### PREAMBLE

Malaria is an infectious disease caused by a malaria parasite (mp), carried by Anopheles mosquitoes (Am) from an infected individual to an uninfected one. It remains a top issue of public health posing a threat to the health and life of human communities. Environmental impacts from hydroelectricity and water resource development projects on Am, and malaria transmission factors i.e., humans, malaria mosquitoes and mp. are of a growing concern to be studied in Vietnam as well as in the world.

In Vietnam, in the last 10 years (2003-2013) malaria has been rolled back step by step; however the "hot spots" of endemic malaria still are the Central and West-Highlands, and Southeastern Vietnam, where there is a rate of 90% malaria incidence and deaths in the entire country, particularly including Gia Lai Province where there are hundreds of hydroelectricity and water resource development projects, large and small, with little knowledge of their extent of impacts on malaria transmission. So, a study to be made on taxonomy and distribution of anopheline mosquitoes; biological characteristics, habitats and disease transmission role of malaria vectors will provide a basis for the identification of appropriate vector control measures to be implemented in the above malaria-endemic regions.

Gia Lai is a province located in the north of Central Highlands, most of which province is highly malaria-endemic as a result of a change in malaria landscaping patterns from the process of socio-economic development over the past several years, involving electricity and water resources projects. Malaria infection exposure primarily focuses on the landscaping pattern of field huts in the forest or on the edge of the forest rather than in the village as has been seen before, along with hard-to-becontrolled patterns of malaria infection from "working in the forest and sleeping there in field huts", all those leading to persistently increased, if not reduced, malaria transmission and a high risk of recurring malaria outbreaks in recent years, thus requiring sustainable interventions.

From the above-raised issues, the research paper entitled " Study on Species Composition of Anopheles Mosquitoes, Biology and Habitats of Malaria Vectors (mvs) for Proposed Control Measures in the Areas of Hydroelectricity and Water Resource Development Projects in Gia Lai Province" aims at achieving the following two objectives:

1) To determine the species composition and distribution of Anopheles mosquitoes, biological and ecological characteristics and transmission role of malaria vectors in the areas of hydroelectricity and water resource development projects;

2) To propose appropriate measures for malaria vector control in the areas of hydroelectricity and water resource development projects in Gia Lai Province.

# SCIENTIFICITY, PRACTICALITY AND NOVELTY OF THE DISSERTATION

**1. Scientificity:** Based on correlation among malaria transmission factors, i.e., humans, malaria mosquitoes and mp, the disseration has clarified a fluctuation in species composition and distribution of Am, malaria vectors and parasite rate in the context of inner-forest and outer-forest landscaping as a high risk of malaria infection exposure for those people working and sleeping in the forest; thus being hard to be controlled in the areas of electricity and water resource development projects under the scope of study.

**2. Practicality:** The results of longitudinal monitoring (2002-2013) and horizontal surveys (2013-2014) in terms of malaria parasite rates, along with KAP surveys have shown that the target populations have not yet turned their awareness into actions of malaria control, which is the cause of exposing themselves to malaria infection from forests and mountain fields. The research paper for dissertation has justified an information, education and communication (IEC) model through "Community's anti-malaria volunteers" campaigns to help the target populations with their self-control of mvs once going to the forest.

**3.** Novelty: There are 23 species of Anopheles mosquitoes identified and determined, including 21 morphologically taxonomized, two (2) determined under PCR method and three (3) others, namely *An. pampanai*, *An. varuna* and *An. notarandai* not found previously in the area of study. Failure to capture a malaria vector (daytime indoor flashlighting) is likely due to a change in its habit of indoor resting after blood feeding. The main vector has only been noted in inner and outer forest habitats, not noted in the village, which is morphologically predominated by forest and field malaria. A pilot program under which volunteers from senior high schools join health workers, along with the implementation of other routine malaria control measures is a sustainable likelihood of helping human communities to control malaria by themselves once they are in the forest.

# **Organization of the Dissertation**

In addition to the Preamble covering two (2) pages, Conclusion two (2) pages and Recommendations one (1) page, the dissertation contains four (4) chapters with 190 pages, 46 tables and 19 figures/pictures. Chapter 1: An overview of relevant documents (48 pages). Chapter 2: Study subjects and methodology (25 pages). Chapter 3: Research study results and findings (48 pages). Chapter 4: Discussions (40 pages). Scientific research projects related to the Dissertation (1 page). Annexes and 173 refrences (80 in Vietnamese, 95 in English).

# Chapter 1. AN OVERVIEW OF RELEVANT DOCUMENTS 1.1 Malaria Situation and Malaria Mosquitoes in the World

According to the WHO, as of 2014 there remained 97 countries and territories endemic for malaria, with a population of 1.2 billion at high risk of malaria infection. In 2013, there were 198 million malaria cases and 548,000 malaria deaths. 90% malaria deaths in Africa were children under 5 years age. The expansion of intervention measures has resulted in malaria incidence being reduced by 30%, deaths from malaria by 47% globally.

According to Mansen (1900), Anopheles is a malaria vector with 545 species of three genera, viz., Anopheles, Bironella and Chagasia Cruz. According to Bruce-Schwatt I.J (1966), there are 422 species of Anopheles, but 70 of which are able to transmit diseases, depending on the environmental condition, separately including Asia where there are some malaria vectors of the two sub-genera Anopheles and Cellia. Manguin S, et al (2008) differentiated main vectors in the Southeast Asia, comprising comlexes of *An. dirus, An. minimus, An. culicifacies, An. leucosphyrus, An. sundaicus, An. fluviatilis* from the species of *An. maculatus*. The change in natural environment, involving hydroelectricity and water resource development projects, have had effects on the variation in species composition of Anopheles and transmission role of malaria vectors

# 1.2 Malaria Situation and Malaria Mosquitoes in Vietnam

According to IMPE Quy Nhon, the malaria incidence of 2014 has reduced, compared with 2003 over the same period, by 21.29% in terms of malaria cases, mp. rate by 8.03%, severe malaria cases by 13.10%, malaria deaths by 16.67%, without malaria epidemic outbreaks. However, the parasite rate remains in the West-Highlands (48.95%), Central (35.29%) and Southern (11.62%), including Gia Lai where there has occurred the highest rate of 27.72% compared with that of the entire country.

In 2008, Vietnam noted 64 species of Anopheles, 15 of which are able to carry malaria. The main vector is *An. minimus* in the mountainous areas of the North, *An. minimus* and *An. dirus* in the mountainous areas of the South, and *An. epiroticus* in the coastal areas of the South. The subsidiary vectors in the mountainous areas of the entire country are *An. aconitus*, *An. jeypriensis*, *An, maculatus*, and in the coastal areas being *An. subpictus*, *An. sinensis* and *An. vagus*. Le Van Sac et al (1985) made a study on malaria-carrying mosquitoes in the area of Dac Uy Hydroelectric Project prior to the commencement date. Bui Dinh Bai et al related mp. to water resource projects in the Central and West-Highlands (1982-1990). Nguyen Xuan Quang (2002) identified environmental impacts on the population of Anopheles in the area of Dac Uy Hydroelectric Project (Kontum Province). Le Dinh Cong et al (2001) determined the epidemiological characteristics of malaria and control measures for the area of Son La Hydroelectic Project. Le Xuan Hoi et al (2011) conducted an investigation on the developments of mosquito species, ecological characteristics of some anopholine species and *An. minimus* in the process of changed habitat conditions in the area of Tuyen Quang Hydroelectric Project (2006-2008). Tran Quoc Tuy et al (2006) determined epidemiological characteristics of malaria and control measures for the area of Son La Hydroelectic Project. These findings are attributed to the identified biological and ecological characteristics and biting behavior patterns of malaria vectors serving as a scientific rationale for the selected measures for malaria control in the areas of hydroelectricity and water resource development projects.

**1.3** Malaria Situation in Gia Lai and Justifications for Research Work

Gia Lai is the "major concern of malaria" of the country with an annual parasite rate of 27.72% (2014), predominated by rural districts with many hydroelectricity and water resource development projects, i.e., Krong Pa District regarded as a "malaria hot spot" making up 50% parasite rate of Gia Lai or Ia Grai District with Se San Hydroelectric Project dominated by a parasite rate not falling down by lower than 6%.

The research findings from some authors have shown that there is a fluctuation in the number of Anopheline species in the West-Highlands indicative of 38 species (Nguyen Duc Manh, 1988); 45 species in Central and West-Highlands (Le Khanh Thuan, 1997); 24 species in Gia Lai (Truong Van Co, 2000), 18 species in Kon Ka Kinh National Parks (Nguyen Xuan Quang, 2012). As per the results of epidemiological and entomological surveys on the areas of Krong Pa and Ia Grai Districts, there were 25 species of malaria mosquitoes for the period 2002-2005, 23 species (2006-2009) and 22 species (2010-2013). Such study focused primarily on the characteristics of the two main vectors, namely *An. dirus* and *An. minimus*. In spite of differing in their living habits and habitats, they are characterized in common by their role in malaria transmission.

Genrally, malaria situation and Am in the world, in Vietnam, in Gia Lai have shown a correlation among transmission factors, namely malaria mosquitoes, mp. and humans at risk from malaria infection, associated with changed environment, habitat condition, and malaria situation in a particular area or region. The study on classification and distribution of Anopheles and their biological and ecological characteristics and role in malaria transmission is a scientific rationale for proposing appropriate interventions, thus contributing to the protection of human communities in the areas of hydroelectricity and water resource development.

## Chapter 2. SUBJECTS UNDER THE STUDY AND METHODOLOGY

**2.1.** Subjects under the study: involving the species of Anopheles, malaria parasites, human communities, environmental factors and landscape, and climatic patterns in the area of study.

## 2.2. Study Sites

- Selected study sites are located in the areas impacted by hydroelectricity and water resource development projects with endemic malaria specific landscape , many malaria cases and complicated developments of malaria transmission.

- Selected are the areas of Krong Pa Hydroelectricity and Water Resource Project, located to the southeast of Gia Lai Province, sharing the boundary with Central Coastal Region, characterized by persistent malaria transmission from 2010 to the present, and of Se San Hydroelectric Project in Ia Grai, located to the southwest of Gia Lai Province, bordering on Cambodia, characterized by low malaria endemicity.

- Selected are also the four (4) sites, namely Chu Rcam and Ia Rsuom in the area of Krong Pa Hydroelectricity and Water Resource Project, and Ia O and Ia Chia in the area of Se San Hydroelectric Project in Ia Grai District. Each site has three (3) malaria specific landscapes selected, i.e., in inner and outer forests and in the village.

Therefore, in the two areas of hydroelectricity and water resource projects there are four (4) study communes with 12 survey sites representative of three (3) landscapes.

## 2.3. Period of Study: from September 2012 to December 2014

- October 2012: field surveys conducted

- April 2013-December 2014: study investigation conducted at a frequency of 4 visits per year in 3 landscaping areas, viz. inner and outer forests and in the village of the four study sites of Chu Rcam, Ia Rsuom, Ia O and Ia Chia, specifically in April (by the end of dry season), August (in the middle of wet season) and October (by the end of wet season).

# 2.4. Study Materials and Equipment

Involving entomological survey equipment, such as light traps, mosquito tubes etc.,. and laboratory apparatuses and supplies, i.e., ELISA, PCR, microscopes, biological specimens, chemicals etc.,.

#### 2.5. Scope of Study

Determination of species composition and distribution of Anopheles, and of biological and ecological characteristics and role in malaria transmission of malaria vectors. Assessment of environmental impacts, parasite rates; knowledge, attitude and practice (KAP) in malaria control and IEC activities for malaria self-control through " Community's anti-malaria volunteers" who are high schoolers from the study sites in the area of hydroelectricity and water resource development projects.

#### 2.6. Study Methodology

2.6.1 Study Design

- Retrospective cohert study:

+ Data and figures for 12 years' period (2002 - 2013) on species composition of Anopheles and parasite rates in the two areas of study, namely Krong Pa and Ia Grai, obtained from the visits of epdemiological investigation and surveillance undertaken by Quy Nhon IMPE and Center for Malaria Control, Parasitology and Entomology of Gia Lai Province

+ Data and figures for 12 years' period (2002 - 2013) on temperature, humidity and rainfall obtained from Center of Meteorology and Hydrology of Gia Lai Province. Data and figgures for 37 years' period (1976 - 2013) on changes in environment, landscape (natural forests, planted forests and coverage of hydroelectricity/irrigation reservoirs) obtained from the Office of Agriculture and the Office of Natural Resources and Environment Management of Krong Pa and Ia Grai districts. Residential areas (established after relocation or previously stablized resettlement, workers' living areas and not-yet-relocated locals)

- Horizontal Study:

+ Entomological investigation: in accordance with WHO (1975), Central IMPE (2011, 1987 and 2008). Eight (8) investigation visits were conducted during the two years (2013 - 2014) in the months of April, June, August and October at four (4) representative sites of the two areas of hydroelectricity and water resource development projects (4 sites x 3 landscapes x 8 visits = 96 visits); thereby continually monitoring species composition, distribution, biological and ecological characteristics, and role in malaria transmission of malaria mosquitoes (1 site/ 4 nights x 6 methods x 3 landscapes x 8 visits x 4 nights = 2,304 nights). Collecting mosquitoes by six (6) mthods involved indoor light traps, outdoor light traps, indoor human baits, outdoor human baits, indoor torching, livestock and poultry shelter torching. Larval collection from catchments/basins, such as creeks, still water areas, rice fields, canals and channels/ditches.

+ The epidemiological study was undertaken once every two months, concurring with the time of entomological study (examination to detect malaria cases, with spleen enlargement and to have their blood tested for malaria parasites: 1 site/400 persons x 4 sites x 7 visits = 12,800 person visits under the investigation)

2.6.2. Method of Sampling

- Size of survey sample for malaria parasite index:

$$N= \begin{array}{c} Z^2{}_{(1-\alpha/2)} \ pq \\ \hline d^2 \\ \end{array} \times k$$

Where  $Z_{(1-\alpha/2)}$ : corresponding to  $\alpha$  value. P: mp. rate of the previous survey. q = 1 - p; d: accuracy; k: design efficiency. Let P = 5% = 0,05; Z = 1.96; d = 0.03 và k = 2. Therefore N = 406  $\approx$  400 persons/study site/1 survey visit

- Size of KAP survey sample: randomly chosen convenient samples with size 400 (representative of 400 households) of 8 hamlets of 4 study communes, with 2 hamplets/commune and 50 HHs/random sample.

- Size of intervention and control samples: ramdomly chosen convenient samples combined with KAP survey samples with a size of 200 persons/200 HHs of 4 hamlets of two intervention communes namely Ia Rsuom and Ia O. Likewise, the size of control sample is 200 persons/200 HHs of 4 hamlets of two control communes namely Chu Rcam and Ia Chia.

- Size of Anopheles mosquito survey by the method of crosssectional survey, depending on the timing of survey.

2.6.3. Techniques involved in the study

Technique for mosquito surveys (WHO, 1975); taxonomy of mosquitoes and larvae (Central IMPE 1971, 1987, 2011); ELISA technique in accordance with Wirtz et al (1985, 1987); PCR technique in accordance with Ngo Thi Huong and asociates (2001), Hoang Kim Phuc et al (2003). 2.6.4. Assessment Indicators

The density of Anopheles mosquitoes collected by using human baits and ligh traps. The density of larvae collected from catchments. The rate of mosquitoes infected with malara parasites (S) by ELISA method; the rate of egg-laying mosquitoes, density of human-biting mosquitos (ma); time of blood digesting cycle and of sporozoite cycle.

2.6.5. Processing of collected survey samples and data

- The number of mosquitoes surviving dissection has their ovaries observed and examined to determine laying mosquitoes in accordance with WHO (1994). Post-dissection and taxonomization mosquito bodies are retained for ELISA and PCR tests.

- Analysis, processing and display of data and figures on Excel spreadsheet and STATA 6.0.

2.6.6. Ethics of Study Approach

The study approach was cleared by the Ethics Council of Quy Nhon IMPE. The persons under the research study are completely voluntary, who are medically examined and treated free of charge for malaria if they have contracted it during the process of being involved in the study. **Chapter 3. RESEARCH STUDY RESULTS AND FINDINGS 3.1 Species composition and distribution of Anopheles, biological and ecological characteristics and transmission role of malaria vectors.** 3.1.1. Species composition of Anopheles (2013 - 2014)

Of 23 species in total of Anopheles (Table 3.1), 21 are taxonomized morphologically and 2 determined by PCR method (*An. pampanai, An. varuana*), including 17 mosquito species and 7 larvae species collected from the area of hydroelectricity and water resource development projects in Krong Pa, and 22 mosquito species and 10 larvae species collected from the area of Se San Hydroelectricity and Water Resource Development Project in Ia Grai.

No.	Species	Krong P	a	Se San		
110.	Species	Mosquitoes	Larvae	Μ	L	
1	An. aconitus	+	+	+	+	
2	An. annularis	+	+	+	+	
3	An. barbirostris	+	+	+	+	
4	An. crawfordi			+	+	
5	An. dirus	+		+		
6	An. jamesi	+		+		
7	An. jeyporiensis	+		+	+	
8	An. kanwari			+		
9	An. kochi	+		+		
10	An. maculatus	+	+	+	+	
11	An. minimus,	+		+		
12	An. montrosus	+		+		
13	An. nitidus			+		
14	An. nivipes			+		
15	An. notanandai			+		
16	An. pampanai			+		
17	An. peditaeniatus	+	+	+	+	
18	An. philippinensis	+		+	+	
19	An. sinensis	+	+	+	+	
20	An. splendidus	+		+		
21	An. tessellatus	+		+		
22	An. vagus	+	+	+	+	
23	An. varuna	+				
	Total	17	7	22	10	

 Table 3.1:
 Species composition of Anopheles (2013 - 2014)

Fully present in the two areas of study are main malaria vectors, namely *An.minimus and An.dirus* and subsidiary vectors viz. *An.aconitus, An.jeyporiensis, An.macalatus*, but the larvae of the main vectors have not been found. The three (3) Anopheline species, *An.pampanai, An.varuna, and An.notanandai* had not appeared before in the areas of study.

3.1.2 Species composition and distribution of Anopheles in Krong Pa At the study sites in Krong Pa District (Table 3.2) have appeared 17
species of Anopheles: Chu Rcam 16 species and Ia RSuom 15 species. *Table 3.2. Species composition and rate of Anopheles at the two study sites of hydrolelectricity and water resource projects in Krong Pa (2013 - 2014)*

		Chư H	RCăm	Ia R	Sươm
No.	Species	Qty	Rate	Qty	Rate
			(%)		(%)
1	An. aconitus	217	3.27	152	2.46
2	An. annularis	209	3.15	606	9.82
3	An. barbirostris	255	3.84	150	2.43
4	An. dirus	116	1.75	52	0.84
5	An. jamesi	60	0.90	0	0.00
6	An. jeyporiensis	131	1.97	94	1.52
7	An. kochi	116	1.75	140	2.27
8	An. maculatus	229	3.45	135	2.19
9	An. minimus	7	0,11	6	0,10
10	An. montrosus	12	0.18	9	0.15
11	An. peditaeniatus	1,154	17.39	1,182	19.15
12	An. philippinensis	931	14.03	512	8.30
13	An. sinensis	1,135	17.11	1,255	20.34
14	An. splendidus	818	12.33	757	12.27
15	An. tessellatus	42	0.63	0	0.00
16	An. vagus	1,203	18.13	1,118	18.12
17	An. varuna	0	0,00	3	0.05
	Total	6,635	100	6,171	100

Table 3.3. Species composition and distribution of Anopheles under landscaping patterns at Chu Rcam study site (2013-2014).

No.	Spacios	Inner forest		Outer	Outer forest		illage
INO.	Species	Q'ty	%	Q'ty	%	Q'ty	%
1	An.aconitus	11	2.73	129	3.91	77	2.60
2	An.annularis	0	0	110	3,36	99	3,34
3	An.barbirostris	15	3.72	142	4.34	98	3.31
4	An.dirus	108	26.80	8	0.24	0	0
5	An. jamesi	0	0	60	1,83	0	0

6	An.jeyporiensis	33	8.19	98	3.00	0	0
7	An.kochi	0	0	116	3,55	0	0
8	An.maculatus	65	16.13	70	2.14	94	3.18
9	An.minimus	3	0.74	4	0.12	0	0
10	An.montrosus	0	0	12	0,37	0	0
11	An.peditaeniatus	69	17.12	426	13.02	659	22.26
12	An.philippinensis	13	3.23	472	14.43	446	15.07
13	An.sinensis	61	15.14	666	20.35	408	13.78
14	An.splendidus	25	6.20	381	11.64	412	13.92
15	An.tessellatus	0	0	42	1.28	0	0
16	An.vagus	0	0	536	16.38	667	22.53
	Total	403	100	3,272	100	2,960	100

Distribution in the habitats of Chu RCam study site, Krong Pa hydroelectric, irrigational area (Table 3.3); inside the forest (10 species), at the buffer zone (16 species), inside the village (9 species).

Distribution under landscaping patterns at the study sites of Ia Rsuom, Krong Pa hydroelectric, irrigational area (Table 3.4): Inner forest (11 species), outer forest (14 species), anc in the village (9 species)

Table 3.4. Species composition and distribution of Anopheles under	^
landscaping patterns at Ia RSuom study site (2013-2014).	

TT	Succession		forest	1	forest		llage
11	Species	Q'ty	%	Q'ty	%	Q'ty	%
1	An.aconitus	27	8.82	58	2.04	67	2.21
2	An.annularis	0	0	147	5.18	459	15.17
3	An.barbirostris	4	1.31	25	0.88	121	4.00
4	An.dirus	52	16.99	0	0	0	0
5	An.jeyporiensis	24	7.84	32	1.13	38	1.26
6	An.kochi	0	0	140	4.93	0	0
7	An.maculatus	58	18.95	77	2.71	0	0
8	An.minimus	3	0.99	3	0.11	0	0
9	An.montrosus	0	0	9	0.32	0	0
10	An. peditaeniatus	67	21.90	520	18.32	595	19.66
11	An.philippinensis	5	1.63	346	12.19	161	5.32
12	An.sinensis	46	15.03	569	20.04	640	21.15
13	An.splendidus	19	6.21	365	12.86	373	12.33
14	An.vagus	0	0	546	19.23	572	18.90
15	An. varuna	1	0.33	2	0.07	0	0
	Total	306	100	2,839	100	3,026	100

3.1.3. Species composition and distribution of Anopheles at Se san (Ia Grai)

There have been found to be 22 anopheline species at the Se San study site (Table 3.6), including 19 species at Ia O and 18 at Ia Chia. *Table 3.5 Species composition of Anopheles at the two study sites in Se San, Ia Grai (2013-2014)* 

		Ia O co	mmune	Ia Chía commune		
No.	Species	Q'ty	Rate	Q'ty	Rate	
		-	(%)		(%)	
1	An. aconitus	171	2.37	110	1.72	
2	An. annularis	412	5.70	584	9.15	
3	An. barbirostris	284	3.93	113	1.77	
4	An. crawfordi	268	3.71	281	4.40	
5	An. dirus	70	0.97	0	0	
6	An. jamesi	315	4.36	339	5.31	
7	An. jeyporiensis	217	3.00	75	1.17	
8	An. karwari	255	3.53	384	6.02	
9	An. kochi	47	0.65	0	0	
10	An. maculatus	193	2.67	84	1.32	
11	An. minimus	11	0.15	0	0	
12	An. montrosus	0	0	1	0.02	
13	An. nitidus	0	0	58	0,91	
14	An. nivipes	149	2.06	146	2.29	
15	An. notanandai	2	0.03	1	0.02	
16	An. pampanai	1	0.01	0	0	
17	An. peditaeniatus	972	13.44	912	14.29	
18	An. philippinensis	738	10.21	675	10.57	
19	An. sinensis	986	13.64	829	12.99	
20	An. splendidus	816	11.29	760	11.90	
21	An. tessellatus	30	0.41	42	0.66	
22	An. vagus	1,293	17.88	990	15.51	
	Total	7,230	100	6,384	100	

Table 3.6 Species composition and distribution of Anopheles under landscaping patterns at the study site of Ia O, Ia grai (2013-2014)

No	No. Species		Inner forest		Outer forest		In village	
INO.	species	Q'ty	%	Q'ty	%	Q'ty	%	
1	An.aconitus	6	1.73	94	3.39	71	1.73	
2	An.annularis	0	0	55	1.98	357	8.69	
3	An.barbirostris	5	1.44	72	2.60	207	5.04	
4	An.crawfordi	0	0	105	3.79	163	3.97	
5	An.dirus	70	20.17	0	0	0	0	

6	An.jamesi	0	0	101	3.64	214	5.21
7	An.jeyporiensis	72	20.75	90	3.24	55	1.34
8	An.karwari	0	0	134	4.83	121	2.94
9	An.kochi	0	0	47	1.69	0	0
10	An.maculatus	70	20.17	85	3.06	38	0.92
11	An.minimus	7	2.01	4	1.04	0	0
12	An.nivipes	0	0	61	2.20	88	2.14
13	An.notanandai	0	0	1	0.04	1	0.02
14	An.pampanai	1	0.29	0	0	0	0
15	An.peditaeniatus	47	13.54	320	11.54	605	14.72
16	An.philippinensis	0	0	394	14.20	344	8.37
17	An.sinensis	50	14.41	387	13.95	549	13.36
18	An.splendidus	19	5.48	340	12.26	457	11.12
19	An.tessellatus	0	0	30	1.08	0	0
20	An.vagus	0	0	454	16.37	839	20.42
	Total	347	100	2,774	100	4,109	100

Distribution under landscaping patterns of Ia O commune, Se San Hydroelectric Project, Ia Grai (Table 3.6): Inner forest (10 species), outer forest (18 species), in village (15 species).

Distribution under landscaping patterns of Ia Chia commune, Se San Hydroelectric Project, Ia Grai (Table 3.7): Inner forest (10 species), outer forest (14 species), in village (16 species).

Table 3.7 Species composition and distribution of Anopheles under landscaping patterns at the study site of Ia Chia, Ia grai (2013-2014)

Ν	G .		r forest		forest		illage
0.	Species	Q'ty	%	Q'ty	%	Q'ty	%
1	An.aconitus	13	6.19	46	1.87	51	1.37
2	An.annularis	0	0	220	8.95	364	9.80
3	An.barbirostris	0	0	0	0	113	3.04
4	An.crawfordi	8	3.81	88	3.58	185	4.98
5	An.jamesi	12	5.71	103	4.19	224	6.03
6	An. jeyporiensis	25	11.90	25	1.02	25	0.67
7	An.karwari	5	2.38	160	6.51	219	5.89
8	An.maculatus	29	13.81	45	1.83	10	0.27
9	An.monstrosus	0	0	0	0	1	0.03
10	An.nitidus	0	0	0	0	58	1.56
11	An.nivipes	0	0	0	0	146	3.93
12	An.notanandai	0	0	1	0.04	0	0
13	An.peditaeniatus	49	23.33	367	14.93	496	13.35
14	An.philippinensis	5	2.28	293	11.92	377	10.15

15	An. sinensis	40	19.05	354	14.40	435	11.71
16	An. splendidus	24	11.43	317	12.90	419	11.28
17	An.tessellatus	0	0	42	1.71	0	0
18	An.vagus	0	0	397	16.15	593	15.96
	Total	210	100	2,458	100	3,716	100

Except for Ia Chia, no main vector was captured/collected. 3 sites Chu Rcam, Ia Rsuom, Ia O have noted main and subsidiary vectors under the landscaping patterns of inner and outer forests, not found in the village. 3.1.4. Seasonal distribution of Anopheles and survey timing.

- The highest density of malaria vectors at the beginning of rainy season is 38% and 32% at the end of rainy season, and lower in the midde of rainy season (20%) and 10% by the end of dry season.

- The study sites in Krong Pa has found 4-8 species in the forest, 8-11 species on the edge of forests and 5-8 species in the village. *An.dirus* is present in the forest at the times of surveys, with the peak in June and October, coinciding with the appearance of *An.minimus*, but its density is lower on the edge of forests. The study sites in Ia Grai has found 4-6 species in the forest, 8-14 species on the edge of forests and 7-15 species in the village. In Ia O, *An.dirus* appeared under forest landscaping patterns between June and August 2013 and August to October 2014, *An.minmus* from April to June 2014. The outer forest was in the absence of *An. dirus*, in the presence of only *An.minimus* in June, August and October 2013. 2 1 5 PCP analysis on species complexes. *An.minimus sl. and dirus sl.* 

3.1.5. PCR analysis on species complexes *An.minimus sl*, and *An.dirus sl*.

- Results of PCR analysis on identical species complexes of *An.minimus sl* appearing at Ia O (Ia Grai): *An.minimus* (87.5%) and *An.pampanai* (12.5%); at Ia Rsuom (Krong Pa): *An.minimus* (62.5%) and *An.varuna* (37.5%); and at Chu Rcam (Krong Pa): *An.minimus* (100%).

- The results of determining *An.dirus sl* species complexes by PCR method have shown 100% *An.dirus* samples as *An.dirus* with the result of electrophoresis being at 120 bp.

3.1.6 . Catchment-based distribution of anopheline larvae

The favorite habitats for anopheline larvae are stagmant water bodies/pools (65%), slow-flowing streams (30%), rice fields and canals and ditches (5%), where no larvae of main vectors but those of subsidiary vectors were found.

3.1.7. Some biological and ecological characteristics of malaria vectors.

- All the study sites have adopted the method of indoor daytime mosquito flashlighting, but not found any mosquitoes resting indoors for blood digestion in the daytime. Other methods enabled malaria vectors to be collected, depending on landscaping patterns.

+ In Krong Pa, the highest density of vectors referred to the landscaping patterns of inner forest, at 2.274 pcs/light trap/night by using

light traps, of outer forest at 5.036 pcs/hr/person by animal shelter night time flashlighting, in-village at 1.804 pcs/hr/person by animal shelter nighttime flashlighting. The density of *An.dirus* collected from inner forests by using indoor light traps was at 1 pc/light trap/night and by outdoor human baits at 0.589 pc/person/night, from outer forests by outer human baits at 0.054 pc/person/night. The density of inner forest *An.minimus* was 0.083 pc/light trap/night by using indoor light traps, outer forest at 0.071 pc/light trap/night by using indoor light traps and 0.036 pc/day/night by using outdoor human baits.

+ In Ia Grai, the highest density of vectors referred to the landscaping patterns of inner forest, at 1.624 pcs/light trap/night by using using outdoor human baits, of outer forest at 2.714 pcs/hr/person by animal shelter nighttime flashlighting, in-village at 3.017 pcs/hr/person by animal shelter nighttime flashlighting. Inner forest *A. dirus* was at 0.464 pc/person /night by using outdoor human baits whilst no *An. dirus* was captured in the outer forest and in village. The density of *An. minimus* collected from inner forests by using indoor light traps was at 0.048 pc/light trap/night and 0.048 pc/light trap/night from the village by using indoor light traps

- Biting bahavior patterns of malaria vectors in the nighttime

+ In Krong Pa: Main vectors tend to bite baits highly from 21:00 hrs to 2:00 hrs in the morning, with the peak at 23:00-24:00 hrs. Subsidiary vectors tend to bite baits earlier from 19:00 hrs to 2:00 hrs in the morning, with the peak at 20:00- 22:00 hrs.

+ In Ia Grai: Main vectors tend to bite baits highly from 19:00 hrs to 1:00 hrs in the morning, with the peak at 23:00-24:00 hrs. Subsidiary vectors tend to bite baits earlier from 19:00 hrs to 3:00 hrs in the morning, with the peak at 21:00-23:00 hrs.

- Bait biting density of malaria vectors before and after 24:00 hrs

+ In Krong Pa, the biting density of mvs before 24:00 hrs is higher than after 24:00 hrs (P<0.05). *An dirus* (7.5 pcs and 0.75 pc/hr/person respectively); *An.minimus* (1.0 pc/hr/person); *An.aconitus* (24.5 pcs and 0.75pc/hr/person respectively); *An.jeyporiensis* (27.75 and 0.25 pc/hr/person); *An.maculatus* (27.75 and 0.5 pcs/hr/person respectively).

+ In Ia Grai, the bait biting density of malaria vectors before 24:00 hrs is higher than after 24:00 hrs (P<0.05). *An.dirus* (10.5 pcs and 0.25 pc/hr/person respectively); *An.minimus* (1.5 pc/hr/person); *An.aconitus* (12.5 pc/hr/person); *An.jeyporiensis* (23 and 0.75 pc/hr/day respectively); *An.maculatus* (22.5 and 0.75 pcs/hr/person respectively)

- Bait biting density of malaria vectors indoors and outdoors

+ In Krong Pa, before 24:00 hrs the human biting density of main vectors outdoors by using outdoor human baits is 10.5 pcs /hr /person, higher than those indoors (8.0 pcs by using indoor human baits), no

significant difference in statistics (P>0.05). Similarly, the density of indoor human biting subsidiary vectors 41.5 pcs/ hr /day is higher than those outdoors (17.75 pcs/hr/person), difference in statistics (P<0.05). After 24:00 hrs, the density of indoor human biting main vectors 0.5 pc is higher than those outdoors (0.25 pc/hr/person) (P>0.05).

+ In Ia Grai, before 24:00 hrs the human biting density of main vectors outdoors by using outdoor human baits is 6.5 pcs/hr/person, higher than those indoors (4.0 pcs/hr/person by using indoor human baits), (P< 0.05). The density of outdoor human biting subsidiary vectors 53.0 pcs/hr /person is higher than those indoors (45 pcs/hr/per), (P<0.05). After 24:00 hrs, the density of main and subsidiary vectors outdoors is 0.25 pc/hr/person and 0.50 pc/hr/person respetively, no indoor human biting vectors found.

- Bahaviors towards preferred hosts:

+ In Krong Pa, main vectors feed on human blood only, not on animal blood, specified by *An. dirus* (19.50%) and *An. minimus* (0.62%). Conversely, subsidiary vectors prefer to feed on animal blood rather than human blood. Let say the density of *An. aconitus* biting animals in comparison with biting humans is 48.04% and 30.65% respectively, (P < 0.05) and of *An. jeyporiensis* being 26.63% and 17.34% (P < 0.05), and of *An. maculatus* 25.33% and 21.89% (P>0.05).

+ In Ia Grai, mvs feed on human blood only, not on domestic animal blood, specified by *An.dirus* (13.99%) and *An.minimus* (1.02%), whilst the density of *An.aconitus* biting animals in comparison with biting humans is 48.29% and 23.55% respectively, (P<0.05) and of *An.jeyporiensis* being 28.97% and 28.33% (P> 0.05) and of *An.maculatus* 22.74% and 33.11% (P<0.05).

3.1.8. Transmission role of malaria vectors: Mean length of life of mvs

+ In Krong Pa: Laying mosquito rate is  $67.25\pm3,2$ : An.aconitus (70.15%), An.dirus (56.55%), An.jeyporiensis (70.55%), An.maculatus (75.38%) và An.minimus (63.63%). Gonotrophic cycle 3.5 days. Survival rate of An.aconitus (89.84%), An.dirus (84.52%), An.maculatus (91.90%), An. minimus (81.81%).

+ In Ia Grai: Laying mosquito rate is  $66,07\pm4,1$ ; An.aconitus (70.70%), An.dirus (61.90%), An.jeyporiensis (70.67%), An.maculatus (64,59%) và An.minimus (62.5%). Gonotrophic cycle 3.3 days. Survival rate of An.aconitus (87,17%), An.dirus (86.24%), An.jeyporiensis (88.25%), An.maculatus (82.45%) và An.minimus (89.23%).

- MP infection rate of MVs from ELISA has not subsidiary vectors as being infected with mps. In Krong Pa, *An.dirus* is positive for P.v 210 (0.61%), *An.minimus* is positive for P.f (14.28%). In Ia Grai *An.minimus* is positive for P.f (11.11%), which has showed that both of the main vectors *An.minimus* and *An.dirus* still play a role in transmitting malaria.

#### **3.2. Proposed measures for malaria vector control**

3.2.1. The relationship between environmental factors and malaria parasite rate in the communities of the study sites over the past several years.

- Retrospective analysis of MP. rate (tab.3.25) has showed that the rate remains unchanged in Se San, Ia Grai; while it has been increasing persistently in Krong Pa, irrigational areas from 2010 up to now.

Irrigational areas (2010-2014)										
		Krong Pa hy		Se San,						
Year	Data	irrigation	nal areas	hydroelectric areas						
		Chư Rcăm	Ia Rsươm	Ia O	Ia Chía					
	Patients	694	391	1,684	1,473					
2010	MP	42	49	48	50					
	Rate(%)	6.05	12.53	2.85	3.39					
2011	Patients	814	739	2.402	1.940					
	MP	80	55	49	40					
	Rate(%)	9.8	7.44	2.04	2.06					
	Patients	2,013	1,587	2,638	2,142					
2012	MP	301	73	88	12					
	Rate(%)	14.95	4.59	3.34	0.56					
	Patients	1,813	2,456	2,647	1,774					
2013	MP	271	163	121	1					
	Rate(%)	14.95	6.64	4.57	0.06					
	Patients	1,751	1,690	1,573	1,228					
2014	MP	257	105	60	2					
	Rate(%)	14.68	6.21	3.81	0.16					

Table 3.25. Malaria parasite rate at the study sites of hydroelectric, irrigational areas (2010-2014)

- MP rate (2013-2014) at Krong Pa (0.25-4.75%) and Ia Grai (0.25-2.5%). No MP were detected at some stages of the investigation period.

- Involved environmental factors over the periods:

Table 3.28. Average temperature, humidity, rainfall (2002-2013)

Study sites	Average annual figures during periods								
	2002-2005			2006-2009			2010-2013		
	Т	Н	R	Т	Н	R	Т	Н	R
Krông Pa	26	74	1146	23	77.5	1196	26	79.2	1294
Ia Grai	22	83	2020	22	82	2216	22	82	2122
	P > 0.05								

- The climatic factors i.e., temperature, humidity and rainfall of each period of four years, from 2002-2013, are not statistically different (P>0.05) but relate to malaria parasite rate of each period.

- The environmental factors, habitats have changed; the forest area is shrinking due to carrying out hydroelectricty and water resource

development projects. In 37 years (1976-2013), in Krong Pa, the area of natural forests was reduced from 60,000 ha to 20,000 ha and planted forests grew larger from 10,000 ha to 25,000 ha. The resevoirs of hydroelectric works have increased 5 times in area, with forest canopy being reduced by 20%. In Ia Grai, the area of natural forests was reduced from 75,000 ha to 20,000 ha and planted forests grew larger from 10,000 ha to 26,000 ha. The resevoirs of hydroelectric works have increased 3 times in area, with forest canopy being reduced by 25%.

3.2.2. Knowledge-Attitude-Practice in preventing malaria by communities in the hydroelectric, irrigational areas

- Knowledge: Proportion of people who has the understanding of the Malaria Control Program (98%), of malaria dangers (89%) and malaria control measures (88%), and mosquitoes as malaria carriers (86%) and that malaria can be prevented (85%).

- Attitude: 45% of the locals have visited the healthcare facilities to be examined and treated when running a fever, 71% usually sleep under bednets and 51% participate in the malaria control program.

- Practice: 70-80% of the locals are forest goers, field-hut sleepers, but only 30% of whom bring bed-nets and 34% bringing precautionary medications with them to the forest, 74% using insecticide treated bed-nets. 3.2.3. Interventions for changing locals' attitude and practice

A study was made interventions for changing locals' attitude and practice in controlling and preventing malaria by the communities of forest/field-hut goers in 3/2014, along with training provided to volunteer groups by December 2014 and monitoring and evaluation of interventions.

- Over 10 months of intervening in Ia Rsuom (Krong Pa), the volunteers had visited target households (80.8% number of visits) and directly communicated with them (78.7% no. of visits). In Ia O (Ia Grai), volunteers had visited target households (71% no. of visits) and communicated with them (68.9 no. of visits). They also worked in coordination with village health workers and detected 218 malaria cases in Ia Rsuom and 45 cases in Ia O, and gave locals instructions on how to access community health facilities to be examined and treated in a timely manner.

- Results of changing locals' attitude/bahaviors in malaria control and prevention in 2 intervening communes (Ia Rsuom, Ia O): The proportion of people going to visit the health clinic when getting a fever is 82-87%, usually sleeping under bed-nets being 80-90% and participating in the MCP 67-70%, bringing with bed-nets and precautionary medications 50-57%, using insecticide treated bed-nets 95-97%, with statistically significant difference (P<0.05) from the two control communes (Chu Rcam, Ia Chia).

### **Chapter 4. DISCUSSIONS**

# **4.1.** Species composition, distribution of Anopheles, biological, ecological and malaria transmission roles of malaria vectors

4.1.1 Species composition of Anopheles at the study sites

The species of Anopheles in this study are more diversed than in some of the previous studies, say, by Nguyen Xuan Quang (2012) in the Kon Ka Kinh National Park, Gia Lai (18 species); Chu Mom Ray, Kon Tum (18 species) and Ea So, Dak Lak (17 species); Nguyen Thi Duyen (2009) in Vinh Thanh District, Binh Dinh (16 species). The above results have been shown to be equal to those of the study conducted by Truong Van Co (2000) in Ia Co commune, Gia Lai (24 species), Ho Dac Thoan (2011) in Gia Lai (22 species), Tran Quoc Tuy (2006) in the area of Son La hydroelectric works (21 species) but lower than those of the study by Ho Dinh Trung (2005) 59 species nationwide, Nguyen Duc Manh (1988) in the Central Highlands 38 species, Le Khanh Thuan (1997) 45 species. The species composition as shown in the results of this study as well as some of the recent studies is lower than in the previous studies. This may be case resulting from the change of natural habitats or the fact that populated areas are no longer in the existence of suitable habitats for malaria mosquitos to reproduce and develop

Of totally 23 Anopheles species collected, 21 were determined by morphology and 2 species determined by PCR (*An.pampanai* và *An.varuna*). Notably, there is an existence of *An.notanandai* of *An. maculatus* complex (Nguyen Duc Manh et al (2001)). Thus, among the total of 23 Anopheles species recorded during the investigation at Krong Pa hydroelectric, irrigational study sites Se San (Ia Grai) hydroelectric area are three species *An. varuna*, *An. pampanai* and *An. notanandai* not found previously at the study sites.

4.1.2. Distribution of Anopheles mosquitos based on the habitats

- The study has chosen 3 landscaping patterns: inner forest areas, outer forest areas and in villages, similar to that of Nguyen Van Tuan (2013) on the composition and distribution of Anopheles in Bu Gia Map National Park (Binh Phuoc and Dak Nong). According to the description of this study, the forest habitat is of narrow areas reclaimed by the locals to plant crops, surrounded by natural forests, isolated from the outside where living houses are small temporary huts by streams to spend time taking care of their crops. The outer forest buffer zone is close to the forests under cultivation. There also appeared on-site living shelters near streams to stay during the crop-planting period. The village and hamlet landscape is characteristic of local residents gathering to live stably in their houses along

either side of the inter-hamlet road, where houses are built of bricks or wood, away from the streams, situated halfway on the hill side or on the hill top, surrounded by cashewnut tree gardens or rubber tree plantations, about 3-4km by air away from the natural forests. The inner and outer forest areas involved in this study have bigger huts and more people living because they earn their living working in mountain fields and sleeping there in their huts.

- The species composition in the study of Nguyen Van Tuan et al (2013) in Binh Phuoc and Dak Nong is lower than that of this study. The latter involves hamlets (10 species), inner-forest areas (6 species), outer-forest areas (2 species), but different in the main vector proportion under 3 landscaping patterns; specified by inner forest with An.dirus (80.2%), An.minismus (73.9%); outer forest with An.dirus (16.3%), An.minimus (17.4%); in village with An.dirus (3.5%), An.minimus (8.7%) while the result of this study showed that the main vectors appeared just in the forest and on the edge of the forest and that only subsidiary vectors are detected in the village. The study of Nguyen Tuyen Quang et al (2011) in Khanh Phu commune (Khanh Hoa) suggested that due to the deforestation activities, the forest has been shrunk from residential areas, which reduced the population of main vectors in hamlets. And according to some foreign authors, habitat conditions with vegetation near residential areas have increased the malaria vector population and malaria infection (O'Loughlin SM et al (2008). In the villages where there are large canopy forests, for instance, in Belize and Bangladesh, the morbidity rate is higher due to a high density of malaria vectors in the forest, as vectors tend to prefer foliage and cavities in the tree to shelter themselves (Haque U et al (2011).

4.1.3. Seasonal distribution of Anopheles

The results have shown that the density of main and subsidiary vectors is both high at the beginning and at the end of rainy season, which is different from those of the study by Nguyen Van Tuan et al (2013) which showed that the density of *An.dirus* at the middle of the dry season was higher than that at the end of rainy season. This result is consistent with the study of Le Khanh Thuan et al (2001) at two pilot sites Van Can (Binh Dinh) and Iakor (Gia Lai) where the rainfall and rainy season has direct effects on developing the peak of vectors during the year. Rainfall, temperature, humidity have direct effects on the vector population and the spreading of malaria; furthermore vectors's ability to transmit malaria depends on terrains, especially landscaping patterns.

4.1.4. PCR result of determining species complexes

- PCR result of the determination of the species complex of *An.minimus* is similar to that of the study by Le Khanh Thuan et al (1991-2000) which

showed that *An.minimus sl.* much preferred to feed on human blood, whilst the rate of feeding on animal blood was at 10-30%; Nguyen Xuan Quang (2011) in Kon Ka Kinh (Gia Lai). According to Ngo Thi Huong et al (2011-2013), the *An. minimus* species complex in the Central Coastal Region and Highlands has two members *An. minimus* and *An. harrisoni*, which is similar to those of the previous studies in the South East Asia.

- PCR result has shown that the *An. dirus* species complex is consistent with that of the study by Nguyen Xuan Quang (2012) in the Kon Ka Kinh National Park (Gia Lai), Ngo Thi Hurong (2014) there is only one *An.dirus* species in 7/8 investigated provinces in the Central Coastal Region and Highlands.

4.1.5. Some biological, ecological characteristics and transmission roles of malaria vectors in the hydroelectrical, irrigational areas

- Malaria vector density through the investigating methods

+ Unlike the study by Le Xuan Hoi et al (2011) in Tuyen Quang hydroelectric area (2006-2008) with 5 investigating methods that recorded 14 Anopheles species at the upstream area of the dam but only identified one main vector *An.minimus*, where the cattle-biting density is high (5.33pcs/hr), human-biting density indoors and outdoors is low (0.03pcs/hr/person), indoor light trap (1.00 pc/light trap/night). In the downstream area of the dam appear 16 species, where only cattle- biting density of *An.minimus* (1.0c/hr/person) is identified to be higher than human biting density both indoors and outdoors (0.02 pc/hr/person), whereas the result of this study has shown that both main vectors *An.dirus* and *An.minimus* only bite humans, not animals with a higher density in both inner and outer forest areas. This may be a different case that leads to higher and more persistent malaria infection exposure than in Tuyen Quang hydroelectric area.

+ Under the method of indoor daytime flashlighting at the 4 study sites, there was no capture of any mosquito resting indoors for blood digestion. This is the case different from the results of the study by Le Xuan Hoi (2011) in Tuyen Quang hydroelectric area with a density of malaria vectors resting indoors for blood digestion: *An.minimus* (0.25pcs/hr), *An.aconitus* (0.04pc/hr) at the upper area of the dam; *An.minimus* (0.04 pc/hr) at the lower area of the dam; however the result of this study is similar to that of the sutdy by Nguyen Xuan Quang (2011) at 3 study sites, namely Chu Mom Ray (Kon Tum), Kon Ka Kinh (Gia Lai) and Ea So (Dak Lak); of Nguyen Thi Duyen et al (2009)\_in Vinh Kinh, Vinh Thuan and Vinh Hoa communes (Vinh Thanh district, Binh Dinh) as well as some other studies in the Central Coastal Region and Highlands, where there was no capture of any MV resting indoors daytime for blood meal digestion. It could be due to the effectiveness of insecticide treated bed-nets and residual spraying chemicals that kept MVs from resting indoors for blood disgestion or the possibility that the major and subsidiary vectors had changed their behaviors and habit of resting after taking a blood meal, though there are endophagic and exophilic species available, which is consistent with results of the study by Rattanarithikul R.(1966) of Thailand.

- The behaviors of choosing a host of malaria vectors are similar to the those of the study by Truong Van Co (1996) in Central Coastal Region and Highlands, where *An.minimus* prefer to feed on human blood (59.88%) than on animal flood (26.18%); by Nguyen Tuyen Quang (1996) in Van Canh (Binh Dinh) precipitin reaction 72.76% *An.minimus* bite human and 27.24% bite castle. Malaria vector (*An.dirus, An.minimus*) in the study habitats only bite human, don't bite castle, which is different from the study of Nguyen Thi Duyen et al (2009) who showed that *An.dirus* favour biting human but *An.minimus* favour biting both human (61.3%) and castle (29.7%).

- The biting behavior of malaria vector at night is similar to the studies of Nguyen Xuan Quang (2011) in some Western Highland National Parks, his study showed that malaria vector bite man at early time (18-19 P.M), peak (21-23 P.M) and the human biting density outdoor is higher than that indoor; of Nguyen Thi Duyen et al (2009) in Vinh Thanh (Binh Dinh) showed that main vectors bite man intensively from 19 to 24 P.M.

- The laying mosquito rate, daily probability of survival and mean length of life of the malaria vector population: similar to the studies of Nguyen Son Hai et al (2001) in Khanh Phu Khanh Hoa, who showed that the laying mosquito rate and mean length of life is 71% and 9.4 days, respectively; *An.dirus* (64% and 7.2 days), Truong Van Co (2001) in Chu Se (Gia Lai) determined the laying mosquoto rate, daily probability of survival and mean length of life of *An.minimus* is 69.15%, 89.6% and 9.17 days, respectively; *An.dirus* (56%, 84% and 5.9 days). Nguyen Xuan Quang (2011) in three Western Highland national park and reservation determined the laying mosquito rate (64.3 ± 7.6); probability of survival is 88 ± 3 and the population's mean length of life is (7.9± 2.4) days.

- The malaria parasite infection rate of the malaria vectors: ELISA results in Krong Pa *An.dirus* is positive with P.v (0.61%), *An.minimus* is positive with P.f (14.28%); in Ia Grai *An.minimus* is positive with P.f (11.11%), which is lower than previous period and didn't reflect the right malaria situation of the locality. According to Nguyen Thai Binh (2009) in Hon Ba (Khanh Hoa), the proportion of *An.dirus* infected with malaria parasite (ELISA) is 2.24%.

#### 4.2. Propose malaria control measures appropriately

4.2.1. Rate of malaria parasite infection

- The length-sectional descriptive study showed that in Chu Rcam, rate of malaria parasite in the period 2012-2014 was about 15%; in Ia Rsuom, rate of malaria parasite did not decrease between 5-6.5% and 3.5-4.5% in Ia Grai. Most of malaria parasite infection were mainly people going to forest, sleeping in field hut or exploiting forest and native products.

- The cross-sectional descriptive study show that rate of malaria parasite infection was low with 0.25%-0.75%; in the peak months of malaria transmission season (October), this rate was between 2.5%-4.5%. There was the difference because the result of surveying 400 people at community for one survey turn, so rate of malaria parasite was lower than surveyed result at health centres with the patients having fever.

4.2.2. The impact of change environmental and social factors to malaria situation in survey areas

- The impact of factors such as temperature, humidity, rainfall to the development of malaria vector: examining the elements of hydrometeorology in the period of 2002 - 2013, there was not significant change. So the apperance of hydroelectric dam, irrigation systems did not affect climate in the areas, but it affected to environment, ecosystem and created favourable condition for the development of malaria parasites and malaria vectors.

- The impact of change environment and ecosystem of forest to malaria vector: the change of forest ecosystem led to the change of vector habit to adapt to exploited forrest or planted forest, together with the difference between activite time, biting habit, capacity of resistance to insecticide and restriction to vector control effectively (Sinka ME and coworkers, 2010)

- The impact of society in building hydroelectric plans, irrigation systems: although the government had policies to affected areas but in real situation, people did not have cultivated land, so they had to go to remote fields, sleeping in field huts and having risk of mosquitoes biting; the balance of ecosystem upset but had not adjusted suitably yet.

- The impact of mobilise people going to malaria-endemic areas: in village, structure of houses and hygiene were improved, fruit-trees were planted around houses, so it was not suitable for malaria vectors. However, model "going to forest, sleeping in field huts" was very difficult to control with applying vector control measures such as spraying insecticides, bednet impregnation), so most of malaria morbidity were mainly in this object.

4.2.3. Proposed malaria vector control measures

The existence of malaria depends on 3 factors: malaria parasite, malaria mosquito and human, therefore apart from studying on classification, distribution, biological and ecological, transmission roles of malaria mosquotos as well as malaria parasite, it is necessary to understand the community clearly (self-protection behaviors in the malaria area)

- The real situation of communication and the awareness of malaria control in the community: after 10 monts of intervention, the number of people coming to the health station to be examined and treated when get fever (82-87%), sleeping under bed-nets (80-90%) and participating in the malaria control program (60-70%); bring bed-nets in to the forest/field-huts (51-60%), bring stand-by treatment (50-57%), using insecticide treated bed-nets (95-97%) compared to the control group. Those showed that the people have changed the awareness to the malaria control practice that is appropriate to the model "malaria detection and management site" for the mobile population of Le Xuan Hung et al in Western Highland, which satisfied the malaria control activities.

- The effectiveness of the model "Community malaria volunteer" to communicate the awareness of malaria self-protection in the community is a stable solution in the National Malaria Control and Elimination Strategy while malaria increase persistently in recents years (2010-2014) in Krong Pa, reducing slowly in Ia Grai as well as study sites when the priority malaria control measures were deployed thoroughly but haven't improve the situation.

- We propose the education communication method in malaria control, implement effectively the communication monitor, mobile population management; increase the proportion of performing knowledge-attitudepractice correctly in malaria control in the high risk population, so that they are good enough to protect themself from malaria; monitoring the spraying, imprenating insecticide in the directed areas.

#### CONCLUSION

1. Species composition, distribution of Anopheles, biological, ecological characteristics and malaria transmission roles of malaria vectors of MVs:

The study identifed the existence of 23 Anopheles species (2 species of *An.pampanai*, *An.varuna were identified by PCR*), including 17 species of mosquitoes and 7 species of larvae in Krong Pa, 22 species of mosquitoes and 10 species of larvae. There were 2 main vectors (*An.dirus, An.minimus*) and 3 minor vectors (*An.aconitus, An.jeyporiensis, An.maculatus*), no main vector of larvae.

- The major vectors (*An.dirus, An.minimus*) were distributed in forests, forest's edge, no appearance in village at 3 study points (Chu Rcam, Ia Rsuom, Ia O), in Ia Chia had no major vectors. Minor vectors distributed with high rate in most of the ecosystem.

- The density of MVs was high at the begining of rainy season (32%), mid-rainy season (20%) dry season (10%). Some areas of Anopheles larvae: stagnated pool (65%), stream flowing slowly (30%), field and canal (5%).

- The biting density in human of major vectors had the peak period from 8 p.m to 2 a.m, minor vectors had the earlier peak time from 7 p.m to 1 a.m, the density of mosquitoes feeding prior to 12 p.m was higher than that after 12 p.m. The density of mosquitoes feeding outdoor was higher than indoor; major vectors tend to bite human much more than cattle, only bit human, but minor vectors showed a reverse tendency.

- The result of ELISA at Krong Pa: *An.dirus* was positive, malaria parasite: P.v 210 (0.61%), An. Minimus: P.f (14.28%); at Se San (Ia Grai) *An.minimus* was positive: P.f (11.11%)

2. Propose vector control measures for people living in hydroelectric and irrigational areas and going to forests, sleeping in field huts.

- Manage malaria for people going to forests, sleeping in field huts.

- Measures for vector self-control: health communication and education for people going to forests, sleeping in field huts; people changed the awareness to behaviour through the program "Volunteer for malaria at community" together with other priority control measures.

#### RECOMMENDATION

- Study tools for personal protection to control vectors suitably (antimosquito cream, anti-mosquito flavouring, bednet and hammock impregnation)

- Study to expand the model "Volunteer for malaria at community" for people living in hydroelectric and irrigational areas and going to forests/fields.