in mosquitoes, it is important to recognize the relevant parasite stages in the stomach (oocysts) of the Anopheles and in the salivary glands (sporozoites).

RESULT

According to the study's findings, no sporozoite was discovered in the salivary glands of the dissected samples. The midgut of the dissected mosquitoes revealed no oocysts. An. philippinensis in Baghaichari had a higher parous rate than nail-parous rate (9 parous and 7 nali-parous among the 16 collected sample). In the same sentinel site as An. vagus, a similar event took place. 13 nali parous and 27 parous were discovered. In Laxmichari, no parous mosquitoes were discovered among the dissected ones. Most of the species were found nail -parous in Thanchi. There were only 3 Anopheles mosquito samples collected in Naikhangchari, so dissection was not done there. There were no suspected or secondary vector species that demonstrated vectorial potential. Due to the absence of sporozoite and oocyst malaria parasite results, none of the suspected or secondary vector species demonstrated vectoral capacity. The pick biting period of An. vagus was recorded 6.15pm to 7.15pm and 7.15pm to 8.15pm in An. phlippinensis.

Table 1: Total number of collected mosquitoes of four sites

SI No	Name of species	Baghaichari	Laxmichari	Thanchi	Naikhongchari
1	*An. philippinesis*	19	186	2	1
2	*An. vagus*	63	365	392	2
3	*An. jamsii*	28	108	29	
4	*An. willmori*	11	0	0	
5	*An. jeyporiensis*	6	0	0	
6	*An. varuna*	3	0	0	
7	*An. nivipes*	47	36	0	
8	*An. kochi*	40	24	15	
9	*An. peditaetanatus*	157	2	0	
10	*An. barbirostris*	14	0	30	
11	*An. nigerimus*	10	26	361	
12	*An. subpictus*	0	7	0	
13	*An umbrosus*	0	0	101	
Total		398	754	930	03

This data shows that from four sites total 2085 Anopheles mosquitoes were collected. Among all of the mosquitoes 13 different types of mosquitoes were found. In Baghaichari, Rangamati total 398 Anopheles mosquitoes were collected and 157 *An. peditaetanatus* species were identified from this sentinel sites. From Laxmichari, Khagrachari total 754 mosquitoes were collected and among them 365 *An. vagus* species were found highest in this area. 186 *An. philippinesis* were found here. From Thanchi, Bandarban total 930 Anopheles mosquitoes were collected and among them *An. vagus* and *An. nigerrimus* were found high number 392 and 361 respectively. Total only 3 Anopheles mosquitoes were found in Nykhongchari, Bandarban. Total 2 species of *An. vagus* were collected from this area.

Table 2: Determination of parous, nulliparous in oocysts and presence of sprozoite in Salivary gland and midgut in Baghaichari, Khagrachari

			Salivary Gland			Midgut		Ovary dissection	
SI No	**Name of species**	**Total Dissected**	**Spor. +ve**	**Spor. -ve**	**Spor. Rate**	**Oocyst. +ve**	**Oocyst -ve**	**Parous**	**Nali-parous**
1	*An. philippinesis*	16	0	16	0	0	16	9	7
2	*An. vagus*	40	0	36	0	0	39	27	13
3	*An. jamsii*	19	0	16	0	0	17	13	6
4	*An. nivipes*	28	0	23	0	0	26	17	11
5	*An. kochi*	26	0	18	0	0	21	17	9
6	*An. jeyporiensis*	3	0	3	0	0	3	3	0
7	*An. varuna*	1	0	1	0	0	1	1	0
8	*An. willmori*	6	0	5	0	0	6	4	2
9	*An. pediateniatus*	101	0	85	0	0	97	78	23
10	*An. barbirostris*	11	0	8	0	0	10	8	3

This table represents that total 251 mosquitoes were dissected in Baghaichari, Khagrachari. But all of them showed negative results while dissecting them to determine the parous, nulliparous in oocysts and presence of sprozoite in salivary gland and midgut.

Table 3: Determination of parous, nulliparous in oocysts and presence of sprozoite in Salivary gland and midgut in Laxmichari, Khagrachari

			Salivary Gland			Ovary	
SI No	**Name of species**	**Total Dissected**	**Spor. +ve**	**Spor. -ve**	**Spor. Rate**	**Parous**	**Nulli Parous**
1	*An. philippinesis*	296	0	148	0	0	90
2	*An. vagus*	657	0	267	0	0	141
3	*An. jamsii*	146	0	82	0	0	31
4	*An. nivipes*	57	0	18	0	0	5
5	*An. kochi*	29	0	28	0	0	18
6	*An. subpictus*	11	0	7	0	0	4
7	*An. nigerrimus*	40	0	20	0	0	14

8	*An. pediateniatus*	3	0	2	0	0	1

Table-3 represents that total 1239 mosquitoes were dissected in Baghaichari, Khagrachari. But all of them showed negative results while dissecting them to determine the parous, nulliparous in oocysts and presence of sprozoite in salivary gland and midgut. In Laxmichari 708 mosquitoes were dissected. Out of them 505 mosquitoes were nulliparous and 203 mosquitoes were parous. Out of the total number of mosquitoes *An. vagus* was highest in number and *An. philippinensis* was in second position. As we know that if the number of naliparous is more than parous, the intervention like indoor residual spraying (IRS), LLIN, adulticide were effective.

Table 4: Determination of parous, nulliparous in oocysts and presence of sprozoite in Salivary gland and midgut in Thanchi, Bandarban

			Salivary Gland			Ovary		Midgut	
SI No	**Name of species**	**Total Dissected**	**Spor. +ve**	**Spor.- ve**	**Spor. Rate**	**Parous**	**Nulli Parous**	**Spor. +ve**	**Spor. -ve**
1	*An. philippinesis*	2	0	2		0	2	0	2
2	*An.vagus*	270	0	270		80	190	0	270
3	*An.jamsii*	27	0	27		6	21	0	27
4	*An.kochi*	26	0	26		8	18	0	26
5	*An.nigerimus*	295	0	295		80	215	0	295
6	*An.umbrosus*	70	0	70		24	46	0	70
7	*An.barbiostris*	18	0	18		5	13	0	18

This table represents that total 708 mosquitoes were dissected in Baghaichari, Khagrachari. But all of them showed negative results while dissecting them to determine the parous, nulliparous in oocysts and presence of sprozoite in salivary gland and midgut. In Thanchi, 708 mosquitoes were dissected. Out of them 505 mosquitoes were naliparous and 203 mosquitoes were parous. Out of the total number of mosquitoes *An. vagus* was highest in number and *An. philippinensis* was in second position.

Table 5: Timewise data collection mosquitoes from night resting in Thanchi, Bandarban

Time	***An. vagus***	***An. kochi***	***An. nigerimus***	***An. umbrosus***
05.15- 06.15pm	12	2	2	6
06.15-07.15	21	0	13	3
07.15- 08.15	14	0	1	3
04.15- 09.15	2	0	0	0
Total	49	2	16	12

This table shows that total 79 number of four different species of mosquitoes An. vagus, An. kochi, An. nigerimus, and An. umbrosus were found. An. vagus were found highest in number, 49. Only 2 species of An. kochi were found.

Table 6: Timewise data collection mosquitoes from night landing in Thanchi, Bandarban

Time	***An. philippinensis***	***An. vagus***	***An. kochi***	***An. nigerimus***	***An. jamesii***	***An. umbrosus***	***An. barbirostris***
5.15- 6.15	0	108	4	140	3	24	7
6.15-7.15	0	86	5	118	7	26	8
7.15- 8.15	2	75	3	65	16	18	7
4.15- 9.15	0	30	1	36	3	6	7
Total	2	299	13	359	29	74	29

This table shows that total 805 number of seven different species of mosquitoes An. philippinensis, An. vagus, An. kochi, An. nigerimus, An. jamesii, An. umbrosus and An. barbirostris were found. An. nigerimus were found highest in number, 359. Only 2 species of An. philippinensis were found and An. vagus were found 299 in number.

The number of Anopheles mosquito species found at the sentinel sites of Baghaichari, Laxmichari (Khagrachari), and Thanchi, Nykhongchari (Bandarban) was counted in the current study. A total of 2085 Anopheles mosquitoes were collected from four sites. There were 13 different varieties of mosquito's total among all the insects.

DISCUSSION

In Baghaichari, Khagrachari, 708 mosquitoes were dissected as part of this study. But when they were dissected to check for parity or nulliparity in the oocysts and the presence of sprozoites in the midgut and salivary gland, all of them produced negative results. The salivary glands of the dissected samples contained no sporozoites. The dissected mosquitoes' midguts had no oocysts visible. With diverse infectivity profiles in the mosquito vector site of development (oocysts) and site of concentration for transmission to the host body, Plasmodium sporozoites offer a remarkable model of parasite infection biology (salivary glands) [12]. 708 mosquitoes were dissected in Thanchi. 505 of them (or more of them) were naliparous, and 203 were parous. An. philippinensis came in second place while An. vagus came in first place overall in terms of mosquito population. As we know that if the number of nulliparous is more than parous, the intervention like indoor residual spraying (IRS), LLIN, adulticide were effective [13].

An. philippinensis, An. vagus, An. kochi, An. nigerimus, An. jamesii, An. umbrosus, and An. barbirostris were all detected in this study in total quantity of 805. A total of 359 An. nigerimus were discovered. Only 2 species of An. philippinensis and 299 different An. vagus species were discovered. As we know that *An. philippinensis* is the primary vector and *An. vagus* is the secondary vector of malaria. The following chart represents their pick biting time from night landing collection of Thanchi, Bandarban. *An. philippinensis* found mostly in between 7.15 - 8.15 pm and *An. Vagus* was found in between 5.15 - 6.15 pm and this time is considered as its pick biting time as the percentage is 36. There is some genetic foundation for where malaria vectors prefer to bite, such as indoors versus outside [14]. Different mosquito species' indoor biting behavior at different times of the night varied from one another [14].

According to this study findings, 43% of *An. nigerimus* specimens were obtained from human landings (collected from night landing in Thanchi, Bandarban). There were 25%, 10%, 1%, 2%, and 19% of *An. jamesii, An. umbrosus, An. kochi, An. barbirostris*, and *An. vagus*, respectively. 80% *An. vagus were* found mostly from human landing collection (collected from night landing in Thanchi, Bandarban). *An. philippinensis* and *An. nigerimus* were found 7% and 13% respectively. From Thanchi, Bandarban total six different species of mosquitoes are collected. Among them *An. Vagus* and *An. nigerrimus* were found mostly from this sentinel area and the percentage were 47% and 44% respectively.

The pick biting hour of *An. Vagus* (collected from night resting in Thanchi, Bandarban) was 6.15 - 7.15 pm. In that pick time total 49 mosquitoes were collected and the percentage was 43. Another study conducted in Bangladesh reported similar findings [15]. Just after dusk, Ameen *et al*., [15] discovered peak biting on a human.

CONCLUSION

This study did not find any direct evidence of the confirmation of vectoral capacity of the primary, secondary or suspected vector mosquitoes which indicates the necessity of further intensive study in the endemic areas at a large scale. The vector longevity is found by the determination of the gonotropic cycle which was detected by parous and nail parous rate. This finding suggest that the mosquitoes might have grown resistance against the insecticides used in the sentinel areas.

RECOMMENDATIONS

- Further study should be conducted to become confirmed about the vectoral capacity of the primary and secondary vectors.
- Whole night mosquito catches should be carried out in the hard to reach areas of the malaria endemic areas to collect and dissect the major primary vector species.
- Epidemiological and entomological surveillance should be carried out simultaneously in the endemic areas.
- Modern vector surveillance technologies should be involved to determine the density of primary vectors in the endemic areas.
- PCR/ELISA tests could be carried out for finding out the parasites among the collected vectors.

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