OVERVIEW OF THE THESIS

1. Urgency

Binh Phuoc and Dak Nong province are high malaria endemic areas. Thousands of patients with malaria parasites are detected each year. In which *P. falciparum* accounts for over 50%, multidrug resistance and artesunate. Current status of malaria vector, the level of resistance and the genetic changes related to the resistance of the parasite has impacted how for malaria disease in this region be essential for the prevention of malaria. Therefore, we conducted research thesis.

2. Objectives

- Determine species composition and distribution of the Anopheles.
- Determine rate of Anopheles be infected sporozoite.
- Detection of mutation points of gen MDR-1 and ATPase6, amino acids of protein Pgh-1 and enzymes ATPase6 are replaced.

3. Novelty and practical

The results of research have identified 19 species of Anopheles distributed at Binh Phuoc, compared with 7 species of previous studies. For the first time confirmed the presence of three malarial vector *An. minimus*, *An. aconitus* and *An. jeporiensis*. The main vector *An. minimus* and *An. dirus* distributed at all habitats of the study site. *An. dirus* infection of sporozoites distributed at the forest edges and deep forest, where people living and working.

Research results show that *P. falciparum* at Binh Phuoc and Dak Nong already resistance to artesunate. The ADN sequence of gene ATPase6 was decoded. There are 4 amino acids N465S, I569L, I654K and T656P was detected be replaced by mutations in P. falciparum resistance to artesunate. Open up an new direction for research drug - resistant parasites in Vietnam.

Result of study are index of molecular epidemiology of the resistant drug malaria significantly for the prevention of vector and prevent the spread of drug-resistant parasites at Binh Phuoc.

INTRODUCTION

Vietnam has made great achievements in the prevention of malaria. However, the prevalence of malarial of Binh Phuoc and Dak Nong provinces are still high in recent years. Natural conditions, economic and social are favorable for development of Anopheles mosquitoes transmit the disease throughout of the year. The migration

of people from areas without malaria come here is complicated. In which the index of Anopheles were not reflected the transmission of malaria, not correlate with epidemiological situation. Therefore to study index of mosquito Anopheles is essential for measures to prevent the spread of malaria at this region

The mechanism to resistant artemisinin of *P. falciparum* is proposed for genes ATPase6 and MDR-1. The result of studies showed that mutations occured at several points, not the same on *P. falciparum* at different places. Viet Nam have been studies gene MDR - 1 of *P. falciparum* resistance to artemisinin, but hasn't yet any study for ATPase6 gene. While injectable artesunate still use to treatment of severe malaria caused by *P. falciparum*. Artemisinin is one of major component of combination therapy (ACT) to treat *P. falciparum*. Therefore research mutations gen resistance to artemisinin is essential to monitor and prevent the development of malaria and spread of resistant genotype now. From the above mentioned problems, we performed project

Study the species composition, prevalence of mosquito Anopheles have infected the sporozoite and mutation gen resistance to artesunate of *P. falciparum* at Binh Phuoc and Dak Nong provinces.

Chapter 1

LITERATURE REVIEW

Currently, there are 41 species of Anopheles in the world have identified role transmit malaria and the distribution of them. In Vietnam, there are 3 main vector and 6 secondary vector. Northern there are one main vector *An. minimus* and 3 secondary vector *An. aconitus*, *An. minimus* and *An. jeyporiensis*. South Western and Coast there are one main vector *An. epiroticus* and 3 secondary vector *An. supictus, An. vagus* and *An. sisnensis*. Central-Highlands and Southeast there are two main vector *An. minimus* and *An. dirus* and 3 secondary vector *An. aconitus*, *An. minimus* and *An. jeyporiensis*. Ecological features and capability to transmit of each species of Anopheles are different. The distribution and abundance of Anopheles depends on biogeographical scene and is determined by the quality of larval ecology. Active of bite and blood consumption indoor or outdoor and ability to change the location to avoid the effects of chemicals

depending on each of the species. Temperature and humidity are two important factors that govern the development of mosquitoes and parasites in mosquitoes, affecting the spread of malaria. Disease of malaria in human caused by 5 species of Plasmodium. Biological cycle of the parasite undergoes two human hosts and mosquito Anopheles. The stage development in human of the parasite caused malaria, in Anopheles transmit malaria. Factors parasite, mosquito Anopheles and human to interact each other in natural environment deduced epidemiology of malaria. In which *P. falciparum* malaria accounts for over 50% and multi-drug resistance both with artemisinin in most of the regions of malaria. Mechanisms of resistance to most antimalarial drugs was determined by mutations gen coding enzyme relate to act of drug. Gene mutations also cause cross-resistance to drugs such as mefloquine and halofantrine of alcool amino groups, or cycloquanil and pyrimethamine of antiforlate groups. For artemisinin, resistance mechanism was proposed by the mutant gene MDR-1 and ATPase6. In which the mutation of gen MDR-1 replaced amino acid at position N86Y of protein Pgh-1 related to resistant chloroquine and quinine, increased susceptibility to mefloquine or artemisinin. The mutations of gen ATPase6 replaced amino acid at position L263E, E431K, A623E and S769N of the enzyme ATPase6 increased value IC50 of artemisinin and artemether...

SUBJECTS AND METHODS

1. Study subjects

- Anopheles mosquitoes collected at the field.
- Sporozoite of Plasmodium infected in Anopheles.
- Malaria patients at the field.
- *P. falciparum* in vivo patients testing with artesunate.

2. Time study

The study was conducted from 2011 to 2014 in 3 years

3. Study sites

- Fieldwork collect mosquitoes and in vivo test is done in two provinces of Binh Phuoc and Dak Nong.
- Analysis samples at laboratory of Molecular Department of National Institute of Malaria Parasitology Entomology

4. Research methodology

4.1 Study design

❖ The cross survey to determine species composition, distribution and prevalence of Anopheles infection sporozoite Plasmodium.

- ❖ Descriptive the relate mutations of gene MDR-1 and ATPase6 of *P. falciparum* for levels of response to artesunate.

4.2. Methods

- ❖ Collect Anopheles on the field at night by
  - + Light traps indoor.
  - + Getting mosquitoes in barn of animal.
  - + Getting mosquitoes landing on human indoor
  - + Getting mosquitoes landing on human outdoor.
- ❖ The response of *P. falciparum* to artesunate was assessed by in vivo test for 28 days.
- ❖ Parasites in blood of patients in vivo test was detected by giemsa method.
- ❖ Parasites in Anopheles and blood of patient absorbed on paper Whatman 3mm were detected by nested - PCR method.
- ❖ *P. faciparum* reappear were distinguished recurrence - reinfection by multiple nested - PCR method.
- ❖ Sequence DNA of gen MDR-1 and ATPase6 were decoded.
- ❖ Mutation gen were detected by softwere MEGA 4.0
- ❖ Difference of vector density in habitats at times was analyzed by software SPSS.

RESULTS

3.1. Survey results Anopheles mosquitoes in the study place.

3.1.1.The composition of Anopheles species

The result of 3 times of survey in 10/2012, 12/2012-1/2013 and 12/2013 were collected 2063 individuals Anopheles, identification of 19 species, the ratios of each species presented in table 3.1

Table 3. 1. The composition of Anopheles species

List	Species of Anopheles	Number mosquito	Rates %
1	*An.(Cell.) aconitus* Dönitz 1902**	15	0,73
2	*An. (Ano.) barbirostris* W*ulp*1884**	3	0,15
3	*An. (Ano.) campestris* Reid 1962**	11	0,53
4	*An. (Ano.) crawfordi* Reid 1953*	8	0,39
5	*An.* (*Nyss.*) *darlingi* Root, 1926**	1	0,05
6	*An. (Cell.) dirus* Peyton & Harrison*	1377	66,75
7	*An. (Cell.) jamesii* Theobald 1901**	15	0,73
8	*An. (Cell.) jeyporiensis* James1902**	2	0,10
9	*An. (Cell.) kochi* Dönitz 1901**	2	0,10

Table 3. 1. The composition of Anopheles species

List	Species of Anopheles	Numbermosquito	Rates %
10	*An.(Cell.) karwari* James 1903**	1	0,05
11	*An. (Cell.) maculatus* Theobald 1901*	62	3,01
12	*An. (Cell.) minimus* Theobald1901**	28	1,36
13	*An. (Cell.) nivipes* Theobald 1903*	11	0,53
14	*An. (Cell.) philippinensis* Ludlow 1902*	489	23,7
15	*An. (Ano.) peditaeniatus* Leicester 1908*	1	0,05
16	*An. (Ano.) sinensis* Wiedemann 1828**	10	0,48
17	*An. (Cell.) splendidus* Koizumi 1920**	23	1,11
18	*An. (Cell.) tessellatus* Theobald 1901**	1	0,05
19	*An. (Cell.) vagus* Dönitz: 1902*	3	0,15
Total mosquitoes number of 19 species		**2063**	**100**

* Anopheles species has been investigated in previous studies.

**Anopheles species investigated first time in this study.

In 19 species of Anopheles, there are 12 species first time investigated in this study marked**, seven species investigated in previous studies marked*. There were 5 species accounted for highest proportion were *An. dirus* accounted for 66.75%, *An. philippinesis* accounted for 23.70%, *An. minimus* 1.36%, *An. minimus*3.01%, *An. splendidus* 1.11% and the remaining 14 species have low of percentage from 0.05% - 0.73%.

3.1.2. Distribution of Anopheles in the habitats

In three habitats,the villages distributed much the most Anopheles species (17 species), accounting for 33.74% the number of mosquitoes. The forest edges distributed 6 Anopheles species, accounting for 11.44% the number of mosquitoes. The deep forest distributed 3 species, accounting for 54.82% the number of mosquitoes as shown in table 3.2.

Table 3.1.2. Distribution of Anopheles at the habitats

List	Species Anopheles	Total	Villages		Dege forest		Deep forest	
			Number	Rate %	Number	Rate %	Number	Rate %
1	*An. aconitus*	1	15	10	0	0	0	0
2	*An. barbirostris*	3	2	66,	1	3	0	0
3	*An. campestris*	1	0	0	0	0	11	100
4	*An. crawfordi*	8	6	75	2	2	0	0
5	*An. darlingi*	1	1	10	0	0	0	0
6	*An. dirus*	1	52	3,7	223	1	110	80

Table 3.2. Distribution of Anopheles at the habitats

List	Species Anopheles	Total	Villages		Dege forest		Deep forest	
			Number	Rate %	Number	Rate %	Number	Rate %
7	*An. jamessi*	1	15	10	0	0	0	0
8	*An. jeyporiensis*	2	2	10	0	0	0	0
9	*An. karwari*	1	1	10	0	0	0	0
10	*An. kochi*	2	2	10	0	0	0	0
11	*An. maculatus*	6	57	91	5	8,	0	0
12	*An. minimus*	2	6	21	4	1	18	64
13	*An. nivipes*	1	11	10	0	0	0	0
14	*An. peditaeniatus*	1	1	10	0	0	0	0
15	*An. philippinensis*	4	489	100	0	0	0	0
16	*An. sinensis*	1	10	100	0	0	0	0
17	*An. splendidus*	2	23	100	0	0	0	0
18	*An. tessellatus*	1	0	0	1	10	0	0
19	*An. vagus*	3	3	100	0	0	0	0
Tổng số cá thể muỗi		**2**	**696**	**33,**	**236**	**1**	**113**	**54,**
Số loài		**1**	**17**		**6**		**3**	

The data in table 3.2 show that at villages distributed more species Anopheles than at forest habitats, but number of mosquitoes collected at least.

3.1.3. Vector malaria at the study sites

Based on the survey results at the study site there are five species of malaria vector. The composition and proportion of each vector species presented in table 3.3.

3.3. The composition of vector malaria at the study sites

List	Vector species	Number of mosquitoes	Rate (%)
1	*An. aconitus*	15	1,0
2	*An. dirus*	1377	92,8
3	*An. jeyporiensis*	2	0,1
4	*An. maculatus*	62	4,2
5	*An. minimus*	28	1,9
Tổng số		1484	100

The data in table 3.3 shows there are five species of malaria vector, but number of mosquitoes accounting more than 2/3 (72.8%) of 19 species of Anopheles (1484 versus 2063). In which main vector *An. dirus* dominates 92.8%. Four other species are low proportion, *An. minimus* accounted for 1.9%, *An. minimus*accounted for 4.2%, *An. aconitus* 1% and *An. jeyporiensis* 0.1%.

3.1.4. The distribution of malaria vector at the habitats

The villages, forest edges and deep forest where have peoples living and working. The composition and proportion of malaria vector species at the habitats provided in table 3.4.

Table 3.4.Vector malaria distribution in the habitats

List	Species of vector	Habitats						Total
		Villages		Forest fringer		Deep forest		
		Number of mosquito	Rate (%)	Number of mosquito	Rate (%)	Number of mosquito	Rate (%)	
1	*An. aconitus*	15	100	0	0	0	0	15
2	*An. dirus*	52	3.78	223	16.1	1102	80.0	1377
3	*An. jeyporiens*	2	100	0	0	0	0	2
4	*An. maculatus*	57	91,9	5	8,06	0	0	62
5	*An. minimus*	6	21,4	4	14,2	18	64,2	28
Total number		132	9	232	16	1120	75	1484
Number species of vector		5		3		2		
Number species of Anopheles		17		6		3		

The results in table 3.4 show that the malaria vector is present in all three habitats and distributed as follows:

The villages distributed 5 vector species, in which main vector *An.dirus* were 3.78% (lowest percentage), secondary vector *An. aconitus* and *An. jeyporiensis* were 100%, *An. minimus*was 91.94%.

The forest edges distributed 3 vector species, in which *An. dirus* and *An. minimus* dominated, only a secondary vector *An. minimus*was 8.06% (lowest percentage).

Deep forest distributed 2 main vector *An. dirus* and *An. minimus* were 80.03% and 64.29% respectively (highest percentage).

Thus 2 main vector *An. dirus* and *An. minimus* distributed at all three habitats, secondary vector *An. maculatus*, *An. aconitus* and *An. jeyporiensis* only distributed at the village, not distributed at forest habitats.

3.1.5. The density of *An. dirus* and *An. minimus*

The The density of *An. dirus* and *An. minimus*at outdoors on 12/21012 -1/2013 (early of dry season) increased compare with 10/2012 (last of rain season) at all three habitats ($p < 0.05$).

Density of *An. dirus* in the last of rainy season at the village was 0.003, forest edges was 0.081 and deep forest was 0.97. Density of mosquito in the early of dry season at the village was 0.015 increase 5 times, the forest edges was 0.55 increase 6.97 times and deep forest

was 5.550, increase 5.72 times compared with the last of the rainy season as table 3.5.

Table 3.5. Density of *An. dirus* at habitats on 2 time of survey

The time of survey	Habitas		
	Village	Forest edges	Deep forest
10/2012 Last of rainy season	0,003 ± 0,01	0,081 ± 0,06	0,970 ± 0,8
12/2012 – 1/2013 Early of dry season	0,015 ± 0.02	0,550 ± 0.27	5,550 ± 5,19
Compare	Increase 5 times	Increase 6.9 times	Increase 5.7 times
p values	<0,05		

Density of mosquito: Individuals/hour/person

An. minimus did not caught any mosquitoes at the end of rainy season (10/2012) at the village and forest edges. The deep forest, density of mosquito was very low (0,006). The early of dry season (12/2012 - 1/2013), density of mosquito at the village and the forest edges were 0,012 (increased compared with the last of rainy season), deep forest was 0,1 (increase 16,67 times) as table 3.6.

Table 3.6. Density of *An. minimus*at habitas on 2 time of survey

The time of survey	Habitas		
	Village	Forest edges	Deep forest
10/2012 Last of rainy season	0	0	0.006 ± 0.02
12/2012 - 1/2013 Early of dry season	0,012 ± 0,016	0,012 ± 0,016	0,100 ± 0,089
Compare	Increase	Increase	Increase 16.67 times
p values	<0,05		

Density of mosquito: Individuals/hour/person

3.2. Results detect sporozoites in the mosquito Anopheles

3.2.1.Mosquitoes be infected sporozoites

Using nested - PCR method to detect the Plasomdium sporozoites in 2063 mosquitoes of 19 species Anopheles. The analytical results presented in table 3.7.

Table 3.7.The proportion of Anopheles be infected sporozoites

Species of vector	Number of mosquito analyzed	Nmuber of mosquito infected	Rate of mosquito infected (%)	Species of parasiste			
				P. falciparum		*P. vivax*	
				Number	Rate %	Number	Rate %
An. dirus	1377	8	0,58	4	50	4	50
An. minimus	28	0	0	0	0	0	0
An. maculatus	62	0	0	0	0	0	0
An. aconitus	15	0	0	0	0	0	0
An. jeyporiens	2	0	0	0	0	0	0
Another species of Anopheles	579	0	0	0	0	0	0

The analytical results showed only detection *An. dirus* for sporozoite infection at the rate of 0.58%. *An. minimus*, *An. maculatus*, *An. jeporiensis*, *An. aconitus* and other Anopheles species did not detect infection sporozoite. In 8 *An. dirus* be infected sporozoite there were 4 mosquitoes be infected sporozoite *P. falciparum* (50%) and 4 mosquitoes be infected of sporozoite *P.vivax* (50%).

3.2.2. *An. dirus* be infected sporozoites distribute at the habitats

An. dirus infected sporozoite distribute in the habitat is presented in table 3.8.

Table 3.8. *An. dirus* be infected sporozoites distribute at the habitats

List	Habitats	The result of analysis		
		Numbers of mosquito analyzed	Numbers of mosquito infected	Rate (%)
1	Village	52	0	0
2	Forest edges	223	1	0,45
3	Deep forest	1102	7	0,64
Total		1377	8	100

Data in table 3.8 shows that *An. dirus* caught in the forest edge and the deep forest be infected sporozoite were 0.45% and 0.64%

respectively. *An. dirus* caught at the village did not detect infect sporozoite.

3.3.Results detect mutation gen MDR-1 and ATPase6 of *P. falciparum*

Prior to detect mutations gen relate to resistance artesunate of *P. falciparum*. The response of *P. falciparum* to artesunate was assessed by in vivo test. Then *P. falciparum* resistant and sensitivity for drug would be select to analyze mutation.

3.3.1. The response of *P. falciparum* to artesunate

Table 3.9.The results of in vivo test to assess the response of *P. falciparum* to artesunate

Results of *in vivo* test	Study sites				Total	
	Binh Phuoc		Dak Nong			
	Number of patients	Rate (%)	Number of patients	Rate (%)	Number of patients	Rate (%)
Sensitive	43	86	31	94	74	89,2
D2 positive	30	60	12	36,3	42	50,6
D3 positive	10	20	6	18	16	19,3
Treatment failure	7	14	2	6	9	10,8
Total	50	100	33	100	83	100

Total 83 patients in vivo testing have gotten the results and presented in table 3.9

Data in table 3.9 showed number of patients positive for *P. falciparum* at D2 was 50.6% (time clean of parasite to 48 hours compared to 32 hours previously), number of patients positive at D3 was 19.3% (reduction in effect) and 10.8% number of patients treatment failure (the parasites reappear within 28 days of follow-up). These indicators were over of threshold to determine of resistance according to the guidelines of WHO, 2010.

3.3.2. Results distinguish *P. falciparum* recrudesce - reinfection

The parasite reappear in patients with treatment failure were analyzed for genotyping of parasite to determine parasite relapse is due not response to treatment or reinfection of new parasite after cure. In 11 pairs of samples analyzed there were 6 samples *P. falciparum* reappear at days D19, D23, D24, D26 and D27 are defined as

recrudesce. Two samples *P. falciparum* reappear at D11 and D28 are determined as reinfection. Four samples *P. faciparum* reappear at D21, D22 and D28 were determined combine recrudesce and reinfection, including one sample at D21, one sample at D22 and 2 samples at D28. These samples are defined as recrudesce. The finally results, *P. falciparum* recrudesce defined 9 samples (82%) and 2 samples reinfection (12%). Results analysis are presented in table 3.10.

3.10. Results distinguish *P. falciparum* recrudesce - reinfection

Genotype of parasite	D0 and D parasite reappear								+	Result of analyze		
	D0 D11	D0 D19	D0 D22	D0 D23	D0 D24	D0 D26	D0 D27	D0 D28		Recru-desce	Re-infection	Mix
Similar		1		1	1	1	1		5	5		
Different	1							1	2		2	
Different and Similar			1					3	4			4
Total	1	1	1	1	1	1	1	4	11	5	2	4

3.3.3.Results to detect mutated gene MDR-1 and amino acid of protein Pgh-1 be replaced

The result of DNA sequencing and analysis comparative fragment 600bp of gen MDR-1 containing code for amino acid at position 86 of protein Pgh-1 of 9 samples *P. falciparum* resistance artesunate and 2 samples *P. falciparum* sensitivity have detected three mutation points, 2 amino acid of protein Pgh-1 be replaced. In which there is 1 amino acid at position I107T1 of *P. falciparum* resistance and 1amino acid at position F170I of *P. falciparum* sensitive. These substitution mutations did not relate to resistant artesunate and have not seen any published studies.

Results of the analysis also identified *P. falciparum* resistant artesunate carrying wild type of amino acid at position N86, same as the result of research in and out country have determined relate to resistant artemisinin and drugs group amino alcohol. Image of analysis of DNA sequence detect mutations gen MDR-1 and amino acid of protein Pgh-1 be replaced in figure 3.1 and 3.2.

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gi.9/D0    AATATTAAAGAACATGAATTTAGGTGATGATATTAATCCTATAATATTATCATTAGTATC 300
gi.24/D0   AATATTAAAGAACATGAATTTAGGTGATGATATTAATCCTATAATATTATCATTAGTATC 300
gi.5/D0    AATATTAAAGAACATGAATTTAGGTGATGATATTAATCCTATAATATTATCATTAGTATC 300
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gi.48/D0  AATATTAAAGAACATGAATTTAGGTGATGATATTAATCCTATAATATTATCATTAGTATC 300
gi.15/D0  AATATTAAAGAACATGAATTTAGGTGATGATATTAATCCTATAATATTATCATTAGTATC 300
gi.K1     AATATTAAAGAACATGTATTTAGGTGATGATATTAATCCTATAATATTATCATTAGTATC 300
gi.29/DO  AATATTAAAGAACATGAATTTAGGTGATGATATTAATCCTATAATATTATCATTAGTATC 300
gi.14/D0  AATATTAAAGAACATGAATTTAGGTGATGATATTAATCCTATAATATTATCATTAGTATC 300
gi.62/D0  AATATTAAAGAACATGAATTTAGGTGATGATATTAATCCTATAATATTATCATTAGTATC 300
gi.1/D0   AATATTAAAGAACATGAATTTAGGTGATGATATTAATCCTATAATATTATCATTAGTATC 300
          ****************:********************************************

gi.9/D0   TATAGGTTTAGTACAATTTATATTATCAATGATATCAAGTTATTGTATGGATGTAATTAC 360
gi.24/D0  TATAGGTTTAGTACAATTTATATTATCAATGATATCAAGTTATTGTATGGATGTAATTAC 360
gi.5/D0   TATAGGTTTAGTACAATTTATATTATCAATGATATCAAGTTATTGTATGGATGTAATTAC 360
gi.48/D0  TATAGGTTTAGTACAATTTATATTATCAATGATATCAAGTTATTGTATGGATGTAATTAC 360
gi.15/D0  TATAGGTTTAGTACAATTTATATTATCAATGATATCAAGTTATTGTATGGATGTAATTAC 360
gi.K1     TATAGGTTTAGTACAATTTATATTATCAATGATATCAAGTTATTGTATGGATGTAATTAC 360
gi.29/DO  TATAGGTTTAGTACAATTTATATTATCAATGATATCAAGTTATTGTATGGATGTAATTAC 360
gi.14/D0  TATAGGTTTAGTACAATTTACATTATCAATGATATCAAGTTATTGTATGGATGTAATTAC 360
gi.62/D0  TATAGGTTTAGTACAATTTATATTATCAATGATATCAAGTTATTGTATGGATGTAATTAC 360
gi.1/D0   TATAGGTTTAGTACAATTTATATTATCAATGATATCAAGTTATTGTATGGATGTAATTAC 360
          ******************* ************************************

gi.9/D0   ATCAAAAATATTAAAAACTTTAAAGCTTGAATATTTAAGAAGTGTTTTTTATCAAGATGG 420
gi.24/D0  ATCAAAAATATTAAAAACTTTAAAGCTTGAATATTTAAGAAGTGTTTTTTATCAAGATGG 420
gi.5/D0   ATCAAAAATATTAAAAACTTTAAAGCTTGAATATTTAAGAAGTGTTTTTTATCAAGATGG 420
gi.48/D0  ATCAAAAATATTAAAAACTTTAAAGCTTGAATATTTAAGAAGTGTTTTTTATCAAGATGG 420
gi.15/D0  ATCAAAAATATTAAAAACTTTAAAGCTTGAATATTTAAGAAGTGTTTTTTATCAAGATGG 420
gi.K1     ATCAAAAATATTAAAAACTTTAAAGCTTGAATATTTAAGAAGTGTTTTTTATCAAGATGG 420
gi.29/DO  ATCAAAAATATTAAAAACTTTAAAGCTTGAATATTTAAGAAGTGTTTTTTATCAAGATGG 420
gi.14/D0  ATCAAAAATATTAAAAACTTTAAAGCTTGAATATTTAAGAAGTGTTTTTTATCAAGATGG 420
gi.62/D0  ATCAAAAATATTAAAAACTTTAAAGCTTGAATATTTAAGAAGTGTTTTTTATCAAGATGG 420
gi.1/D0   ATCAAAAATATTAAAAACTTTAAAGCTTGAATATTTAAGAAGTGTTTTTTATCAAGATGG 420
          ************************************************************

gi.9/D0   ACAATTTCATGATAATAATCCTGGATCTAAATTAAGATCTGATTTAGATTTTTATTTAGA 480
gi.24/D0  ACAATTTCATGATAATAATCCTGGATCTAAATTAAGATCTGATTTAGATTTTTATTTAGA 480
gi.5/D0   ACAATTTCATGATAATAATCCTGGATCTAAATTAAGATCTGATTTAGATTTTTATTTAGA 480
gi.48/D0  ACAATTTCATGATAATAATCCTGGATCTAAATTAAGATCTGATTTAGATTTTTATTTAGA 480
gi.15/D0  ACAATTTCATGATAATAATCCTGGATCTAAATTAAGATCTGATTTAGATTTTTATTTAGA 480
gi.K1     ACAATTTCATGATAATAATCCTGGATCTAAATTAAGATCTGATTTAGATTTTTATTTAGA 480
gi.29/DO  ACAATTTCATGATAATAATCCTGGATCTAAATTAAGATCTGATTTAGATTTTTATTTAGA 480
gi.14/D0  ACAATTTCATGATAATAATCCTGGATCTAAATTAAGATCTGATTTAGATTTTTATTTAGA 480
gi.62/D0  ACAATTTCATGATAATAATCCTGGATCTAAATTAAGATCTGATTTAGATTTTTATTTAGA 480
gi.1/D0   ACAATTTCATGATAATAATCCTGGATCTAAATTAAGATCTGATTTAGATTTTTATTTAGA 480
          ************************************************************

gi.9/D0   ACAAGTGAGTTCAGGAATTGGTACGAAATTTATAACAATTTTTACATATGCCAGTTCCTT 540
gi.24/D0  ACAAGTGAGTTCAGGAATTGGTACGAAATTTATAACAATTTTTACATATGCCAGTTCCTT 540
gi.5/D0   ACAAGTGAGTTCAGGAATTGGTACGAAATTTATAACAATTTTTACATATGCCAGTTCCTT 540
gi.48/D0  ACAAGTGAGTTCAGGAATTGGTACGAAATTTATAACAATTTTTACATATGCCAGTTCCTT 540
gi.15/D0  ACAAGTGAGTTCAGGAATTGGTACGAAATTTATAACAATTTTTACATATGCCAGTTCCTT 540
gi.K1     ACAAGTGAGTTCAGGAATTGGTACGAAATTTATAACAATTTTTACATATGCCAGTTCCTT 540
gi.29/DO  ACAAGTGAGTTCAGGAATTGGTACGAAATTTATAACAATTTTTACATATGCCAGTTCCTT 540
gi.14/D0  ACAAGTGAGTTCAGGAATTGGTACGAAATTTATAACAATTTTTACATATGCCAGTTCCTT 540
gi.62/D0  ACAAGTGAGTTCAGGAATTGGTACGAAATTTATAACAATTTTTACATATGCCAGTTCCTT 540
gi.1/D0   ACAAGTGAGTTCAGGAATTGGTACGAAAATATAACAATTTTTACATATGCCAGTTCCTT 540
          ****************************:*:****************************
```

Figure 3.1. DNA sequencing of gene MDR-1 *P. falciparum* of patients and the strain *P. falciparum* K1.
Mutated nucleotide underlined in bold black

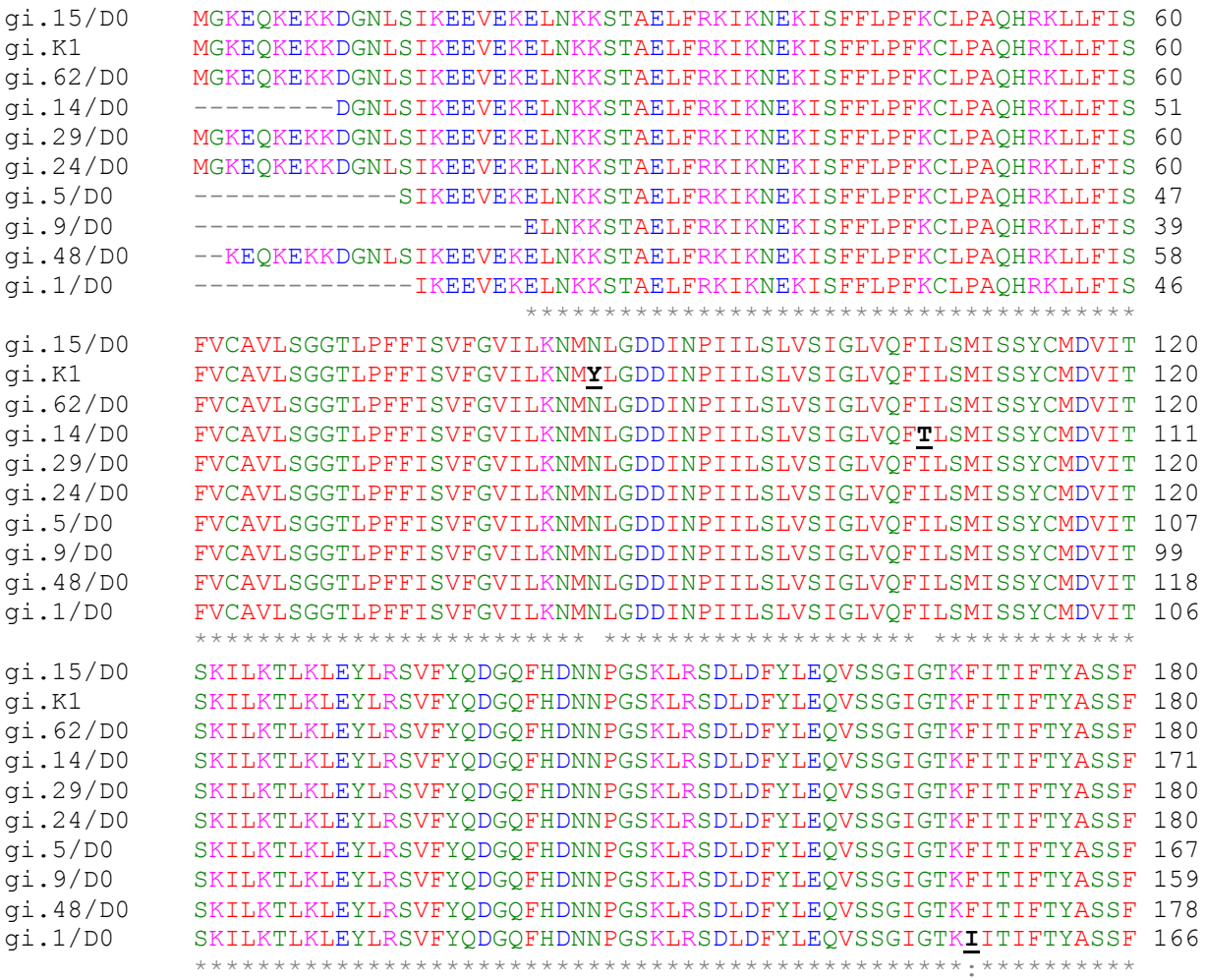

Figure 3.2.The sequence of amino acid of protein Pgh-1 of *P. falciparum* of patients and sample *P. falciparum* K1. Amino acids be mutated in black, bold and underlined.

3.3.3. Results detect gene mutations ATPase6 and amino acid of enzyme ATPase6 be replaced.

The results to analyze sequence gen ATPase6 *P. falciparum* of patients in vivo and compared with *P. faciparum* strain 3D7 have found 83 mutation points in12 samples *P. falciparum* as shown in table 3.10.

Table 3.10. Results detect mutation gene ATPase6 of P. falciparum

Results of analysis	The level of response of *P. falciparum* in vivo				Total
	Sensitive	Reinfection	Recrudescense	D3 positive (Loss)	
Mutated	2	2	7	1	12
No mutated	4	0	2	2	8
Total	6	2	9	3	20

in table 3.10 shows that among 12 samples of *P. falciparum* have mutated, there are 7 samples *P. falciparum* resistant, 2 samples of

new infections, 2 samples sensitivity and 1 sample of patients remain *P. falciparum* at D3 (loss so not identified result of in vivo test). 8 samples *P. falciparum* remaining did not find mutations, in which there were two samples *P. falciparum* resistant, four samples sensitivity and two samples of patients remain *P. falciparum* at D3.

Comparative analysis of amino acid sequence of the enzyme ATPase6 were detected 49 amino acid be replaced. In which, there were 33 amino acids appear in *P. falciparum* resistant, 16 amino acids appear in both *P. falciparum* sensitivity, resistant and reinfection. Amino acids be replaced distributed into 5 sections as shown in figure 3.12.

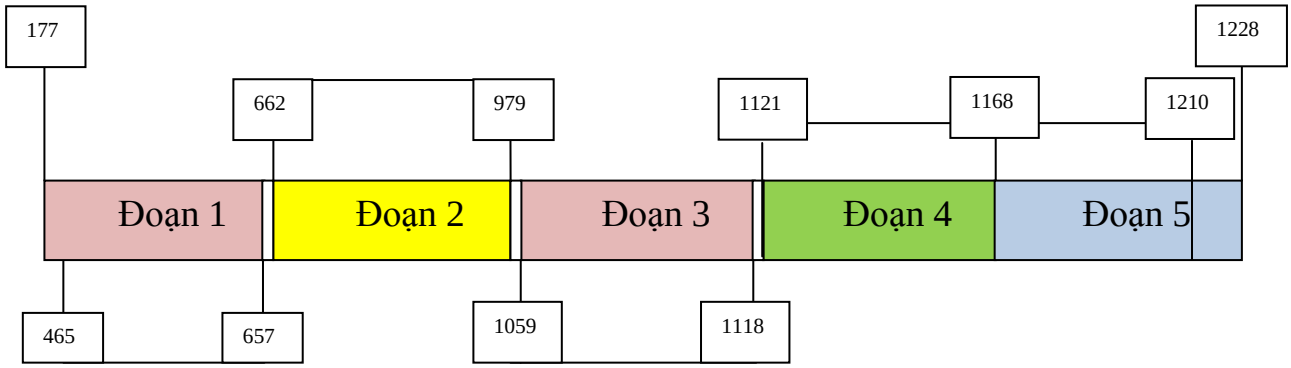

Figure 3.12. Model of enzyme ATPase6 of *P. falciparum* in patients. Small box upper and lower shown nucleotide position of the amino acid segment has been replaced.

In 33 amino acids be replaced in *P. falciparum* resistant, there were 14 amino acids distributed in fragment 1 (from position 403 - 657), in which four amino acids at positions N465S, I569L, T654K and T656S appear in 3 samples *P. falciparum* resistance is most concern. Two amino acids in fragment 2 (from positions 662-979) and 17 amino acids in fragment 3 (from positions 1059 to 1118).

In 16 amino acids be replaced in both *P. falciparum* sensitivity, resistance and re-infection there were 9 amino acids in *P. falciparum* sensitivity distributed in fragment 1 and 2, 2 amino acids in *P. falciparum* reinfection distributed in fragment 2, 4 amino acids in *P. falciparum* resistant and sensitivity distributed in fragment 2 and 3, 1 amino acid in both *P. falciparum* resistant, reinfection and sensitivity distributed in fragment 2.

In addition there were sequence of 90 amino acid of 20 samples *P. falciparum* are different compared with strain of *P. falciparum* 3D7. In which there are 48 amino acids are lost completely, corresponding with positions 1120 - 1168 (the fragment 4, dashes) and 42 amino acids are completely different corresponding to positions

1169 to 1210 compared with *P. falciparum* strain 3D7 (the fragment 5) as shown in figure 3.4.

```
gi.20D0   -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYIYIYIYIYIYIYIYF 1210
gi.34D0   -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYIYIYIYIYIYIYIYF 1210
gi.33D0DN -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYIYIYIYIYIYIYIYF 1210
gi.29D0DN -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYIYIYIYIYIYIYIYF 1210
gi.36D0   -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYMYIYIYIYIYIYIHIYIYF 1210
gi.62D0   -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYIYIYIYIYIYIYIHLYIYF 1210
gi.48D0   -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYIYMYIYIYIYIFIYFFF 1210
gi.24D0   -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYMYIYMYIYIYIYIYFFI 1210
gi.9D0    -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYIYIYIYIYIYIFLYIYFFI 1210
gi.44D0   -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYIYIYIYIYIYIYIYLYI 1210
gi.29D0BP -------V-------------------------------------INIVHTHKHTHTHIYIYIYIYIYIYMCVCVYVYIYKYTYIYIYI 1210
gi.15D0   -------V-------------------------------------INIVHTHKHTHTHIYIYIYVYLR------IYMYIFIYIHLFIYF 1210
gi.17D0   -------V-------------------------------------INIVHTHKHTHTHIYIYIYVYLR------IYMYIFIYIHLFIYF 1210
gi.46D0   -------V-------------------------------------INIVHTHKHTHTHIYIYV--YLR------IYMYIFIYIHLFIYF 1210
gi.33D0BP -------V-------------------------------------INIVHTHKHTHTHIYIYV--YLR------IYMYIFIYIHLFIYF 1210
```

Figure 3.4. The sequence of amino acid of the fragment 4 and 5 of enzyme ATPase6 in *P. falciparum* of patients in vivo and 3D7 strains from gene banks.
The sequence of red amino acids on the top line of *P. falciparum* 3D7
The sequence of amino acid under of *P. falciparum* in patients
The dashes of amino acids deleted.

Some amino acids of enzyme ATPase6 are replaced shown in figure 3.5.

```
>>3d7            NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.32D0        NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.5D0          NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.9D0          NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.15D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.17D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.46D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.33D0BP       NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.14D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.1D0          NNNNNNNNNNSNSVPSECISSWRKECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.48D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.24D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.36D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.29D0DN       NINYHHNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.44D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.62D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.33D0DN       NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.20D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.34D0         NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
>>gi.29D0BP       NNNNNNNNNNSNSVPSECISSWRNECKQIKIIEFTRERKLMSVIVENKKKEIILYCKGAPE 720
                  * * ::****************:************************************
```

Figure 3.5. The sequence of amino acids of enzyme ATPase6 of *P. falciparum*
Amino acids were replaced with black, bold and underlined

The position of 49 amino acid replaced were shown in table 3.12.

Table 3.12. Amino axits of enzym ATPase6 be replaced

TT	Vị trí aa thay thế	Axit amin bị thay thế ở *P. falciparum*				
		Thất bại	Đáp ứng	Tái nhiễm	Tái phát và	Tái phát
1	403		T403A			
2	**465**	**N465S**				
3	478	K478E				
4	504	Y504C				
5	519	N519S				
6	522	N522H				
7	526	N526D				
8	**569**	**N569K**				
9	578	K578T				
10	593	K593Q				
11	594	N594D				
12	652	N652Y				
13	**654**	**I654K**				
14	**656**	**I656S**				
15	657	I657P				
16	662		N662I			
17	664		N664Y			
18	665		N665H			
19	666		N666H			
20	683		I859T			
21	859		Y862S			
22	870			F870S		
23	889			C889Y		
24	927	I927L				
25	936		T936S			
26	939				A939S	
27	940					K940N
28	941	E941Q				
29	944				D944N	
30	978				S978I	
31	979		S979I			
32	1059	A1059D				
33	1064	F1064Y				
34	1065	V1065M				
35	1066	Y1066C				
36	1073				D1073E	
37	1077	H1077P				
38	1079	L1079W				
39	1084	Q1084L				
40	1089	N1089I				
41	1094	W1094R				
42	1098	R1098C				
43	1100	N1100Y				
44	1106	S1106C				
45	1107	E1107V				
46	1108	D1118G				
47	1109	H1109L				
48	1115	A1115P				
49	1118	I1118K				
Axit amin		**33**	**9**	**2**	**4**	**1**

DISCUSSION

4.1.The composition of species Anopheles and vector Anopheles malaria in the study

The survey results identified 19 species of Anophele to distribute at the study site, much more 12 species compared previous studies. The density of most species of Anopheles are very low. In which 5 species of Anopheles have the ratio over 1%, those are *An. dirus* 66.75%, *An. philippinesis* 23.7%, *An. minimus*3.01%, *An. minimus* 1.36% and *An. splendidus* 1.11%. Remaining 14 species were very low rate from 0.05% to 0.73%. Main vector *An. dirus* are most dominant at the study site accounting for 66.75% of the total mosquitoes of 19 species of Anopheles. Many other Anopheles species have low rate so the previous studies that have not investigated, including the main vector *An. minimus*. In three habitat at the study site, the villages distributed the most much species Anopheles with 17/19 species, but the number of mosquitoes caught only to occupy 1/3 number of mosquitoes of 19 species (33.74%). The forest habitats distributed less Anopheles species than villages, but the number of mosquitoes occupy 2/3 number of mosquitoes of 19 species (66.26%). These distribution are in accordance with natural law, where there are many species, the number of individuals of each species are low. The distribution of Anopheles at the forest less than at the villages shows that the ecological environment between habitats now is distinctly different. The ecological at villages suitable for many species of Anopheles development but not suitable for *An. dirus* development. Findings consistent with of Ho Dinh Trung and Nguyen Tuyen Quang about distribution of Anopheles in Ninh Thuan and Binh Dinh has also the number of Anopheles species at the forest reduced. Similar to the results of the investigation of Nguyen Van Chau at forest of Cat Tien Lam Dong have only 8 species of Anopheles, or natural conservation forest Can Gio, Ho Chi Minh City have 3 species Anopheles.

The survey results at the study site distributed five species of malaria vector. Includes two main vector *An. dirus* and *An. minimus*, 3 secondary vector *An. maculatus*, *An. jeyporiensis* and *An. aconitus*. This is the first time, main vector *An. minimus*and 2 secondary vector *An. jeyporiensis* and *An. aconitus* was determined distribution at Binh Phuoc that studies published previously have not seen. Malaria vector distributed in the study area only 5 species of Anopheles but accounted for 72,8% number mosquito of 19 species (1484/2063). In which the number of main vector *An. dirus* was highest, accounting for 55,67%

number mosquito of 19 species and 92,8% number mosquito of 5 species vector. The percentage of 4 vector species remaining were low (7,28%). Thus with dominance and capable to transmit 4 species plasmodium human and malaria monkey and affinity with drug-resistant parasites of the main vector *An. dirus* those are factors favorable for the development of malaria at study site. Main vector *An. minimus* accounted for only 1,9%, but live close human and ability to transmit malaria strong after *An. dirus*. So that should not be overlooked in the presence of this specie vector despite low density.

The distribution of the vector are different at three habitats. The village distributed 5 species of malaria vector, but the number of mosquitoes was at least (9%). The most of secondary vector are distributed at the village, *An.minimus*was 91.94% opposite to distribution of *An. minimus* in Ninh Thuan predominated in all three habitats, density of *An. minimus*in forest fold 1.2 times and 2.7 times the village. The remaining 2 secondary vector *An. aconitus* and *An. jeyporiensis* negligible proportion (0.73% and 0.1%) and just got in the village, did not found at forest habitats. The main vector *An. dirus* and *An. minimus* had low percentage and collected at besides houses in the garden of rubber near the stream. At the village, density of *An. dirus* only from 0.003 to 0.015 units/hour/person (depending on the time of the survey). This shows *An. dirus* still can development with low density at ecosystem of artificial forest replaced natural forests. Similar of study of Van Bortel in Thailand, *An. dirus* distribute in the garden of rubber with low density. *An. minimus* at the village did not collect at the last of rain season, but the early of dry season, density of *An. minimus* was 0.012units/hour/person. At the forest edge distributed 3 vector species, accounting for 16% number mosquito of 5 species vector and the main vector be mainly. The secondary vector *An. minimus*has low percentage. Deep forest distributed 2 main vector *An. dirus* and *An. minimus* accounted for 75% number mosquito of 5 species vector (3/4). In that *An. dirus* accounted for 80.3%,*An. minimus* 64.29% number mosquito of each species. No investigation found any species of secondary vector at deep forest. Main vector *An. dirus* and *An. minimus* distributed at all three habitats, but very differently densities. Density of *An. dirus* at the deep forest was fold 10 times at forest edges and 370 times at the village, higher than some malaria endemic areas such as Phu Khanh Hoa Khanh, density of *An. dirus* at deep forest fold 2 times at forest edges and 10 times at the village. In Ninh Thuan, density of *An. dirus* at deep forest fold 1.3 times at forest edges and 7.9 times at the village. Dak O, 2005 also has ratio of density

of *An. dirus* at the villages: forest edges: deep forest were 1: 6.7: 19.8 compared to density of *An. dirus* at the villages: forest edges: deep forest of this study were 1: 27: 323 at the end of the rainy season, or 1: 37: 370 at the early of the dry season. In addition, thousands of people from other places came to harvest of agricultural products and sleep there, especially at the dry season in the time that density of *An. dirus* higher to make increases the risk for malaria transmission. The cause for malaria prevalence were higher in annual dry season. This shows the danger of the group *An. dirus*, a species of vector living wild outdoor and abundance in natural forests Bu Gia Map. So that, who sleep at the forest if have been mosquitoes bite certainly be the main vector. Chaveepojnkanjorn study in Thailand showed that the risk of malaria of who regularly sleep at the forest higher fold 6-13 times at the village. The research results of Chambers at the Central and Southeast Vietnam also similar, who often to sleep at the forest have the risk for malaria fold 2 to 4 times sleep at residential areas. Based on the distribution and density of vector at the study site showed that the risk of malaria at the villages now was decreased compare to 2005, but at the forest habitats is still very high. While the fluctuations of population at study site is often complicated with thousands of people from other places come to look for jobs in the dry season. Many of them have bought anti-malaria drug by themself when malaria so often take drug did not enough or incorrect regimen dose. It is one of the main causes of drug resistance of the parasite and difficult for the management of malaria patients of medical stations. More dangerous when they return residential carrying resistant parasite, creating opportunities for malaria back development and spread drug-resistant parasites. The process of follow up and interview malaria patients at study site for 2 years showed the most of them certified that be infected malaria at the forest. While forest habitat occupies a large part of the land of local people. Such they will still continue to sleep at the forests even possible be malaria. So prevent malaria vector effectively for people sleep at the forests and management of malaria patients will be contribute reduce malaria and prevent the spread of drug resistance parasites

The main vector *An. dirus* and *An. minimus* distributed at 3 habitats of 2 times investigation in 10/2012 (end of rain season) and 12/2012-1/2013 (early of dry season). However mosquito density increases rapidly between two time of survey in less than two months. The villages, density of *An. dirus* in early dry season increased 5 times compared to the end of rainy season. The forest edge, density of

An. dirus increased 6.79 times, deep forest increased 5.72 times. *An.minimus* did not investigate at the village and forest edges on the end of rain season, but in early of dry season were 0.012 mosquito/ hour/person, at deep forest increased 16.67 times compare at the end of rainy season. So on early dry season density of *An. dirus* and *An. minumus* at all three habitats increased compare with 10/2012, despite at the study sites had sprayed insecticide before investigate on early of dry season. However density of mosquito is still increased, even at the village where the chemicals are sprayed periodically in accordance with the direction of the national malaria program. The datas showed the effective of this solution was not high, especially at the forest habitat. Therefore, research of solution to monitor and prevention vector effectively for people in this habitat, especially in the dry season is necessary. Because this is time many people concentrated product harvest of agricultural and correlated with malaria prevalence increased high every years. Statistics of Dak O medicine station in 4 months dry season of each years from 2010 to 2012 accounted 60% number of malaria patients of the all year, similar Dak O commune was 57%. So controlling the vector and objects at the time of high density of mosquito will be reduce drastically of number and limited the spread of malaria. This was demonstrated by indicator of malaria of Dak O commune fall 60% of 6 months of 2014 compared with 2013 and 65% over 4 years from 2010 - 2013. Because since the end of 2013 the entire habitats from the villages to the edge of forest at the study sites area has been enhanced and closely monitor 2 times spraying insecticide. The effect of chemical beside killing adult mosquitoes also pressure *An. dirus* back into deep forest, increased the distance between mosquito and human also contribute to limit strongly the spread of malaria. There's also a objective factors as feel of local population in 2013 the amount of people came to this region reduced because the crop loss. Therefore if this solution continue to maintain, the malaria prevalence will be decline, not grow back

4.2. Findings the sporozoite in Anopheles

Results PCR analysis only to detect *An. dirus* infection parasite with the rate of 0.58%. The proportion infected parasite of An.dirus is low, but distributed at the forest edges and deep forest, in the end of the rainy season and early dry season, collected at commune Dak O of Binh Phuoc, Dak Ngo and Quang Truc of Dak Nong provinces, where many people from other places came to work on dry season each year. Statistics of medicine station of Dak O from 2011 to 2013 annual average there were 600 patients with malaria parasites are detected. In

which over 30% people came from Dak Nhau, Bom Bo, Road 10 of Bu Dang District and other provinces. From 2011 to 2013 annual average had 127 patients with malaria parasites are detected at Dak Nhau commune there were 85% of patients confirmed be infected malaria during their time working at forest Bu Gia Map of Dak O communes or forest Quang Truc, where collected the mosquito infection parasite. *An. minimus*, *An. aconitus*, *An. minimus*and *An. jeyporiensis* did not detect be infect parasite because little of number mosquito analyzed. However *An. minimus*is a main vector with capable of transmitting malaria strong. Secondary vector also have a low prevalence infection in the previous study. A recent study of Ron, 2011*An. minimus*infection parasite at Khanh Phu Khanh Hoa was 0.012%.

4.3. Research results and detection of drug resistance mutations

Artemisinin and derivatives has been highly effective to *P. falciparum* drug resistant in the early 90s of the last century. However by 2003, doses of 7 days artesunate with *P. falciparum* have been decrease effective. At some study points as Phu Trung, Dak Nhau and Dak O, Binh Phuoc province had 16.4% patients *P. falciparum* malaria not clean parasite at D3. The study results showed that *P. falciparum* at Binh Phuoc and Dak Nong provinces resisted artesunate. There were 50.6% of patients prolong time cleaning parasite more 48 hours (D2 still has parasites) compared with 36 hours (1.9 days) at Ninh Thuan and Gia Lai ($p < 0.01$). The effective of artesunate reduced 15% at Dak Nong, 20% of Binh Phuoc compared to 2003 and 2009 were13.2% and 16%. There were 9.1% and 14% patients of Dak Nong and Binh Phuoc treatment failure were over the threshold of 10% to determine resistance drug and not used to drug prescribed of Ministry of Health.

4.3.1. Results detection of gene mutations MDR-1 and amino acids have been replace

Before DNA sequencing to detect the mutation of gene relate to resistant artesunate. The parasites reappear of fail treatment patients had been distinguish recrudesce or reinfection. The result show that among of 11 samples *P. falciparum* reappear there were 9 samples relapse, 2 samples reinfection. Results the analysis of DNA sequences gen MDR-1 of 9 samples *P. falciparum* (7 resistance and 2 sensitivity samples) only 3 mutation points be detected, 2 amino acid had been replace at position 107 (I107T) of 1 sample *P. falciparum* resistant and position 170 (F170I) of 1 samples *P. falciparum* sensitivity. These mutations appear in P. falciparum sensitive and resistance with low frequency and have not yet seen any published studies, so only are

random mutations of *P. falciparum* at the field. Results of analysis have seen only wild type of amino acid in position 86 of protein Pgh-1 at all sample *P. falciparum* resistance artesunate. Similar the results of Ngo Thanh 2003 in Viet Nam or Maja 2013 in Tanzania, *P. falciparum* resistance artemether combine lumefantrine have wild type of amino acid in position 86. The other foreign authors also have had similar results and considered it's a sign molecular determine resistance ACT of *P. falciparum*. Reed and Durasinght believed that the replace amino acid of protein Pgh-1 at position 86 relate to increase the sensitivity of *P. falciparum* to artemisinin and mefloquine is due the change of level acid in the digestive vacuole of the parasite not related to the absorption and excretion of drug. The mutation of gene MDR-1 in P. falciparum only increase the level of resistance, not decided the resistance such as mutation gen CRT at position 76 to decide the resistance with chloroquine or mutation gene CYT-b at position 263 to decide the resistance with autovaquion...The inversely relate between the wild type of amino acid at position 86 of *P. falciparum* to resistance artemisinin can be explain by assuming reverse mutation mechanism. *P. falciparum* resistance chloroquine, gen MDR- is mutated, amino acid of protein Pgh-1 at position 86 had replacement. When artemisinin is used to treatment *P. falciparum* resistance chloroquine, gen MDR-1 is mutated, amino acid at position 86 had replacement return wild type of original

4.3.2. Results detect mutation gen ATPase6 of *P. falciparum*

Results analysis of DNA sequences of gen ATPase6 found 83 mutation points, 49 amino acids of enzyme ATPase6 replaced. Of which only 4 amim acids at positions N465S, I569K, I654K and I656S of 3 samples *P. falciparum* resistant is concern. These amino acids are distributed in fragment 1 from amino acid at position 403 to 657 of the function region of the enzyme as Lionel had been detected in P. falciparum at Dak Nong Vietnam, 2010, Mallika Imwong detected in *P. falciparum* of Thailand or Jambou had been detected in *P. falciparum* of Americas, Asia and Africa.

Thus of the 49 amino acids of the enzyme ATPase6 have had replace there were 4 amino acid at position N465S, I569L, T656S and I654K appeared in three samples *P. falciparum* resistant to artesunate as some of studies has been published. However, the sample size in this study is small, so it is necessary continue to research with sample number much more and refer to the strain of *P. falciparum* resistance is generated from sensitive strains in the laboratory to conclude for more accurate.

CONCLUSION

1. There were 19 species Anopheles distribution at the study site. In which *An. dirus* accounted for 66.75%, *An. philippinensis* 23.7%, *An. minimus*3.01%, *An. minimus* 1.36%, *An. splendidus* 1.11% and the remaining 14 other species have low rate from 0.05% - 0.73%. The village have had distribute 17 species of Anopheles, 5 vector species in which 2 main vector and 3 secondary vector. The forest edge have had distribute 6 Anopheles species, 3 vector species in which 2 main vector and 1 secondary vector. The deep forest have had distribute 3 species Anopheles, 2 main vector. Main vector *An. dirus* and *An. minimus* distributed at all habitats villages, forest edges and deep forest. Secondary vector no found in forest habitats, the most at the village. Density of main vector An.dirus and *An. minimus*at deep forest is highest, then the forest edges, the village is lowest. Density of mosquitoes at the early of dry season is higher at the end of rainy season from 5 to 7 times depend on the habitats.
2. Detection infection soprozoite of *An. dirus* was 0.58%. There was 0.45% *An. dirus* collected at the forest edge be infected sporozoite, at the deep forest was 0.64%. *An. dirus* collected at the village did not detect. *An. dirus* collected at the end of the rainy season be infected sporozoite was 0.44%, at the early of the dry season was 0.61%. *An. minimus*and other species of Anopheles did not detect infect sporozoite.
3. Determined wild type of amino acid at position 86 in protein Pgh-1 related to resistance artesunate of *P. falciparum* and 3 mutation points of gen MDR-1 to make 2 amino acid had been replace at position 107 (I107T) and 170 (I170F) in protein Pgh-1. These mutations are not related to resistance artesunate of *P. falciparum.* .

   Detected 83 mutation points gen ATPase6 to make 49 amino acids of the enzyme ATPase6 had been replace. In which 4 amino acids at positions N465S, I569L, T656P and I654K of *P. faciparum* resistance artesunate.

RECOMMENDATIONS

Research has achieved some significant results for the scientific and practical contributions of the prevention of malaria, but still some exists recommendation for further research:

1. Continue to research gen ATPase6 and development for gen K13 of *P. falciparum* to detect the mutations relate to resistant the group sesquiterpene lactone drug.
2. Analyze samples collected at three time points D0 - D3 - D reappear *P. falciparum* to distinguish recrudesce - reinfection and detection of gene mutations for more accurate.