STUDY ON THE SPECIES COMPONENT AND ACTIVITY BEHAVIOR OF ANOPHELES MOSQUITOES IN IA DREH COMMUNE, KRONG PA DISTRICT, GIA LAI PROVINCE

NGUYEN QUANG THAI MD.,Ph.D., NGUYEN DUC MANH MD., PhD.,
PHAM XUAN VINH MD., PhD., DINH THI KHUYA Tech.
NGUYEN TIEN CONG Tech., LY HONG VAN Tech.
Military Institute of Preventive Medicine
RO MAH HUAN MD., MSc., PHAN VU HO MD., NGUYEN HAI BINH BSc.
Gia Lai Provincial Center for Disease Control
NGUYEN QUOC VIET Tech.
National Institute of Malariology, Parasitology and Entomology
LE DINH VINH Tech.
Institute of Malariology, Parasitology and Entomology Quy Nhon

Scientific reviewer: (1) Assoc.Prof. LE VAN DONG MD., Ph.D.
(2) NGUYEN CHINH PHONG MD., Ph.D.

ABSTRACT: *Surveys and collect mosquitoes in the village and forestal environment in the dry season (01/01-28/01/2019) and the rainy season (29/6-24/7/2019) in Ia Dreh commune, Krong Pa district, Gia Lai province. The surveys to study species component and activity behavior of Anopheles mosquitoes in the endemic malaria areas.*

Results: *A total of 9,567 mosquitoes of 16 Anopheles species had recorded, in which 1 primary and 7 secondary malaria vectors. The time biting peak of Anopheles mosquitoes was recorded at 6-8 p.m. in the dry season and 8-9 p.m. in the rainy season, in which the primary malaria vector, Anopheles dirus, biting peaked at 6-7 p.m. in the dry and 10-11 p.m. in the rainy season.*

Keywords: *Anopheles*, Gia Lai.

Responsible for the content: Nguyen Quang Thai MD., Ph.D., Email: quangthaisvc@gmail.com

Receipt date: July 01, 2021; invite scientific reviewer: July, 2021; accept: August 15,2021.

1. INTRODUCTION.

Malaria has occurred in many regions of Vietnam, including the Northern, Central, and Southern regions. Before the 1990s, malaria in Vietnam was a public health issue of great concern [6]. With the international support, along with the internal efforts of the people, the government system, and the health sector, Vietnam has achieved great successes in malaria control, contributing to significantly reducing the spread of malaria in the community, to protecting health [6, 12]. However, until 2017, Vietnam still has more than 63 million people living in malaria- endemic areas (accounted for 66% of the population), of which more than 6 million people (accounted for 6.3% of the population) live in malaria-endemic areas with high risk of malaria infection. According to the World Health Organization (WHO), in 2016, Vietnam recorded 4,161 malaria cases and 2 malaria cases deaths [17].

Malaria is caused by the bite of an *Anopheles* mosquito that contains the parasite *Plasmodium*. There are more than 444 species of *Anopheles* mosquitoes in the world [4]; among them, about 40 species are vectors of transmission of malaria parasites to humans. In different geographical areas, the role of malaria transmission in humans is by different mosquitoes species. Some species are considered primary vectors - playing a major role in the transmission of malaria and others are considered secondary vectors - playing a lesser role in the transmission of malaria to humans [14].

In Vietnam, 64 species of *Anopheles* have been recorded [9], of which, only 15 *Anopheles* species are considered malaria vectors, including 3 primary malaria vector (*Anopheles dirus, An. minimus*

and *An. sundaicus*) and 12 species-secondary vectors (*An. vagus, An. indefinitus, An. aconitus, An. jeyporiensis, An. maculatus, An. sinensis, An. barbirostris, An. campestris, An. nimpe, An. nivipes, An. philippinensis, An. subpictus*) [16]. The Central Highlands is considered a key malaria area of Vietnam, with a high prevalence of malaria [1, 8, 14]. Here, recorded primary malaria vectors such as *Anopheles dirus, An. minimus* and some malaria sub-vectors such as *An. maculatus, An. sinensis, An. acconitus, An. jeyporensis, An. barbirostris, An. vagus, An. subpictus* and *An. philippinensis* [8].

Gia Lai province is located in the North of the Central Highlands, Vietnam; is considered one of the provinces with the most number malaria cases in the country. Like other provinces in the Central Highlands, Gia Lai also attracts a large number of migrant workers, contributing to the increased risk of malaria parasite transmission from one area to another. In the recent years, the number of malaria cases recorded in Gia Lai province has decreased significantly (4,424 cases in 2014, 2,245 cases in 2015 and 575 cases in 2016). However, the number of malaria cases is tending to increase again when 845 cases were recorded in 2017; 1,106 cases in 2018 (including 3 cases of malignant malaria). The regions that have recorded many malaria cases in Gia Lai province include the districts of Ia Pa (113 cases), Chu Prong (65 cases), Duc Co (95 cases), Krong Pa district (563 cases); in which, Ia Dreh commune (Krong Pa distrist) has 130 cases (according to Gia Lai Provincial Center for Disease Control). At present, climate change and changes in farming habits of local people have changed environmental conditions, affecting species component and *Anopheles* mosquitoes activity behavior.

We carried out this surveys in order to study species component and activity behavior of *Anopheles* mosquitoes in the endemic malaria areas, contributing to help managers devise measures for effective malaria vector control, prevent and push back malaria in the coming time.

2. SUBJECTS, METHODS OF THE STUDY.

2.1. Subjects of the study:

16 species of the *Anopheles* were collected in the village and forest sites, in Ia Dreh commune, Krong Pa district, Gia Lai province. Collect mosquitoes in two periods: in the dry season, from January 1 to January 28, 2019; and in the rain season, from June 29, 2019 to July 24, 2019.

Species identification by the methods of molecular biology and analysis of results in the laboratory, at the Military Institute of Preventive Medicine.

2.2. Methods of the study:

- Study design: experimental, descriptive sudy.

- Collect mosquitoes in the village habitat by 2 types of traps and mouth aspirator:

+ Human-baited Double Net trap (HDN): collection time from 18:00 to 24 hrs daily. Place one indoor HDN trap and one outdoor HDN trap (minimum 100m apart).

+ CDC light traps (LT): collection time from 6 p.m the night before to 6 a.m the next morning, collecting traps once in the early morning. Put 2 light traps indoors and 2 light traps outdoor (minimum 100m apart).

+ Mosquitoes collection around cattle shed by mouth aspirator (CS): collection time from 18 h 00 to 24 h 00 daily;

Specimens collected during different time frames were placed in different paper cups, which were changed every 1 hour of collection.

- Collect specimens in the forest sites: set up a similar HDN trap to collect specimens with outdoor HDN traps in the village.

- Management specimens in the laboratory:

+ Morphological identification of the specimens by the Olympus SZ binocular magnifying glass, according to the identification key of NIMPE (2008) [10]. The head and thorax of specimens belonging to the sibling species complex and closely related species group including *Funestus, Maculatus* and *Dirus* was extracted for DNA by the QIAamp DNA kit according to the manufacturer's instructions.

+ Species identification by molecular biology Multiplex PCR method: identification of *Dirus* sibling species complex according to the process of Huong et al (2002) [7]. Identification of *Funestus* group according to the process of Garos et al (2004) [2]. Identification of *Maculatus* group according to the process of Walton et al (2007) [15].

- Data processing: by Excel software and medical statistical methods.

3. THE RESULTS OF THE STUDY.

3.1. *Anopheles* diversity:

A total of 9,567 mosquitoes, belonging to 16 species of *Anopheles* were collected. Of which, There is one primary malaria vector (*Anopheles dirus* s.s.*)* and 7 secondary malaria vectors *An. aconitus, An. campestris, An. barbirostris, An. maculatus* s.s., *An. philippinensis, An. sinensis,* and *An. vagus*.

Table 1. Species component and seasonal distribution of collected *Anopheles* mosquitoes.

No.	Species name	Dry season	Rainy season	Total
1	#An. aconitus*	53 (1.87%)	2 (0.03%)	55 (0.57%)
2	An. campestris*	17 (0.6%)	0	17 (0.18%)
3	#An. dirus s.s.**	70 (2.48%)	21 (0.31%)	91 (0.95%)
4	An. barbirostris*	0	3 (0,04%)	3 (0.03%)
5	An. crawfordi	0	6 (0.09%)	6 (0.06%)
6	An. jamesi	8 (0.28%)	1 (0.01%)	9 (0.09%)
7	An. kochi	73 (2.58%)	0	73 (0.76%)
8	#An. sawadwongporni	8 (0.28%)	0	8 (0.08%)
9	#An. maculatus s.s.*	0	16 (0.24%)	16 (0.17%)
10	An. monstrosus	182 (6,44%)	0	182 (1.90%)
11	An. peditaeniatus	292 (10.33%)	81 (1.20%)	373 (3.90%)
12	An. philippinensis*	3 (0.11%)	4 (0.06%)	7 (0.07%)
13	An. sinensis*	361 (12.77%)	173 (2.57%)	534 (5.58%)
14	An. splendidus	12 (0.42%)	2 (0,03%)	14 (0,15%)
15	An. tessellatus	159 (5.62%)	4 (0.06%)	163 (1.70%)
16	An. vagus*	1,590 (56.22)	6,426 (95.36%)	8,016 (83.79%)
Total		2,828 (100%)	6,739 (100%)	9,567 (100%)

** Secondary malaria vector; ** Primary malaria vector in Vietnam (According to WHO, 2017); # species belonging to the sibling species complex or closely related species, identification by molecular biology method; other species by Morphological identification.*

3.2. Seasonality:

Among 16 species collected (table 1), there were 9 species occurring both in the rainy and dry seasons, including *An. aconitus, An. dirus s.s., An. jamesi, An. peditaeniatus, An. philippinensis, An. sinensis, An. splendidus, An. tessellatus* and *An. vagus*. There were 3 species that appear only in the rainy season (*An. barbirostris, An. crawfordi* and *An. maculatus s.s.*) and 4 species that appear only in the dry season (*An. campestris, An. kochi, An. monstrosus* and *An. sawadwongporni*). Most species collected in the dry season had more specimens than in the rainy season, including *An. dirus s.s.* with 70 samples in the dry season and 21 samples in the rainy season. Meanwhile, species *An. vagus* collected 6,426 samples in the rainy season, more than 1,590 samples collected in the dry season. In both seasons, species *An. vagus* was always the dominant species in the Ia Dreh commune: 56.22% of samples in the dry season and 95.36% of samples in the rainy season. Among the remaining species, *An. sinensis* accounted for the highest proportion of 12.77% in the dry season and 2.57% in the rainy season. Species *Anopheles dirus s.s.*, the primary vector of malaria, accounting for only 2.48% of the total number of samples collected in the dry season and 0.31% in the rainy season. The difference in the number of *Anopheles* mosquitoes between the two seasons was statistically significant (with $p < 0.05$).

3.3. Activity behavior:

Table 2. Results of *Anopheles* mosquitoes collection in forest/vilage sites, collection locations and collection methods.

No.	Species name	Collection sites		Collection methods			Collection locations		Total
		Forest	Village	HDN	LT	CS	Outdoor	Indoor	
1	*An. aconitus**	11	44	11	7	37	55	0	55
2	*An. campestris**	0	17	0	1	16	17	0	17
3	*An. dirus* s.s.**	91	0	91	0	0	91	0	91
4	*An. barbirostris**	2	1	2	0	1	3	0	3
5	*An. crawfordi*	1	5	0	1	5	6	0	6
6	*An. jamesi*	0	9	0	0	9	9	0	9

7	*An. kochi*	0	73	1	5	67	73	0	73
8	*An. sawadwongporni*	5	3	5	0	3	8	0	8
9	*An. maculatus* s.s.*	16	0	16	0	0	16	0	16
10	*An. monstrosus*	1	181	1	7	174	182	0	182
11	*An. peditaeniatus*	4	369	8	38	327	367	6	373
12	*An. philippinensis**	1	6	1	2	4	7	0	7
13	*An. sinensis**	20	514	4	21	509	534	0	534
14	*An. splendidus*	1	13	1	4	9	14	0	14
15	*An. tessellatus*	23	140	22	12	129	163	0	163
16	*An. vagus**	258	7,758	47	69	7,900	8,000	16	8,016
Total		434	9,133	210	167	9,190	9,545	22	9,567

In the forest sites, 434 samples (4.54%) were collected, belonging to 13 species (including one primary vector and 6 secondary vectors); meanwhile, in the village sites, 9,133 samples (94.46%) were collected, belonging to 14 species (6 secondary vectors, no primary vector). The difference in the number of *Anopheles* mosquitoes between the forest and village sites was statistically significant ($p < 0.05$).

The method of CS collected 9,190 samples (96.06%), belonging to 14 species. While the HDN method collected 210 samples (2.20%), belonging to 13 species; The light trap method collected 167 samples (1.75%), belonging to 11 species. The number of *Anopheles* mosquitoes caught by the CS method compared with the HDN and the LT, the difference was statistically significant ($p < 0.05$), while between the LT method and the HDN, the difference was not statistically significant ($p > 0.05$).

At the outdoor traps 9,545 samples (99.77%) were collected, belonging to 16 species; meanwhile 22 samples (0.23%) were collected at Indoor traps, belonging to 2 species. The number of *Anopheles* mosquitoes collected between theindoor and outdoor was statistically significant ($p < 0.05$).

Among a total of 16 species collected in the Ia Dreh commune, there were two species of *An. dirus s.s.* and *An. maculatus s.s.* only collected in the forest habitats, by outdoor HDN traps.

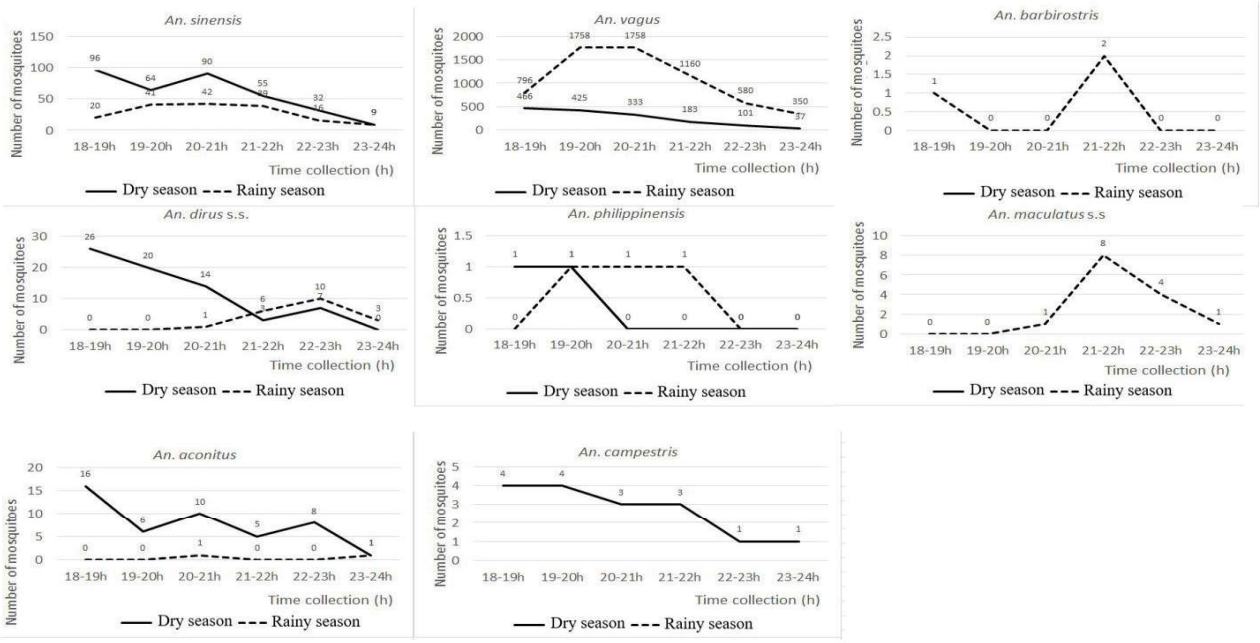
Table 3. Species component and number of samples collected in each habitat by the HDN method.

No.	Species name	Collection sites by the HDN method			Total
		Vilage - Outdoor	Forest - Outdoor	Village - Indoor	
1	*An. aconitus**	0	11	0	11
2	*An. campestris**	0	0	0	0
3	*An. dirus s.s.***	0	91	0	91
4	*An. barbirostris**	0	2	0	2
5	*An. crawfordi*	0	0	0	0
6	*An. jamesi*	0	0	0	0
7	*An. kochi*	1	0	0	1
8	*An. sawadwongporni*	0	0	0	0
9	*An. maculatus s.s.**	0	21	0	21
10	*An. monstrosus*	0	1	0	1
11	*An. peditaeniatus*	3	1	4	8
12	*An. philippinensis**	0	1	0	1
13	*An. sinensis**	4	0	0	4
14	*An. splendidus*	1	0	0	1
15	*An. tessellatus*	0	22	0	22
16	*An. vagus**	27	7	13	47
Total		36	157	17	210

Compare the results collected by HDN traps in different sites, it showed that the number of samples and the number of species collected by outdoor HDN traps - in the forest sites dominated with 157 samples, belonging to 9 species (one primary vector and 5 secondary vectors); the outdorr HDN trap - in the village sites with 36 samples, belonging to 5 species (2 secondary vectors, no primary vector); The lowest collection was by indoor HDN traps in the village sites with 17 samples, belonging to 2 species (one secondary vector, no primary vector). The number of *Anopheles* mosquitoes collected by HDN traps between the three methods was statistically significant ($p < 0.05$).

Among 210 specimens collected by HDN traps, *An. dirus* s.s. accounted for the majority with 91 samples (43.33%), followed by *An. vagus* with 47 samples (22.38%), *An. tessellatus* with 22 samples (10.48%), *An. maculatus* s.s. with 21 samples (10.0%). The remaining species accounted for less than 10% of the total collected samples.

The host-seeking activity of malaria vectors different between species and between the two seasons (Figure 1). In general, in the dry season, malaria vectors have a peak of early predation (18-19 h 00) and reduced until midnight. Meanwhile, in the rainy season, the peak host-seeking activity of malaria vectors started later than in the dry season. The primary vector *An. dirus* s.s. have peak host-seeking activity in the rainy season at 22-23 h 00, other secondary vectors have peak activity usually after 19 h 00, such as *An. vagus* (peak host-seeking activity in the frame 19-21 h 00), *An. sinensis* (peak activity between 19-22 h 00)…



Peak of host-seeking activity of malaria vectors in Ia Drehcommune.

4. DISCUSSION.

4.1. *Anopheles* diversity:

According to Garros (2008), in Kong Chro district, Gia Lai province (about 45 km from Ia Dreh commune), 11 species of *Anopheles* have been collected [3]. However, the authors based on morphological identification only, and thus it did not separate members on sibling species complex were found in their study.. Compared with Garros' study, this study added more 7 morphological species of *Anopheles* for Gia Lai province, including *An. campestris, An. barbirostris, An. crawfordi, An. monstrosus, An. peditaeniatus, An. splendidus* and *An. tessellatus.* In addition, we found two sibling species *An. macualtus* s.s. and *An. sawadwongporni* in complex *An. maculatus* s.l.. In our study, three species were absented in comparing to the Garros et al (2008) results, including *An. minimus* s.l., *An. jeyporiensis* and *An. nivipes*; this result posible due to the difference in collection time [3]. Thus, by combining the results of two studies, the component of *Anopheles* mosquitoes in Gia Lai recorded so far was 19 species.

4.2. Seasonality:

Normally, in the rainy season, the number of *Anopheles* mosquitoes collected will increase because of increasing of the number of breeding sites, as studies by Rosenberg in Bangladesh or by Sungvornyothin in Thailand [11, 13]. In this study, the results of specimens collection from 18 h 00 to 24 h 00 daily, showed that the total number of *Anopheles* mosquitoes collected in

the rainy season was higher than in the dry season, mainly due to the sudden increase in the number of samples of *An. vagus* in the rainy season (6,426/6,739) compared to the dry season (1,590/2,828). Moreover, it was also due to the absent of 3 species in the dry season. The remaining species had quite obvious decline in numbers: *An. dirus s.s.* reduced from 70 samples in the dry season to 21 samples in the rainy season; *An. sinensis* reduced from 361 samples in the dry season to 173 samples in the rainy season… This result may be related to the host-seeking time of *Anopheles* mosquitoes in the study area. The peak host-seeking time of *Anopheles* in the dry season was recorded from 18-19 h 00 (781 samples) and then completely decreased at nearly midnight (68 samples). Meanwhile, in the rainy season, their hos-seeking time peaks around 20-21 h 00 (1,824 samples), however, up to midnight they were still quite active (367 samples). According to the Central Highlands Hydro-meteorological Station, in Gia Lai province in 2019, the rainy season came late, the rainfall was lower, and the outdoor temperature was higher than the average for many years. According to our personal records, from the beginning of the rainy season to the time of the study, the weather was hot and dry in Ia Dreh commune, Krong Pa district, no rain for long . These weather conditions have greatly reduced the number of breeding sites for *Anopheles* mosquitoes. There for, reducing the number of mosquitoes collected. Meanwhile, at the beginning of the dry season (January), breeding sites still exist quite a lot in the Ia Dreh commune, so *Anopheles* mosquitoes still have breeding places and maintain population density in the study area.

4.3. Host-seeking behavior:

Seasonal weather changes have a great influence on the species component and distribution of *Anopheles* mosquitoes. In the dry season, the peak of host-seeking time of *Anopheles* usually came before 20-21h 00 , *An. dirus s.s., An. sinensis* and *An. vagus* were most active at 18-19 h 00. At this time, the local people were still active outdoor. therefore, the using of long-lasting insecticide-treated net (LLIN) as the recomment of National Malaria Control Program (NMCP) in areas where vectors bite outdoors and/ or early in the evening will not be very efective.. In addition, the almost of species and numbers of samples were collected outdoor compared to indoors (include *An. dirus s.s.*). This behavior of *Anopheles* mosquitoes seems to be a way to avoid the effects of indoor residual spraying (IRS). Thus, in the Ia Dreh commune, the behavior of *Anopheles* mosquitoes had now reduced the protective effectiveness of the measures to prevent mosquitoes bites in the "National malaria control program", such as LLIN and IRS for people in endemic areas, especially in the dry season. Perhaps, the use of other individual malaria control methods such as insect repellant applied on the skin and/or chemical impregnated clothing for people in the Ia Dreh commune when outdoor activities at dusk should be studied and supplement.

Although, 96.06% of the mosquitoes collected in this study were around the cattle shed, but still 2.20% of the *Anopheles* mosquitoes were collected by HDN traps; of which, there were 12 species (one primary vector and 6 secondary vectors). Thus, most of *Anopheles* species (12/16) in the Ia Dreh commune were anthropophilic behavior. , there were different roles of malaria transmission between them. *An. dirus s.s.* was the primary vector of malaria transmission in the region; 100% of samples collected by HDN trap showed its anthropophilic behavior. In the village sites, cattle were often kept close to people's houses, which was a condition to increase *Anopheles* density and suck human blood if given the opportunity. The fact that the results obtained, 53 samples belonging to 5 species (2 secondary vectors) collected by HDN traps in the commune habitat have partly shown that. Forest site occured 74.74% collected samples by HDN method, belonging to 9 species (one primary vector and 5 secondary vectors), showed a high risk of malaria transmission in forest site. Especially the primary malaria vector *An. dirus* s.s*. were found only in* the forest sites, outdoors, early host-seeking time and anthoropophilic behavior have shown their main role in malaria transmission in the Ia Dreh commune. In this study, *An. dirus* s.s. found only in forest site. This explained why most local malaria patients have a history of going to and working in the forest for a period of time before being infected (as noted by the Krong Pa District Health Center). This result can be used to help policy makers of malaria control and prevention in the prevention and control of disease vectors by paying more attention to those who were living and working related to forest environment, helping people take extra precautions or change their activity habits, avoid working outdoors, in the forest, especially at 18-20 h 00 in the dry season.

The amount of specimens collected by LT in this study accounted for only 1.75% of the total samples, including 11 species; of which there were 5 secondary vectors. This was a convenient in installation and use method. This showed that light traps was still effective in assessing the component of *Anopheles* species in the study area.

5. CONCLUSION.

This study collected 16 species of *Anopheles* in the Ia Dreh commune, Krong Pa district, Gia Lai province. Supplementing the previous research

results of Garros et al. in Gia Lai province (additional 7 morphological species and 1 sibling species), bringing the total number of species recorded in Gia Lai to the time of completion of the study to 19 species. *Anopheles* mosquitoes in the Ia Dreh commune had peak of host-seeking time at 18-19 h 00 in the dry season and 20-21 h 00 in the rainy season. They were active mainly outdoor and around the cattle shed in the commune. Most *Anopheles* species have a anthropophilic behavior, although the majority of samples were caught around cattle shed. *An. dirus s.s.* played a very important role in malaria transmission in the Ia Dreh commune, especially in forest habitat.

From the results of this study, we believe that, in order to improve the effectiveness of malaria prevention and control, better protect the health of people in the Ia Dreh commune in particular and malaria-endemic areas in general, we should continous to follow the recoment of NMCP as apply LLIN and IRS. Beside, it was recommended that people avoid outdoor activities from 18-20 h 00 in the dry season, using more personal preventive measures such as mosquito repellant, chemical-impregnated clothing, etc., especially, in forest environment when there were outdoor activities in the dusk of the dry season.

REFERENCES:

1. Do Manh C, Beebe N.W, Van V.N, Quang T.L, Lein C.T, Nguyen D.V, Xuan T.N, Ngoc A.L, Cooper R.D (2010), "Vectors and malaria transmission in deforested, rural communities in north-central Vietnam", *Malar J*, 9: 1-12.

2. Garros C., Koekemoer L.L., Coetzee M., Coosemans M., Manguin S. (2004), "A single multiplex assay to identify major malaria vectors within the African *Anopheles funestus* and the Oriental An. minimus groups", *Am. J. Trop. Med. Hyg*, 70: 583-590.

3. Garros C, Nguyen C.V, Trung H.D, Bortel W.V, Coosemans M, Manguin S (2008), "Distribution of *Anopheles* in Vietnam with particular attention to malaria vectors of the *Anopheles minimus* complex", *Malaria Journal*, 7: 11.

4. Harbach R.E (2004), "The classification of the genus Anopheles (Diptera: Culicidae): A working hypothesis of phylogenetic relationships", *Bull. Entomol*, Res. 94: 537-553.

5. *http://www.who.int/malaria/publications/country-profiles/profile_vnm_en.pdf*

6. Hung L.Q, de Vries P.J, Giao P.T, Nam N.V, Binh T.Q, Chong M.T, Quoc N.T.T.A, Thanh T.N, Hung L.N, Kager P.A (2002), "Control of malaria: a successful experience from Viet Nam", *Bull World Health Organ*, 80: 660-665.

7. Huong N.T, Sonthayanon P, Ketterman A.J, Panyim S (2001), "A rapid polymerase chain reaction based method for identification of the *Anopheles dirus* sibling species", *Southeast Asian J Trop Med Public Health*, 32 (3), pp. 615-20.

8. Ngo C.T, Dubois G, Sinou V, Parzy D, Le H.Q, Harbach R.E, Manguin S (2014), "Diversity of Anopheles mosquitoes in Binh Phuoc and Dak Nong Provinces of Vietnam and their relation to disease", *Parasit Vectors,* 7: 316.

9. Nguyen Van Dung (2015), "Checklist of the known species of Culicidae found in Vietnam. National Scientific", *Conference on Ecology and Biological Resources*, 6: 504-509.

10. NIMPE (2008), *Keys to Identify the Anopheles Mosquitoes* (Adults - Pupae - Lavae), Hanoi, Department of Entomologie - Institute of Malariology, Parasitology and Entomology.

11. Rosenberg R, Maheswary N.P (1982), "Forest malaria in Bangladesh. II, Transmission by Anopheles dirus", *Am Journal Trop Med Hyg*. 31: 183-91.

12. Schuftan C (2000), *A story to be shared: the successful fight against malaria in Vietnam.* WHO WPRO and the global Roll Back Malaria Program, *http://www.afronets.org*.

13. Sungvornyothin S, Kongmee M, Muenvorn V, Polsomboon S, Bangs M.J, Prabaripai A et al (2009), "Seasonal abundance and blood feeding activity of *Anopheles dirus* sensu lato in western Thailand", *Journal Am Mosq Control Assoc*, 25: 425-430.

14. Trung H.D, Van Bortel W, Sochantha T, Keokenchanh K, Briët O.J.T, Coosemans M (2005), "Behavioural heterogeneity of *Anopheles* species in ecologically different localities in Southeast Asia: a challenge for vector control", *Trop Med Int Health*, 10: 251-262.

15. Walton C, Somboon P, O'Loughlin S.M, Zhang S, Harbach R.E, Linton Y-M et al. (2007), "Genetic diversity and molecular identification of mosquito species in the *Anopheles maculatus* group using the ITS2 region of rDNA", *Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis*, 7: 93-102.

16. World Health Organization (2007), "Anopheles species complexes in South and South East Asia", *SEARO Technical Publication*, 57. 102 pp.

17. World Health Organization (2017), *http://www.who.int/malaria/publications/country-profiles/profile_vnm_en.pdf.* ❑