MINISTRY OF EDUCATION AND TRAINING

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CURRENT SITUATION OF MALARIA PARASITIC INFECTION AND EFFECTIVENESS OF SUPERVISION, DETECTION, TREATMENT IN BU GIA MAT DISTRICT, BINH PHUOC PROVINCE, 2018-2019

SUMMARY OF PhD THESIS ON PUBLIC HEALTH

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LIST OF PUBLISHED SCIENTIFIC ARTICLES RELATED TO THE THESIS

1. Nguyen Van Khoi, Le Thanh Dong, Le Thi Phuong Mai (2018), "Situation of malaria parasite infection in Bu Gia Map district, Binh Phuoc province in 2018", *Journal of Vietnam Preventive Medicine*, Vol. 28 (11): 110-119.
2. Nguyen Van Khoi, Le Thanh Dong, Le Thi Phuong Mai (2019), "Prevalence of malaria parasite infection and some related factors in Dak O commune, Bu Gia Map district, Binh Phuoc province", Journal of Practical Medicine. Ho Chi Minh City, Vol 23 (5): 192-197.
3. Nguyen Van Khoi, Le Thanh Dong, Le Thi Phuong Mai (2020), "The effectiveness of intervention on communication, surveillance and detection of people infected with malaria parasites in Bu Gia Map district, Binh Phuoc province, 2018-2019", *Journal of Vietnam Preventive Medicine*, Vol. 30 (10), 2020.
4. Nguyen Van Khoi, Le Thanh Dong, Le Thi Phuong Mai (2020), "Effectiveness of intervention of the direct supervised treatment for people infected with malaria parasites in Bu Gia Map district, Binh Phuoc province", *Journal of Vietnam Preventive Medicine*, Vol. 30 (10), 2020.

INTRODUCTION

Malaria is an infectious disease that can cause epidemics by transmission of Plasmodium (P.) via the mosquito Anopheles (An.). According to the World Health Organization (WHO), in 2018 globally, about 228 million people were infected and 405,000 died from malaria. Most malaria cases and deaths were mainly in Africa, accounting for 93.0% of the total cases.

Vietnam is one of the countries with a successful malaria control program (MCP), which has achieved great achievements since 1991 when it switched from a malaria eradication program to a malaria control program. Currently, the scope of disease circulation has narrowed, concentrating mainly in the provinces of Central region – Tay Nguyen and some provinces in the Southeast region. Binh Phuoc is a province with high endemicity, high population mobility and where the first malaria artemisinin-resistant parasite strains were detected in Vietnam. Although measures to monitor, detect and treat malaria patients were defined by the Ministry of Health, but these are mainly carried out passively and patients are provided with home treatment drugs under the guidance of medical staff. Therefore, whether malaria patients were compliant with treatment, cured and eliminated from parasites, monitoring and management data are still unavailable.

The detection technique of malaria parasites by microscopy is considered the gold standard in malaria diagnosis. The Real-Time PCR has not been applied in surveillance and detection of malaria parasites except of request or in scientific research. The application of Real-Time PCR technique has great advantages in detecting malaria parasites, especially among people infected with parasites of low density, below the detection threshold for microscopy or rapid diagnostic test for malaria

antigen and among those infected with malaria parasites with or without clinical symptomatic.

Objectives of the thesis

1) To determine the malaria parasite infection rate in the community by Real - Time PCR technique, rapid test and blood smear microscopy in some malaria-endemic communes of Bu Gia Map district, Binh Phuoc province in 2018.

2) To evaluate the effectiveness of surveillance, detection and direct supervised treatment of people infected with malaria parasites in some malaria-endemic communes of Bu Gia Map district, Binh Phuoc province, 2018-2019.

New contributions and practical value of the thesis

Currently, there are no studies to fully evaluate the status of malaria parasites among the communities in high endemic areas by microscopy, rapid diagnostic tests, and Real-Time PCR on the same subjects. The effectiveness of interventions on surveillance, detection and direct supervised treatment of people infected with parasites in the community with or without clinical symptoms and the cases infected with malaria parasites detected by PCD or ACD. The research results are valuable in practice and provided the scientific evidence supporting health sector to develop plans and orientations towards successful implementation of the malaria control and elimination strategy in areas of severe endemicity in accordance with the current local situation.

STRUCTURE OF THE THESIS

Thesis consisted of 126 pages (not including references and appendixes), 28 tables, 9 figures and 1 scheme. The Introduction includes 3 pages; 37 pages for Chapter 1 (Literature Review); 20 pages for Chapter 2 (Study subjects and methods); 27

pages for Chapter 3 (Research Results); 35 pages for Chapter 4 (Discussion); 3 pages for Conclusion 1 page for Recommendation.

Chapter 1. LITERATURE REVIEW

1.1. General introduction on malaria

1.1.1. *Malaria pathogens and immunity*

Malaria is an infectious disease caused by the Plasmodium parasite. The disease is transmitted via blood, mainly by Anopheles mosquitoes. The disease has typical clinical manifestations: chills, fever, sweating. There are 5 species of malaria parasites that cause disease in humans: *P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi*. Everyone can get malaria and can be infected with one or more species of the parasite. Immunity to malaria is unstable immunity to acquired malaria parasites, without cross-immunity. Humans have natural immunity to Plasmodium species of birds, reptiles and rodents. In communities in severe malaria endemic areas (MEA), most people are immune to the disease, accounting for 65.0-90.7%.

1.1.3. *Malaria parasite infected case with no symptoms*

People living in malaria endemic areas (MEA) may have the parasite in their blood, below the threshold of detection by microscopy or rapid diagnostic tests. Molecular biology techniques are becoming increasingly important for malaria control and elimination programs in each country and territory. Current molecular biology techniques have not been applied in routine surveillance and detection of parasites, but only used in some special cases or research. According to the survey results at several locations in the forest area of Central Vietnam, the rate of malaria parasites detected by PCR technique accounted for 29.14%, especially at Nam Tra My district, Quang Nam province,

the rate of malaria parasites detected by blood microscopy was accounted for 7.8% and by PCR accounted for 22.6%.

1.2. Malaria situation in the world and in Vietnam

1.2.1. *In the world*

Globally, it is estimated that in 2018 there were about 228 million people infected and 405,000 deaths from malaria, compared with 2010 the number of cases has decreased by 9.16%, of deaths decreased by 30.77% and the mortality rate was mainly in children of under 5 years old (67.0%). The composition of malaria parasite species caused by P. falciparum is concentrated mainly in sub-Saharan Africa, accounting for 99.70%, in Southeast Asia accounted for 50.0%, in Mediterranean accounted for 71.0%, and in Pacific, accounted for 65.0%. Parasite infections caused by P. vivax accounted for 75.0% in the Americas, about 53.0% in Southeast Asia, of which India accounted for 47.0%.

1.2.2. *In Vietnam*

Since 1991, Vietnam has implemented the malaria control strategy. After 10 years of program implementation (from 1991-2010), the number of malaria cases in 2010 compared with 1991 decreased by 73.1%, patient deaths decreased by 98.5%. The malaria control program has achieved certain success, but the results are not really sustainable. Malaria still threatens people's health in mountainous, mobile, remote and isolated areas, border exchanges and where many ethnic minorities live.

1.3. Testing techniques for malaria parasites

1.3.1. *Blood smear microscopy technique*

The microscopy technique has high sensitivity and specificity with a detection threshold of about 50-100 parasites/µl

of blood. Results is available soon within 2 hours. If the first test was negative but still suspect that the patient has malaria, it must be tested 2-3 more times, 8 hours apart or at the time the patient is sick. Specimens are fingertip or venous blood samples preserved in anticoagulant. Each thick-drop blood slide requires 6 µl of blood while a thin-drop smear requires 2µl. Blood samples should be used for smear slide preparation immediately or stored at 2-8°C for 48 hours. The technique of microscopy blood smear test has the advantages of simplicity, low cost, and is widely applied in medical facilities. But the quality of testing depends on the qualifications of the technician, when not practicing regularly can lead to errors.

1.3.2. *Rapid diagnostic test for malaria antigens*

Malaria antigen rapid diagnostic test (RDT) is convenient in areas where microscopy is not available and gives quick results. No need for laboratory conditions, others but not health staff can also perform this test. Each test requires 5 µl of fingertip or venous blood to be performed immediately after sample collection or stored with anticoagulant at 2-8°C for 3 days. RDT test gives results after 15 minutes, does not read results after 30 minutes.

1.3.3. *Real-Time PCR technique*

Real-Time PCR technique is applied in the detection and quantification of human pathogens such as viruses, bacteria, fungi, and parasites for diagnosis and treatment monitoring. However, this technique has not been widely applied because of the high cost per test, expensive equipment, and more complex community deployment compared to other techniques. Real-Time PCR

technique has high sensitivity and specificity, the detection threshold is approximately 1 parasite/µl of blood and the limitation is that it cannot distinguish the pathogen alive or dead.

The application of Real-Time PCR technique in detecting malaria parasites is of great significance in malaria prevention and elimination. It can be used for detecting malaria parasites in infected people with a low density, below the detection threshold of microscopy or rapid diagnostic test.

1.4. Monitoring, detection and treatment of malaria parasites

1.4.1. *Monitoring, detection of malaria parasite infected case*

Active and passive surveillance and case detection measures have been evaluated as valuable tools in disease prevention in general and malaria prevention in particular. Active case detection (ACD) method has advantages in early detection of malaria cases in the community, especially cases without clinical symptoms and cases undetected at the local health facility (PCD).

1.4.2. *Directly supervised treatment of malaria parasite infected cases*

This study did not investigate new regimens in the treatment of malaria. Cases infected with parasites receiving treatment according to the protocol of the Ministry of Health specified in Decision No. 4845/QD-BYT dated September 8, 2016 were surveyed and followed under the direct supervision of health staff that supervising and treating each case on a daily basis at the household. family or at workplace. These staff were also responsible for taking blood sample for malaria testing after the end of treatment.

Chapter 2. STUDY SUBJECTS AND METHODS

2.1. Study subjects, site and time

2.1.1 *Study subjects*

For Objective 1: People living and working at Dak O and Bu Gia Map commune, Bu Gia Map district, Binh Phuoc province in 2018.

For Objective 2: People living and working at Dak O and Bu Gia Map commune, Bu Gia Map district, Binh Phuoc province in a period of 2018-2019.

2.1.2. *Study sites*

The study was conducted at two communes: Dak O and Bu Gia Map communes, Bu Gia Map district, Binh Phuoc province. These are the two communes of severe malaria area defined according to the results of epidemiological zoning in 2014.

2.1.3. *Study time*

The study has been carried out from 2018 till 2019.

2.2. Research methods

2.2.1. *Study design*

For Objective 1: Descriptive cross-sectional study

For Objective 2: Intervention design using control group.

2.2.2. *Sample size and sampling method*

Sample size and sampling method for Objective 1:

Investigate the rate of malaria parasite infection: The sample size was calculated according to the formula to estimate a proportion in the population with relative accuracy. The minimum sample size was calculated as 676 people, but in fact the study has investigated 750 people. Subjects were selected for the study by systematic random sampling method.

Sample size and sampling method for Objective 2:

Passive detection of people infected with malaria parasites: All 1,193 people tested for malaria parasites at Commune Health Center (CHS) at Dak O commune that have residence or workplace in Bu Khon village and 1,016 people tested at CHS of Bu Gia commune that has a residence and working place in the Bu Lu village, from September 2018 to August 2019.

Active detection of people infected with malaria parasites: At Bu Khon village, Dak O commune, for each case of index disease detected passively by the CHS, 25 households around the house of the indexed case was actively investigated. For Bu Gia Map commune, the number of households to be actively surveyed was determined based on the commune's annual survey plan. During the study period from September 2018 to August 2019, in Bu Khon village, the total number of people tested was 1,339 and in Bu Lu village, 487 were tested.

Cross-sectional survey before and after the intervention: The sample size was calculated according to the formula to estimate the difference of the two rates. The minimum sample before and after the intervention in the intervention group and the control group was counted as 196 people. Expecting a sample loss of 10%, the minimum sample size to be collected for each group was 216 people. In fact, 240 people were investigated before the intervention and 280 people were investigated after the intervention from the intervention and control groups. Subjects selected for the study were matched by sex, age group and occupation.

Supervised treatment of people infected with malaria parasites: All detected cases infected with malaria parasites by

passive/active detection were selected for treatment under the direct supervision for cross-sectional investigated before intervention.

2.2.3. *Method and information collecting tools*

Method and information collecting tools for Objective 1

Research subjects were selected by systematic randomization according to the sample frame. Venous blood samples were taken for malaria testing by microscopy, RDT, Real-Time PCR. Information was collected through interviews, observations and retrospective data according to a prepared questionnaire form.

Method and information collecting tools for Objective 2

*Method of data collection: A*ll subjects infected with parasites that were passively and actively detected according to the medical examination list at the CHS enrolled for study. For investigating the knowledge, attitudes, and practices on malaria prevention, subjects were selected randomly from the available sample frame and matched by sex, age group, occupation before and after the intervention in two groups (intervention and control group). People infected with parasites are treated under direct supervision at the household or at workplace.

Techniques used in the study: Interviewing knowledge, attitudes, practices and observations, taking blood samples for malaria testing after treatment on days D3, D7, D14, D28 at household or at workplace.

2.3. Data analysis and processing

Collected data was cleaned, processed, coded and entered using Epidata 3.1 software and analyzed using Stata 12.0 software.

Inferential statistics were performed through an estimate of 95% confidence interval, the odds ratio (OR) was calculated in the analysis of the relationship between cause and effect with statistical significance at p <0.05. Using the Chi-squared test to test the difference of the intervention group and the control group at two time points: before the intervention - after the intervention and to test the effectiveness of the intervention.

2.4. Research ethics

The study design was approved by the Ethics Committee for Biomedical Research, National Institute of Hygiene and Epidemiology under code IRB-VN1057/IORG 0008555.

Chapter 3. RESULTS

3.1. Rate of malaria parasite infection at the studied sites

Table 3.1. Rate of malaria parasite infection at studied sites

Detection technique	n	**Malaria parasite infection**	
		Yes (%)	No (%)
Microscopy	750	16 (2.13)	734 (97.87)
Rapid test (RDT)	750	10 (1.33)	740 (98.67)
Real-Time PCR	750	179 (23.87)	571 (76.13)

The rate of malaria parasites infection detected by blood smear microscopy test was accounted for 2.13%, by RDT accounted for 1.33% and by Real-Time PCR technique accounted for 23.87%.

Table 3.2. Species composition of malaria parasites at the study site (n=750)

Detection technique	n	**Malaria parasite species**			**Total**
		P. falciparum	*P. vivax*	*P. falciparum +P. vivax*	

Microscopy					
Dak O	399	08(1.07)	01(0.13)	00(0.0)	09(1.20)
Bu Gia Map	351	05(0.67)	02(0.27)	00(0.0)	07(9.33)
Total	**750**	**13(1.73)**	**03(0.40)**	**00(0.0)**	**16(2.13)**
RDT					
Dak O	399	03 (0.27)	00(0.0)	00(0.0)	03(0.40)
Bu Gia Map	351	05 (0.67)	02(0.27)	00(0.0)	07(0.93)
Total	**750**	**08(1.07)**	**02(0.27)**	**00(0.0)**	**10(1.33)**
Real-Time PCR					
Dak O	399	61(8.13)	07(0.93)	04(0.53)	72(9.60)
Bu Gia Map	351	55(7.33)	30(4.0)	22(2,.3)	107(14.27)
Total	**750**	**116(15.47)**	**37(4.93)**	**26(3.47)**	**179(23.87)**

The rate of malaria parasites detected by Real-Time PCR was accounted for the highest, of which P. falciparum was detected in 15.47%, P. vivax accounted for 4.93% and those infected with a combination of P. falciparum + P. vivax were accounted for 3.47%.

Table 3.3. Factors related to infection of malaria parasite

(n= 750)

Factors		**Malaria parasite infection**		OR	CI 95%	p
		Yes (%) n=179	No (%) n=571			
Sex	Male	91(27.08)	245(72.92)	1.38	0.97-1.95	0.06
	Female	88(21.26)	326(78.74)			
Age group	<5	05(17.86)	23 (82.14)	1		

	5 - 15	39(22.94)	131(77.06)	1.37	0.49-3.84	0.55
	>15	135(24.46)	417(75.54)	1.49	0.56-3.99	0.43
Ethnic	Kinh	74(22.16)	260(77.84)	1		
	S'tiêng	76(24.28)	237(75.72)	1.13	0.78-1.62	0.52
	Tay, Nung	19(36.54)	33(63.46)	2.02	1.09-3.76	0.03
	Others	10(19.61)	41(80.39)	0.86	0.41-1.79	0.68
Occupation	Farming	85(28.72)	211(71.28)	1		
	Make forest	21(39.62)	32(60.38)	1.63	0.89-2.98	0.11
	Small business	04(15.38)	22(84.62)	0.45	0.15-1.35	0.15
	House-wife	05(17.24)	24(82.76)	0.52	0.19-1.40	0.19
	Students	31(22.14)	109(77.86)	0.71	0.44-1.31	0.15
	Others	33 (16.02)	173(83.98)	0.47	0.30-0.74	0.001

The rate of malaria parasite infection among the Tay, Nung, and Mo Nong ethnic groups was found 2.02 times higher than that of the Kinh group and this rate among people with other occupations was only about 0.47 times higher than that of those working as the farmers, $p<0.05$.

Table 3.4. Relation between some epidemiological factors, malaria history and malaria parasite infection rate (n= 750)

Factors		Malaria parasite infection		OR	CI 95%	p
		Yes (%) n=179	No (%) n=571			
Place to stay within 14 days	At home	31(24.03)	98(75.97)	1		
	In the field	90(22.17)	316(77.83)	0.90	0.56-1.44	0.66
	In the forest	18(47.37)	20(52.63)	2.85	1.34-6.05	0.007
	Other	40(22.60)	137(77.40)	0.92	0.54-1.58	0.77
Length of stay	<1 year	02(15.38)	11(84.62)	0.87	0.09-4.07	0.61
	≥1 year	177(24.02)	560(75.98)			
Border Exchange	Yes	28(32.94)	57(67.06)	1.67	0.10-2.78	0.04
	No	151(22.71)	514(77.29)			
Use mosquito net at night	Yes	177(23.82)	566(76.18)	0.78	0.13-8.28	0.30
	No	02(28.57)	05(71.43)			
Sleep in the forest	Yes	32(47.76)	35(52.24)	3.33	1.92-5.74	0.001
	No	147(21.52)	536(78.48)			
Sleeping at farm slopes	Yes	49(31.82)	105(68.18)	1.67	1.11-2.51	0.009
	No	130(21.81)	466(78.19)			
Had malaria	Yes	67(31.90)	143(68.10)	1.80	1.23-2.59	0.001
	No	112(20.74)	428(79.26)			

Rate of malaria parasite infection among the people who have been in the forest within 14 days before the survey showed to be 2.85 times higher than those stayed at home; this rate among

the people who used to stay in forest for several days was 3.3 times higher than those who did not and those who sleep at the farm fields had a parasite infection rate of 1.67 times higher than those who sleep at home; among the people with a previous history of malaria, rate of malaria parasite infection was found 1.8 times higher than that of people who have never had malaria.

3.2. The effectiveness of surveillance, detection and directly supervised treatment of people infected with malaria parasites

3.2.1. Passive and active detection of parasite infection

Table 3.5. Rate of parasite infection actively detected

Variable	Intervention gr.		Control gr.		p
	Number	%	Number	%	
Yes	02	0.15	00	0.0	
No	1,337	99.85	487	100.0	>0.05*
Total	**1,339**	**100.0**	**487**	**100.0**	

*: *Fisher's precision correction*

Rate of parasite infection detected actively in the intervention group was accounted for 0.15%, while none of case was found in the control group by this detection method.

Table 3.6. Rate of parasite infection detected passively

Variable	Intervention gr.		Control gr.		p
	Number	%	Number	%	
Yes	15	1.26	30	2.95	
No	1,178	98.74	986	97.05	p <0.05
Total	**1,193**	**100**	**1.016**	**100**	

Rate of parasite infection detected passively through the surveillance system among the intervention group was accounted for 1.26% and it was accounted for 2.95% among the control group.

Table 3.7. Malaria parasite composition detected passively and actively

Malaria parasite	**Intervention gr.**		**Control gr.**		**p**
	Number	(%)	Number	(%)	
P. falciparum	10	58.82	13	43.33	
P. vivax	07	41.18	17	56.67	p>0.05
Total	**17**	**100**	**30**	**100**	

The detected rate of *P. falciparum* and *P. vivax* species were not significantly different among intervention group and control group *(*p>0,05).

3.2.2. Surveillance result obtained before and after an intervention

Table 3.8. Rate of malaria parasite before and after an intervention

Variable	Intervention group					Control group					Intervention Effectiveness p
	Before n=240		After n=280		Efficiency Index	Before n=240		After n=280		Effect. Indicator p	
	#	%	#	%		#	%	#	%		
RDT											
Yes	03	1.25	00	0,0	100.0	07	2.92	0	0.0	100.0	0.0
No	237	98.75	280	100.0	>0.05*	233	97.08	280	100.0	<0.05*	//
Microscopy											
Yes	06	2.5	00	0.0	100.0	05	2.08	00	0.0	100.0	0.0
No	236	98.33	280	100.0	<0.05*	235	97.92	280	100.0	<0.05*	//
Real-Time PCR											
Yes	53	22.08	06	2.14	90.31	57	23.75	10	3.57	82.46	7.85
No	187	71.92	274	97.86	<0.05	183	76.25	270	96.43	<0.05	>0.05

Rate of parasites detected after intervention by Real-Time PCR in the intervention group decreased from 22.08% to 2.14% with the efficiency index (EI) reached 90.31%, in the control group this rate has decreased from 23.75% down to 2.57% with the efficiency index reaching 82.46%, the intervention effectiveness (IE) of 7.85.

Table 3.9. Effectiveness of improving the knowledge, attitude, practice on malaria prevention

Index		**Intervention group**			**Control group**			**IE**
		Before n=240 (%)	After n=280 (%)	EI p	Before n=240(%)	After n=280(%)	EI p	**p**
Knowledge	Good	83 (34.58)	203 (72.50)	109.66 < 0.05	82 (34.17)	128 (45.71)	33.77 < 0.05	75.89 < 0.05
	Not good	157 (65.42)	77 (27.50)		158 (65.83)	152 (54.29)		
Attitude	Good	151 (62.92)	367 (95.36)	51.56 < 0.05	148 (61.67)	195 (69.64)	12.92 > 0.05	38.63 < 0.05
	Not good	89 (37.08)	13 (4.64)		92 (38.33)	85 (30.36)		
Pratice	Good	154 (64.17)	256 (91.43)	42.48 < 0.05	91 (37.92)	132 (47.14)	24.31 < 0.05	18.17 < 0.05
	Not good	86 (35.83)	24 (8.57)		149 (62.08)	148 (52.86)		

Communication to improve knowledge, attitude and practice on malaria prevention among research subjects in the

community showed to be an effective measure of intervention, it significantly improved the knowledge with an intervention effectiveness (IE) of 75.89%, p<0.05; the attitude (IE=38.63%, p<0.05) and practice on malaria prevention (IE=18.17%, p<0.05).

3.2.3. Evaluation of the effectiveness of directly supervised malaria treatment at the study site

Table 3.10. The efficiency of treatment among people infected with malaria parasites detected passively and actively by microscopy

Parasite infection Test result		**Intervention gr. n=17**		**Control gr. n=30**		p
		SL	%	SL	%	
Day D3	(-)	17	100.0	10	83.33	>0.05*
	(+)	00	0.0	02	16.67	
Day D7	(-)	17	100.0	10	100.0	//
	(+)	00	0.0	00	0.0	
Day D14	(-)	17	100.0	10	100	//
	(+)	00	0.0	00	0.0	
Day D28	(-)	17	100.0	10	100	//
	(+)	00	0.0	00	0.0	

*: *Fisher's precision correction*

Rate of parasite clearance in the intervention group on day D3, D7, D14 and D28 have reached 100.0%, while in the control group the positive for parasites on day D3 was detected in 16.67% subjects and those who were negative on day D3 were retested on day D7, D14, D28 at commune health centerers (CHC), results showed 100.0% negative.

Table 3.11. Treatment efficiency among people infected with malaria parasites detected by cross-sectional investigation microscopy before intervention

Parasite infection	**Intervention gr.**	**Control gr.**	p

	Test result	n=6		n=5		
		SL	%	SL	%	
Day D3	(-)	06	100.0	04	80.0	>0.05*
	(+)	00	0.0	01	20.0	
Day D7	(-)	06	100.0	03	100.0	//
	(+)	00	0.0	00	0.0	
Day D14	(-)	06	100.0	03	100.0	//
	(+)	00	0.0	00	0.0	
Day D28	(-)	06	100.0	03	100.0	//
	(+)	00	0.0	00	0.0	

*: *Fisher's precision correction*

Treatment efficacy among people infected with malaria parasites detected by microscopy in the intervention group was approved by the negative result obtained from 100.0% of subjects the on days D3, D7, D14, D28. In the control group, 20.0% of subjects showed positive with malaria parasites infection on day D3, those who were negative on day D3 were resampled on day 7, D14, and D28, results showed all 100.0% negative.

Table 3.12. Evaluation the treatment efficacy among people infected with malaria parasite detected by Real-Time PCR

Parasite infection / Test result		**Intervention gr. (n=53)**		**Control gr. (n=57)**		**IE p**
		#	Rate (%)	#	Rate (%)	
P. falciparum		**43**	**81.13**	**43**	**75.44**	
D3	(-)	43	100.0	128	65.12	41.67
	(+)	00	0.0	15	34.88	<0.05*
P. vivax		**10**	**23.26**	**12**	**21.05**	
D14	(-)	05	100.0	00	0.0	//
	(+)	00	0.0	00	0.0	
P. falciparum* + *P.		**00**	**0.0**	**02**	**3.51**	

vivax						
D14	(-)	00	0.0	00	0.0	//
	(+)	00	0.0	00	0.0	

**: Fisher's precision correction*

Rate of parasite-clearance by treatment on day D3 in the intervention group was found in 100.0% subjects while in the control group it was 65.12%, the efficiency index was 41.67%. People infected with *P. vivax* in the intervention group were monitored and treated accounted for 50.0%. These were all negative (100%) on day D14. In the control group, treatment was not monitored and not retested after treatment.

Chapter 4. DISCUSSION

4.1. Rate of malaria parasite infection at some malaria-endemic communes of Bu Gia Map district, Binh Phuoc province in 2018

4.1.1. Prevalence of malaria parasite infection detected by microscopy, RDT and Real-Time PCR

In this study, blood samples collected from venous blood were tested simultaneously with 3 techniques: smear microscopy, RDT, and Real-Time PCR. The rate of malaria parasites detection by microscopy blood smear test accounted for 2.13%, by RDT accounted for 1.33% and by Real-Time PCR accounted for 23.87%. The rate of samples negative by microscopy blood smear test turned to be positive by Real-Time PCR technique was accounted for 22.28% and those who were negative by RDT technique were positive by Real-Time PCR with the rate of 22.84%. The rate of parasites detected by Real-Time PCR technique was 11.19 times higher than that detected by microscopy blood smear test and 17.90 times higher than parasites detected by RDT technique. Rate of parasite infection detected by blood smear microscopy test was found 1.6 times higher than by RDT technique. Compared with the results obtained by Nguyen Xuan Xa et al. (2012) (the rate of malaria parasite infection was accounted for 3.6%), and results found by Pham Vinh Thanh et al. (2015) (7.8%), the rate of malaria parasites detected by microscopic blood smear test in this study was lower. This rate also was lower than the results obtained by Jung Mi Kang et al. (2017) in Myanmar (23.20%) and the rate found in the study of Lek D. et al (2016) in Cambodia (2.74%). In Vietnam, this rate was lower than the results obtained from the study of Dao Van Hue, Pham Van Ky

et al (2004) in Ninh Thuan (the rate of malaria parasites detected was found of 13.02% by microscopy blood smear, 16.25% by RDT). In this study, the rate of malaria parasites detected by Real-Time PCR technique was higher than that obtained by Pham Vinh Thanh's study (22.60%) and by Nguyen Thi Huong Binh's study (2009) (17.65%), but lower than the results found by Nguyen Hong Van et al. (29.14%). Our results were higher than that obtained from the research of Lies Durnez, Myrthe Pareyn, Vanna Mean et al (2018) in Ratanakiri, Cambodia (8.40%) and higher than that (13.71%) found by the study of Daniel M. Parker, Stepphen A., Matthews et al (2015).

4.1.2. Some factors related to infection rate of malaria parasite at research sites

In this study, a number of factors was found related to the prevalence of malaria parasite infection detected by Real-Time PCR. Parasite infections were found related to mobile population, frequent going to the forest, sleeping at the farm fields, exchanging border sometime at the harvesting season, preparing for a new crop. These are the factors contributing to malaria prevalence persistent growth and circulation. Research results showed that the rate of malaria parasite infection among those who frequently stay in the forest for some days was 3.33 times higher than those who do not stay in the forest. This rate was higher than the results obtained by Le Thanh Dong and colleagues at Binh Thuan and Quang Binh (2005) which showed the rate of malaria parasite infection among the subjects who went frequently go to the forest, slept in the fields, and the subjects often crossing border for exchange was 1.67 times higher than that among other subjects. According to

the results of research conducted by Ho Van Hoang and colleagues in Huong Hoa district, Quang Tri province, the rate of malaria parasite infection in people with frequent border exchanges was 5.37 times higher than that in people without border exchanges, the difference was statistically significant. On the other hand, the rate of malaria parasite infection in subjects with a history of malaria was 1.8 times higher than in subjects without previous malaria. The results of this study may be consistent with the actual situation because people infected parasites were being treated according to the regimen prescribed by the Ministry of Health, but whether the patient adheres to the treatment, recovers from the disease and is free of parasites, is not well supervised and managed.

4.2. Effectiveness of surveillance, detection and treatment of people infected with malaria parasites

4.2.1. Monitoring and detecting people infected with malaria parasites

Surveillance is the backbone of disease prevention and control, collecting and providing information on disease situation and recommending effective interventions. Passive surveillance and case detection (PCD) and active case detection (ACD) play an important role in early detection and timely treatment of infected patients that having symptoms or those suspected malaria at health facilities and in community. However, the ACD measure has an important role in the early detection of malaria cases in areas where health services are difficult to access and those who are missed by PCD or clinically asymptomatic cases with malaria infections.

In this study, rate of parasites detected by PCD in the control group was 2.39 times higher than that found in the intervention group and the parasite infection rate in the

intervention group accounted for 0.15%, the control group had not yet detected parasites infected subjects. The ACD is implemented as soon as the case is detected through the routine surveillance system. Our result is consistent with the results obtained from the study of Neeru Singh (2016), R. Wongsrichanalai C. (2007), Wesolowski A. (2012), Christopher Lourenco (2019), Chand G., (2015), Chand S.K. (2013). In our study, we did not detect gametocyte-carrying parasite infections while in the study of Bousema JT (2004), the proportion of subjects detected by ACD with gametocytes was accounted for 12.0%.

The effective communication to improve knowledge, attitude and practice on malaria prevention among the people at the study site has been found significantly improved with the intervention effectiveness (IE) of 75.89% for knowledge, 38.63% for attitude and 18.17% for correct practice improvement. Results of our study were found in consistent with the results obtained in the studies of Tran Thanh Duong (2015), Nguyen Xuan Xa (2015), Le Xuan Hung (2008) and Wongsrichanalai C. (2007), Sharma S.N. (2000).

4.2.2 *Effectiveness of treatment of people infected with malaria parasites*

This study was designed and conducted for the first time in a severe malaria area of Binh Phuoc province, therefore was considered as an active measure to monitor the effectiveness of under direct supervision antimalarial treatment (iDES) in subjects infected with malaria parasites caused by *P. falciparum, P. vivax,* symptomatic or asymptomatic associated malaria infection, detected by passive (PCD) or active case detection (ACD). Cases of malaria parasites caused by *P. falciparum* in the intervention group were treated with arterakine under direct supervision at the household on days D0, D1, D2, D3 and tested after treatment with microscopy

blood smears on days D3, D7, D14, D28 all were 100% negative, the treatment efficiency reached 100.0% compared with the control group. Meanwhile, results of the study conducted by Wichai Satimai, Prayuth Sudathip, Saowanit Vijaykadga et al. (2012) showed the rate of malaria parasites on day 3 accounted for 14.0%. Results obtained by the study of Bui Quang Phuc et al. (2013) showed this rate on day 3 accounted for 38.50% among treated people infected with malaria parasites caused by *P. falciparum* but did not have complications. According to the results found by the study of Quach Ai Duc et al. in 2013, rate of parasites on day 3 accounted for 30.60%. This rate was found of 14.80% according to the study of Bui Quang Phuc in 2015 in Kon Tum, 17.40% in Khanh Hoa and 36.0% in Binh Phuoc province. The results of our study were consistent with data found by the study of Nguyen Chinh Phong (2019) at Phuoc Chien commune, Ninh Thuan province: no malaria parasites were detected on day D3 of the treatment and all remained negative with malaria parasite testing after on days D7, D14, D28 of treatment. Our results were also consistent with the results obtained by the research of Wichai Satimai (2012) and Lwidiko E. Mhamilawa (2020).

For the malaria parasite infected cases detected by Real-Time PCR technique, results of following and post-treatment test of parasite infection were found negative in 100% cases on day D3. The treatment effect for the subjects enrolled in this study was higher than that obtained by the study of Ogutu B. (2010); Baraka j. Nzobo (2015), Khatib R.A. (2012) and the rate of malaria parasite infection caused by *P. falciparum* after treatment was 0.0% while that obtained by Lwidiko E. Mhamilawa, Billy Ngasala, Ulrika Morris, Eliford Ngaimisi Kitabi, Bory Barnes (2020) showed the rate of malaria parasite infection on day D3 of 60.0%. Among the subjects infected with malaria *P. viva*, results of testing after treatment with

chloroquine and primaquine showed the rate of parasite infection of 0.0%, lower than that found by the study of Nguyen Chinh Phong (2019) which was 13.33%.

CONCLUSION

1. The prevalence of malaria parasite infection in the community of communes Dak O and Bu Gia Map, Bu Gia Map district, Binh Phuoc province in 2018

Rate of malaria parasites detected by Real-Time PCR technique was 23.87%, accounted for 17.95 times higher than rate obtained by RDT (1.33%) and 11.21 times higher than that detected by blood smear microscopy (2.13%). The composition of malaria parasite species detected by Real-Time PCR showed mainly *P. falciparum* (64.80%), *P. vivax* (20.60%). Those infected simultaneously with *P. falciparum* + *P. vivax* were accounted for 14.53%.

Rate of malaria parasite infection among the subjects of Tay, Nung, and Mo Nong ethnic groups was found 2.02 times higher than that of the Kinh group. The infection rate found in people of other occupations was only 0.47 times higher than that among the farmers. People who have been in the forest 14 days before the survey were found being infected with malaria parasites with the rate of 2.85 times higher than that of people who stayed at home; the parasite infection rate among the people that frequently border exchanged was found 1.67 times higher than of those who did not; this rate among people who used to stay long time in the forest was found 3.33 times higher than found in the subjects thus did not sleep in the forest and this rate of people used to sleep on the farm field was found 1.67 times higher than that in people who sleep at home. People with a history of malaria infection had the infection rate of 1.8 times higher than those who never had, the differences were statistically significant ($p<0.05$).

2. The effectiveness of intervention on surveillance, detection and treatment of malaria parasite infected people at Bu Gia Map district, Binh Phuoc province in 2018-2019

- Effect of health education communication:

Rate of subjects with good knowledge was found increased after the intervention in both intervention and control group with intervention efficacy of 75.89%, $p<0.05$.

Rate of subjects gained good attitude was found increased after the intervention in both intervention and control group with intervention efficacy of 38.63%, $p<0.05$.

Rate of subjects having good practice was found increased after the intervention in both intervention and control group with intervention efficacy of 18.17%, $p<0.05$.

- Effect of case detection by passive and active method during period of 9/2018 to 8/2019:

Rate of parasite infection case actively detected in the intervention group was accounted for 0.15%, while in the control group, none of case was actively detected during study period.

Rate of parasite infection passively detected at Commune Health Station after intervention in the control group was accounted for 2.95%, higher than that of the intervention group (1.26%), $p<0.05$.

- The effect of anti-malaria parasite treatment under direct supervision:

Rate of parasite clearance among cases detected passively and actively by microscopy in the intervention group on day D3, D7, D14 and D28 have reached 100.0%, while in the control group the positive result for parasites on day D3 was detected in 16.67% subjects, all (100%) were turned negative only on day D7, D14, D28.

People being positive with malaria parasites in the intervention group detected by blood smear microscopy in a

cross-sectional investigation before the intervention showed to have the rate of parasite clearance of 100.0% on days D3, D7, D14, and D28 of treatment courses, higher than that of the control group. In the control group, the parasite clearance rate on day D3 was accounted for 80.0% and has reached to 100% on days D7, D14, and D28.

People infected with *P. falciparum* in the intervention group detected by Real-Time PCR technique in the pre-intervention cross-sectional investigation were treated and showed to have parasite clearance on day D3 of 100.0%, higher than that in the control group (parasite positivity on D3 was accounted for 65.12%). The proportion of people infected with *P. vivax* parasite in the intervention group was monitored and treated was accounted for 50.0% and the results of parasite eradication after treatment among these subjects have reached 100.0% compared with the people infected with *P. vivax* parasites treated without direct supervision in control group. In this study, in the intervention group, no cases of combined malaria infection were detected.

- The effectiveness of intervention to reduce the rate of malaria parasite infection at the study site

After the intervention, rate of malaria parasite infection detected by Real-Time PCR technique in the intervention group was found decreased from 22.08% to 2.14% with the efficiency index of 90.31%. In the control group, this rate has been decreased from 23.75% to 3.57% with the effective index of 82.46%. The intervention efficiency reached 7.85%.

RECOMMENDATION

1) Research develops a procedure of monitoring and treating under direct supervisor for people infected with malaria parasites in the community during the malaria eradication period.

2) Recommend the Ministry of Health to add the content of direct supervised treatment and parasite testing after D3, D7, D14, D28 in the field to the routine surveillance program during the malaria prevention and elimination phase.

3) It is necessary to conduct further studies on surveillance, detection and treatment under direct supervision of subjects infected with malaria parasites on a broader scale among forest-sleeping, farm-sleeping and border crossing populations in endemic areas. Propaganda to raise public awareness about adherence to treatment when infected with parasites caused by *P. vivax* and with the combination of *P. falciparum* + *P. vivax*.