MINISTRY OF EDUCATION AND TRAINING MINISTRY OF HEALTH
NATIONAL INSTITUTE OF MALARIOLOGY, PARASITOLOGY, AND ENTOMOLOGY

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STUDY ON THE MALARIAL PARASITE FORMULA AND EFFICACY OF CHLOROQUIN REGIMEN WITH *Plasmodium vivax* IN KRONG PA, GIA LAI PROVINCE

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FOREWORDS

For over a decade, the world has made significant progress in the fight against malaria. Since 2000, malaria mortality has decreased by more than 25% with nearly 100 countries with malaria approaching the World Health Assembly's 2015 target of reducing malaria morbidity by over 75%. Global malaria report data from the World Health Organization (WHO) for 2020 showed that in 2019 there were 229 million cases of malaria in 87 endemic countries, of which 409,000 were malaria-related deaths, primarily focused in children under 5 y/o in the sub-Saharan Africa region. Despite progress, there are still approximately 3 billion people at risk of contracting malaria.

In Vietnam, although malaria morbidity and mortality rates have decreased significantly due to proactive interventions, there are still many challenges to face such as mobile and migrant populations, forest goers and sleepers, border exchange in malaria-endemic areas, mosquito resistance to chemicals, and drug-resistant malaria parasites. The success of malaria control has been significantly contributed to by highly effective malaria drugs, but under drug pressure and resistance, regular monitoring of drug sensitivity-resistance is a crucial step. Not only *P. falciparum* became resistant to multiple drugs, including artemisinin combination therapies (ACTs) widespread in the Western Pacific region, which poses technical barriers to drug selection, but *P. vivax* has also become less sensitive or resistant to chloroquin (CQ) in many countries such as Cambodia, Thailand, Myanmar, Indonesia, East Timor, India and may affect Vietnam where CQ is currently the first-line drug. Some sentinel sites have seen a decrease in *P. vivax*'s response to CQ in places such as Binh Thuan (Phan Tran Giao *et al.*, 2002), Quang Nam, Gia Lai (Huynh Hong Quang *et al.*, 2017).

During the implementation of malaria control and elimination measures, the malaria parasite composition changed in favor of *P. vivax* which was recorded in the direction of increasing or nearly equal to *P. falciparum* in some areas. Moreover, the use of CQ for over 70 years in Vietnam for various purposes such as prophylaxis, stand-by treatment, and treatment has now recorded some cases of late parasitological failure due to *P. vivax*, which is a critical warning sign. Therefore, monitoring the efficacy of current drugs and testing new drug candidates on *P. vivax* malaria patients is necessary to

supplement data and act as a fundamental for sketching new malaria drug policies in Vietnam.

Based on that, the study "Study on the malaria parasite formula and the efficacy of chloroquin regimen with *Plasmodium vivax* in Krong Pa district, Gia Lai province" was conducted to achieve the objectives:

1. To describe the malarial parasite species formula progress in Krong Pa district, Gia Lai province in period 2010-2019;

2. To evaluate the drug efficacy of chloroquine phosphate in treatment of *Plasmodium vivax* patients at study sites (2018-2019).

STRUCTURE OF THESIS

The thesis has totally 120 pages (not include references and annex parts). With Foreword (2 pages), Medical literature review (33 pages), Subjects and methods (27 pages), Results (26 pages), Discusions (30 pages), Conclusions (2 pages) and recommendations (1 page). Total figures (26 figures), 35 tables. The references included 138 (19 Vietnamese and 119 English references, and in which 62/138 update references in recent 5 years).

Chapter 1. GENERAL OVERVIEW

1.1. The parasite composition changes worldwide and Vietnam

Among the five species that are responsible for human malaria, four common ones are *P. falciparum, P. vivax, P. malariae, P. ovale,* and the species *P. knowlesi*, which was previously found in long-tailed macaque monkeys (*Macaca* spp.), has now been found in humans as well. Among these, *P. falciparum* is the most dangerous as it can cause complications and death in patients, cause malaria outbreaks in communities, and has developed drug resistance on a large scale. The advantages of each species depend on each geographical region. In tropical regions, *P. falciparum* and *P. vivax* are usually more prevalent, while *P. ovale* is more dominant in temperate or cold regions. *P. malariae* is sporadically distributed and has a low infection rate. However, the majority of reported cases (193.5 million cases/year) are caused by *P. falciparum*. *P. vivax* is the second most common cause, with approximately 14.3 million cases per year.

1.1.2. In the World

The analysis report on the parasite composition before 2010 in most malaria-endemic regions worldwide showed that *P.falciparum* accounted for a higher proportion compared to *P. vivax*, especially in key malaria areas (except for North Korea, Indonesia, and some South American countries where the proportion of *P. falciparum* is lower or equal to that of *P. vivax*). According to Joel G Breman, malaria occurs in most tropical regions around the world, with *P. falciparum* causing the greatest disease burden, followed by *P. vivax*. *P. falciparum* predominates in Africa, New Guinea, Haiti, and Dominica, while *P. vivax* is much more common in the Americas and the Western Pacific.

The WHO malaria report in 2020 showed that in 10 years (2010-2020), the parasite formula had significant changes, with a lot of hyperendemic areas showing an increasing trend of *P. vivax* in the composition, even equal to the proportion of *P. falciparum*. This change in malaria species composition can be seen as a positive signal, as the decrease in *P. falciparum* implies a reduction in the number of complicated malaria cases or deaths, and demonstrates the effectiveness of malaria prevention and elimination measures. However, the increase of *P. vivax* in the parasite formula is also a technical challenge in the implementation of strategic interventions for malaria elimination and control due to the biological cycle of *P. vivax*, which can persist in the liver cells in a dormant or latent form, causing continuous relapse, overlapping episodes, and chronic infection. Treatment of *P. vivax* with the antimalarial drug chloroquin (CQ) in combination with gametocyte and hypnozoite-targeting drugs such as primaquin (PQ) for 14 consecutive days is difficult to achieve patient adherence and can lead to failure in killing hypnozoite.

1.2.3. In Vietnam

In addition to the two common species *P. falciparum* and *P. vivax*, some provinces also have rare species such as *P. malariae* (Dak Lak, Ninh Thuan, Khanh Hoa, Binh Thuan) and *P. ovale* (Binh Thuan, Ninh Thuan) or recently there is also the species *P. knowlesi* transmitted from monkeys to humans (Quang Tri, Ninh Thuan, Khanh Hoa, Binh Phuoc, Phu Yen) accounting for a low rate of <1%, mix-infection between different *Plasmodium* spp. is also common in hyperendemic areas.

P. falciparum is a species that commonly causes complications and deaths in patients, leading to seasonal outbreaks and widespread multi-drug resistance. *P. vivax* has a wider geographic distribution than *P. falciparum*, ranging from latitude 37^0 North to 25^0 South, and causing up

to 20 million cases of malaria each year. However, studies on this species are still limited due to its mild pathogenic characteristics and low threat to life, and also because it has not been able to be cultured *in vitro* for long periods of time due to its preference for invading young red blood cells. Recently, a special strain of *P. vivax* called *P. vivax muchatum*, which has the ability to cause complicated malaria, multiple complications, and death was discovered in China. *P. malariae* is sparsely distributed with low prevalence, while *P. ovale* is rare in Vietnam.

According to the National Institute of Malariology, Parasitology, and Entomology, in some districts of the malaria endemic areas, *P. falciparum* has a high prevalence. Especially since 2010, the national data on malaria have shown a change in the parasite composition, with the national rate of *P. falciparum* being 61%, but with regional variations. Over 5 years (2016-2020) in the Northern region (*P. falciparum* 58.32%; *P. vivax* 36.87%), there was a clear "reversal" in 2019 (*P. falciparum* 37.0%; *P. vivax* 58.3%); in 2020 (*P. falciparum* 12.8%; *P. vivax* 81.4%). The Central Highlands region (*P. falciparum* 58.3%; *P. vivax* 36.9%) did not differ significantly from the national rate (*P. falciparum* 61.6%; *P. vivax* 36.8%) because malaria cases accounted for nearly 70% of the total cases in the country. Although there are different interpretations by region, the parasite composition is tending to shift toward higher rates of *P. vivax* (compared to 25% in 2010). In particular, although *P. falciparum* is still dominant in the Central Highlands, the trend in the parasite composition over 40 years (1976-2015) by the Institute of Malariariology, Parasitology, and Entomology Quy Nhon showed a decreasing trend of *P. falciparum* and an increasing trend of *P. vivax*.

1.2. Efficacy of CQ drugs used in treating *P. vivax* malaria

P. falciparum and *P. vivax* are the two main species in the parasite composition in almost all malaria-endemic regions in Vietnam. Although *P. falciparum* still dominates (65-70%), the increasing presence of *P. vivax* (30-35%) in the Central Highlands region compared to before is happening in the context of decreasing response of *P. vivax* to CQ globally. Additionally, *P. vivax* has been neglected for some time, resulting in a lack of research on its response to other drugs. Therefore, it is important to pay attention to this matter in malaria elimination in the near future.

Some studies have shown that chloroquine (CQ) is highly effective against *P. vivax* in Vietnam, but the follow-up period after short-term treatment (28 days) is not sufficient to draw a complete conclusion about

recrudescence/relapse, reinfection, or slow development of resistance. Trieu Nguyen Trung and colleagues (2001) evaluated the drug-resistant development and efficacy of treatment regimens in the Central Highlands region (1996-2000) for 85 cases of *P. vivax* malaria followed for 28 days. They found a 100% sensitivity rate with no cases of relapse or reduced sensitivity to CQ. The time to fever clearance and parasite clearance was not significantly different from the results of standard treatment, so the CQ regimen for eradicating dormant parasites, killing gametocytes, and eradicating hypnozoites for 14 days can be continued to achieve radical cure. In a study in Binh Thuan (1997-2000), up to 11% of *P. vivax* were resistant to CQ at the R_I level (Phan Tran Giao *et al.*, 2002), and subsequently reduced CQ sensitivity was detected in Ninh Thuan, Quang Nam, which is a new concern. These rates were determined by measuring the concentration of CQ+DCQ with a general failure rate of less than 5% in the surveyed locations. Huynh Hong Quang (2020) evaluated the efficacy of the CQ regimen in treating *P. vivax* malaria in some Central Highlands locations (2014-2017) with 174 malaria cases (*in vivo*) over 28 days. They found that CQ efficacy against *P. vivax* was still high in Dak Nong (41 cases), Phu Yen (59 cases), and Ninh Thuan (28 cases), with a 100% ACPR rate and no LCF, ETF or LPF. However, in Gia Lai (46 cases), CQ efficacy decreased by 95.24%, and the late parasitological failure rate was 4.76%.

Under the circumstances that showed signs of decreased sensitivity and resistance to CQ by *P. vivax* in some areas, finding new drug candidates and testing new drugs for the treatment of *P. vivax* malaria is extremely urgent. Studies on the efficacy of ACTs in the treatment of *P. vivax* have shown faster clearance of parasitemia than CQ, and a high cure rate in the ACTs has been recorded in Afghanistan, Indonesia, Thailand, and Vietnam. While early intervention with artemisinin in *P. falciparum* can limit the development of gametocytes, these drugs are much less effective against *P.vivax* because gametocytes can exist before treatment. In Vietnam, especially in the Central Highlands, studies on the efficacy of CQ against *P. vivax* have shown a decrease in its efficacy, especially in the late parasitological failure rate below 10%, as an alarm indicator when *P.vivax* species are increasing resistance to CQ in some countries globally.

Chapter 2. SUBJECTS AND METHODOLOGY

2.1. Methodology for Objective 1: *Describe the malarial parasite species formula progress in Krong Pa district, Gia Lai in period 2010-2019*

2.1.1. Study subjects

Reporting data from all malaria confirmed cases and malaria parasite that storaged in Institute of Malariology, Parasitology, and Entomology Quy Nhon, Center for diseases control and prevention of Gia Lai province, Krong Pa district health center, and all communal health stations in period 2010-2019.

2.1.2. Study timeframe

From Jan.2018 to Dec.2019;

2.1.3. Study sites

All selected communal health stations of Krong Pa district, Gialai.

2.1.4. Study design

A retrospective (2010-2017) and prospective (2018-2019) study design.

2.1.5. Samples size

Total sample size included of monthly, quaterly, and yearly reporting data at communal health stations (CHS) and district health center (DHC) that belonging to study area in 10 years (2010-2019).

2.1.6. Study techniques

Collect and synthetase of total data of malaria cases in Krong Pa from 2010-2019 via monthly, quaterly, and yearly reports;

2.2. Methodology of Objective 2: *Evaluation of the drug efficacy of chloroquine phosphate in treatment of Plasmodium vivax patients at study sites (2018-2019).*

2.2.1. Study subjects

Inclusion criteria

- Age between 5 ys/o to under 70 ys/o;
- Mono-infection with *P. vivax* detected by light microscopy;
- Parasitaemia of 500 to under 100.000 asexual forms/µl blood;
- Axillary temperature ≥ 37.5°C or history of fever past 24h);
- No any symptom of severe vivax malaria, and ability to swallow oral medication;
- Ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule;
- Informed consent from the patient or parents in the case of children.

Exclusion criteria

- A positive pregnancy test, intention of pregnancy/ on breastfeeding;
- Presence of general danger signs in children aged under 5 years or signs of severe falciparum malaria;
- Mixed or mono-infection with another *Plasmodium* species;
- Presence of severe malnutrition, febrile conditions due to diseases other than malaria (acute lower respiratory tract infection, severe diarrhoea) or other known underlying chronic or severe diseases, severely vomitting, or psychological disorders;
- Patient used any antimalarial drugs pre-study.

2.2.2. Drug and dosage in clinical trial study

Chloroquin 250mg tablet with 3-day regimen as followed by day 1 (10mg base/kg), day 2 (10mg base/kg), day 3 (5mg base/kg).

2.2.3. Study timeframe

From Jan.2018 to Dec.2019;

2.2.4. Study sites

All of communes where malaria endemic zone and Krong Pa district health center, Gia Lai province.

2.2.5. Study design

Non-randomised controlled study design. *In vivo* TES with follow-up 42 days for the clinical and parasitological clearance response on patients via WHO protocol (WHO, 2009).

2.2.6. Sample size

With the previous CQ failure rate of 3.7% (Pham Vinh Thanh, 20150, select p≈5% a confidence interval of 95%, and a precision level of 5%, a minimum of 73 patients should be enrolled

Table 2.1. Treatment failure-based minimum sample size (WHO, 2009)

Estimated population proportion (p), confidece interval 95%										
d	0,05	0,10	0,15	0,20	0,25	0,30	0,35	0,40	0,45	0,50
0,05	**73**	138	196	246	288	323	350	369	380	384
0,10	18	35	49	61	72	81	87	92	95	96

To avoid loss of sample, plus more 20%, then final estimated sample size n # 90 cases.

2.2.7. Study code method in study patient

- GLCQ: Gia Lai chloroquin phosphat;
- In order from the GLCQ01, GLCQ02,... to the GLCQ90.

2.2.8. Study techniques

- Evaluation of patients' clinical parameter, body temperature, weight, urinalysis for antimalarial drug or pregnant status detection,
- Giemsa slide examination and malaria parasite counting;
- CareStart^{TM} Malaria *Pf/Pv* (HRP2/ pLDH) Ag rapid test;
- Measurement of the CQ plus desethylchloroquin metabolite (DCQ) concentration by intergrated HPLC/MS analyis in whole blood;
- Haematological testing at Krong Pa district health center;
- Standard operation procedure of potential molecular markers that involved in antimalarial resistance *P. vivax*;

2.3. Ethic consideration aspects in study

Approval by the official ethical and scientific committee of the Institute of Malarology, Parasitology, and Entomology Quy Nhon with the Decision No. 906/VSR-QLKH on dated 05 Apri 2017.

Chapter 3. RESULTS

3.1. Progress of malarial parasite formula in Krong Pa district, Gia Lai province, period 2010-2019

3.1.1. Characterictics of malaria parasite distribution in Krong Pa

Table 3.1. Overall characteristics of study malaria patients

#	Patient's characteristics (n=10.100)		Number	Proportion (%)
1	**Gender**	Male	8304	*82.22*
		Female	1796	*17.78*
2	**Age group**	<5	225	*2.23*
		≥5-< 15	740	*7.33*
		≥ 5	9135	*90.45*
3	**Ethnic group**	Ja Rai	9242	*91.50*
		Tay	151	*1.49*
		Nung	64	*0.64*
		Kinh	643	*6.37*

Male gender occupied predominently (82.22%) to female (17.78%); age gouped 15 and over (90.45%), and mainly Ja Rai ethnic group (91.50%).

Table 3.2. Residene location distribution of study malaria patients

#	Place of residence of patients (N = 10.100)	Malarial patients	
		Number	Proportion (%)

1	Ia R'sai commune	691	*6.84*
2	Ia R'Sươm commune	627	*6.21*
3	Chư R'Căm commune	1294	*12.81*
4	Phú Cần commune	758	*7.50*
5	Krong Nang commune	345	*3.42*
6	Phu Tuc commune	434	*4.30*
7	Đat Bang commune	753	*7.46*
8	Chu Gu commune	1091	*10.80*
9	Chu Ngoc commune	522	*5.17*
10	Ia R'mok commune	751	*7.44*
11	Ia M'Lah commune	775	*7.67*
12	Chư Drăng commune	437	*4.33*
13	Uar commune	504	*4.99*
14	Ia Drech commune	1118	*11.07*

Several communes have high infection proportion, such as communes of Chư R'Căm (12.81%), Ia Drech (11.07%), Chư Gu (10.80%), Ia M'Lah (7.67%), Phu Can (7.50%), Dat Bang (7.46%), Ia R'mok (7.44%).

3.1.2. Malaria parasite formula in Krong Pa district in 2010-2019

Table 3.3. Overal parasite formula in Krong Pa district (2010-2019)

#	***Plasmodium* spp.**	**Number**	**Proportion (%)**
1	*P. falciparum*	5761	*57.05*
2	*P. vivax*	4099	*40.58*
3	*P. malariae*	3	*0.03*
4	Mixed *P.falci* plus *P. vivax*	237	*2.34*
Total		**10.100**	***100.00***

In the paraste formula, *P. falciparum* occupied of 57.05%, *P. vivax* (40.58%), *P. malariae* (0.03%), mixed-infection (2.34%).

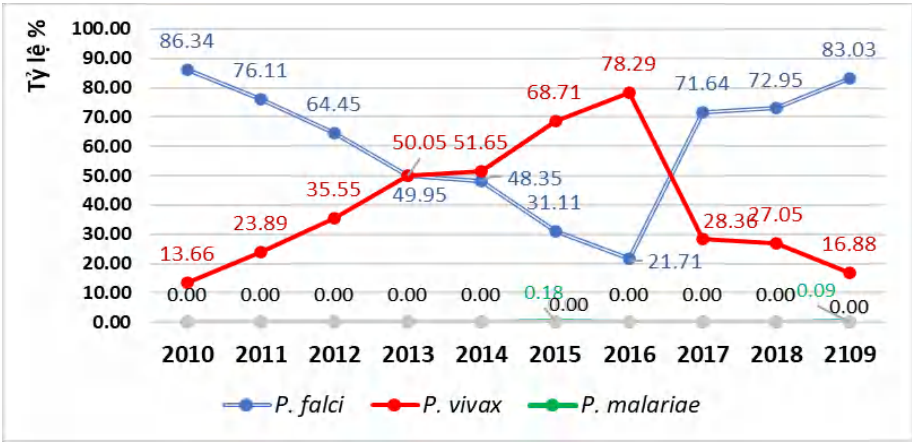

Figure 3.1. Parasite formula in each year in Krong Pa (2010-2019)

In the three years period 2010-2012, proportion of *P. falciparum* highly occupied than *P. vivax* (86.34% vs., 13.66%; 76.11% vs., 23.89% and 64.45% vs. 35.55%), in period (2013-2016), the propotion of converted *P. vivax* occupied higher than *P. falciparum* (49.95% vs., 50.05%; 48.35% vs. 51.65%; 31.11% vs. 68.71%; 21.71% vs., 78.29%). In the next three years 2017-2019, *P. falciparum* proportion was higher than *P.vivax.*

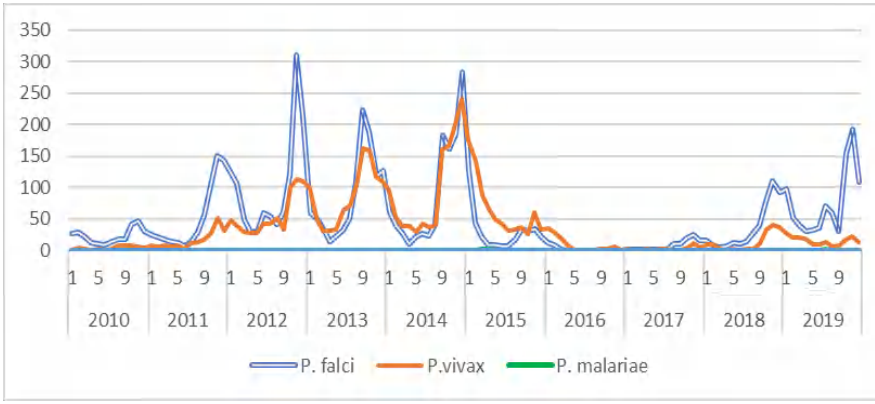

Figure 3.2. Malaria infected proportion in Krong Pa by month (2010-2019)

In the period (Jan-Jun, dry season), number of patients is lower than period Jul-Dec (wet season).

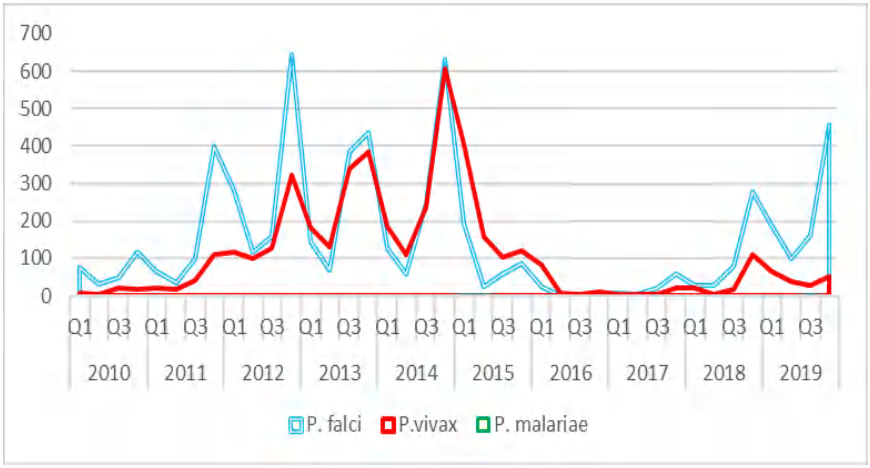

Figure 3.3. Proportion of malaria parasites in Krong Pa by quater

In the first and second quarters of each year, number of patients and parasites were decreased vice-versa third-fourth quarters (high forest activity).

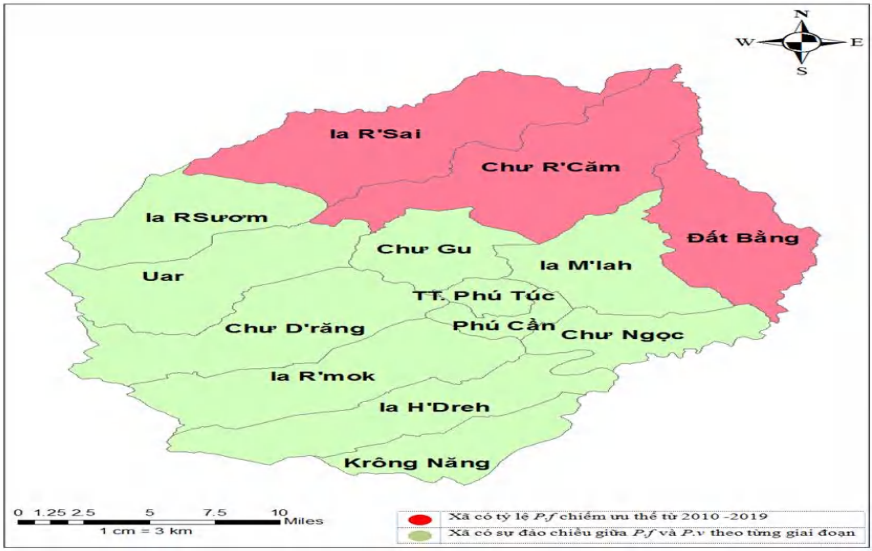

Figure 3.4. *Plasmodium* species distribution in Krong Pa by commune

Data showed that communes of Ia R'Sai, Chư R'Căm, Dat Bang in some continuous years with *P. falciparum* were high in forrmula vs. *P. vivax*, while at the communes of Uar, Ia R'Sươm, Ia R'mok, Ia Dreh,

Krong Nang, Chư D'răng, Ia M'lah, Chư Gu, Phu Can, Chu Ngoc have *P.falciparum* and *P. vivax* proportion converted in some periods.

3.2. Evaluation of chloroquine efficacy in the treatment for vivax malaria in sentinel sites (2018-2019)

3.2.1. Overal characteristics of enrolled drug efficacy evaluation

Table 3.4. Some characteristics of study patients' population

#	Study patients characteristics	Number	Proportion (%)
1	**Total of screening cases**	**1908**	
	Malaria parasite postive cases (+)	453	*23.7*
	Malaria parasite negative case (-)	1455	*76.3*
2	**Malaria parasite formula**	**453**	
	P. falciparum	337	*74.4*
	P. vivax	109	*24.1*
	P. malariae	01	*0.2*
	P. falciparum + P. vivax	06	*1.3*
3	**Total of positive *P. vivax***	**109**	
	No. of cases in clinical trial	96	*88.1*
	Excluded positive cases in TES	13	*11.9*
4	**Total of *P. vivax* cases in TES**	**96**	
	- No. of case that un-meet criteria	6	*6.25*
	- No. of case that meet TES criteria	90	*93.75*

Patients with *P. vivax* monoinfection met inclusion criteria was 96, but after ICF consultation, more 6 cases refused participation as they can not follow to study timeframe.

Table 3.5. Some clinical manifesttions and fever history on patients

#	Study patients characteristics	Number	Proportion (%)
1	**Body temperature and weight**		**Min - Max**
	Mean temperature (SD)^{0}C	39,0 (0,6)	37,5 - 40,6
	Mean body weight (SD)kg	60,3 (7,6)	25 - 72
	No. of days fever before D_0		
	1 day	54	*60.0*

	2 days	17	*18.9*
	3 days	19	*21.1*
2	**No. case fever or fever history**	**Number**	**Proportion(%)**
	Tympanic temp. at $D_0 \geq 37.5^0C$	57	*63.3*
	Faver history (in 48 hrs)	78	*86.7*
3	**Tình trạng lách**	**Number**	**Proportion(%)**
	Spleenomegaly (≥ II level)	16	*7.7*
	No spleenomegaly	74	*82.3*
	Spleenectomy	0	*0*

Patient's mean temperature 39.0 ± 0.6^0C, body weight 60.3±7.6 kg. From patients have malaria symptom to approach to sentinel sites varied 1-3 days, on-site fever with 57 cases (63.3%) or fever history in 48 hrs was 78 cases (86.7%).

Table 3.6. The *P. vivax* parasites characteristics & hematological aspects

Study patients characteristics	**At the pre-dose (D_0)**	
	Number	**Min - Max**
Average parasite density		
- MĐKST thể vô tính/µl	7.151	690 - 60.041
- Số bệnh nhân có giao bào	73/90	*81,1%*
- Số bệnh nhân không có giao bào	17/90	*18,9%*
Thông số huyết học (TB)		
- Nồng độ haemoglobine (g/dL)	11,7 (2,78)	9,2 - 12,3
- Tỷ lệ haematocrite (%)	36,8 (7,27)	35,9 - 42,2

Asexual parasite density/µL at pre-dose (D_0) was 7151 (690-60.041) and proportion of gametocytemia of *P. vivax* was (18.9%). Mean Hb concentration at D_0 was 11.7 g/dL and haematocrite (36.8%).

3.2.2. Prolonging asexual *P. vivax* clearance after CQ treatment

Table 3.7. Case analysis of day 3 positive of asexula *P. vivax* on D_3

Total of analysed cases	**Number**	**Proportion (%)**
No. of cases with positive asexual parasite D_1	82	*91.1*
No. of cases with positive asexual parasite D_2	10	*11.1*
No. of cases with positive asexual parasite D_3	0	*0*
No. of cases with positive asexual parasite D_4	0	*0*

Parasite clearance progress of asexual *P. vivax* showed that after 24 hrs was still have 82 cases (91.1%) positive, then by 48 hrs only 10 cases (11.1%), and by 72 hrs or D3 without positive cases.

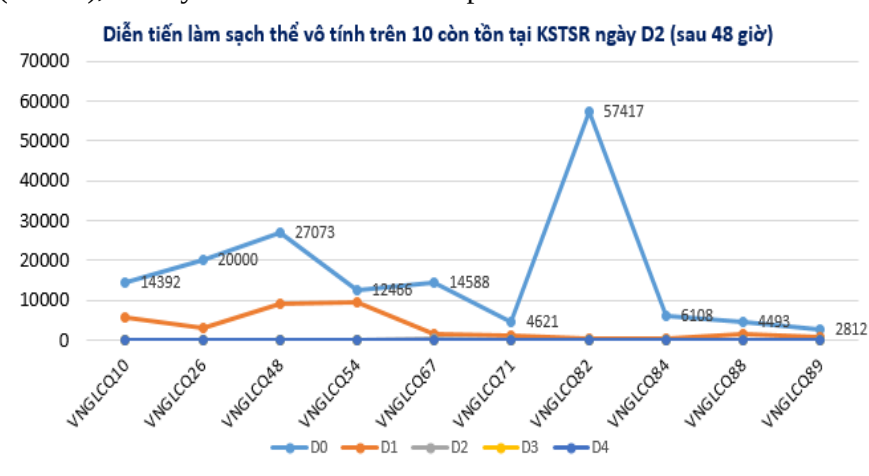

Figure 3.5. Progress of *P. vivax* parasite density before and after 3 days using CQ regimen

Table 3.8. Time and *P. vivax* parasite clearance speed from D_0 to $\geq D_3$

Patients code					***P. vivax* parasite clearance progress**				
#	**Code**	**Age**		**Body weight**	**D_0**	**D_1**	**D_2**	**D_3**	**D_4**
		M	**F**						
1	GLCQ10		15	46	14392	5742	96	0	0
2	GLCQ26	24		61	20000	3009	16	0	0
3	GLCQ48	40		65	27073	9281	119	0	0
4	GLCQ54	24		55	12466	9472	79	0	0
5	GLCQ67	14		48	14588	1584	239	0	0
6	GLCQ71	15		52	4621	1025	159	0	0
7	GLCQ82	25		60	57417	277	80	0	0
8	GLCQ84	20		63	6108	239	80	0	0
9	GLCQ88	16		49	4493	1560	118	0	0
10	GLCQ89	33		62	2812	749	119	0	0
Mean parasite/µL→ gradualy decreasing					16.397	3.293	110	0	0

In total of 90 analysed cases by 42 days, there were 10 cases (11.1%) with positive asexual *P. vivax* on day D_2, but on day D_3 without parasite.

Table 3.9. Efficacy of CQ in the *P. vivax* parasite clearance on patients

#	Total of analysed cases (n = 90)	Mean parameters
1	Average parasite density/µl at D_0	7.151 (690 - 60.041)
2	Mean parasite clearance time (median [IQR]-PCT)	36 (30 - 48)
3	Average body temperature at D_0 (^{0}C)	39.0 (0.6) 37.5 - 40.6
4	Mean fever clearance time (median [IQR]-FCT)	24 (12 - 35)

After CQ treatment with total dose 25mg/kg, PCT and FCT were 36 hrs and 24 hrs, respectively.

Table 3.10. Time and asexual *P. vivax* parasite clearance progress

Parameter	Slope half-life	Progress of asexual *P. vivax* parasite clearance after treatment				
		PC50	PC75	PC90	PC95	PC99
	3.50	8.86	12.61	16.39	18.82	23.59

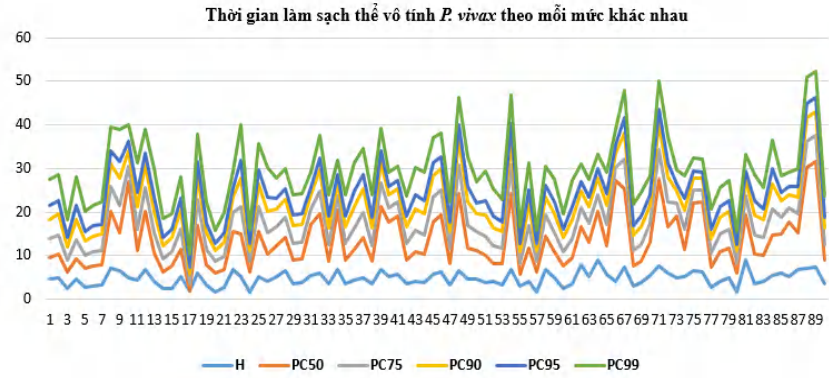

Figure 3.6. Progress of asexual *P. vivax* parasite clearance at level of PC50, PC75, PC90, PC95, PC99 thresholds

Data analysis after CQ using on the clinical and asexual *P. vivax* parasite clearance progress showed that the K index of 0.1979, time for 50% overal parasite biomass of *P. vivax* was 3.5024 hours (hrs) (<5,2 hrs). Afterward, the parasite biomass PC50, PC75, PC90, PC95, and PC99 time

after CQ using were 8.86 hrs, 12.61 hrs, 16.39 hrs, 18.82 hrs and 23.59 hrs, respectively.

3.2.3. Some potential molecular markers that possible related to antimalarial drug resistance of *P. vivax* population

Table 3.11. Potential molecular markers that possible related in resistance of *P. vivax* population

#	Molecular marker(s)	Number	Proportion (%)
1	*Pvmdr1*	0	*0*
2	*Pvcrt1*	0	*0*
3	*Pvcrt-o*	6	*6.67*
4	V552 *Pvk12*	9	*10.0*
5	*Plasmepsine 4* (>1.5 copy)	0	*0*

In total of 90 *P. vivax* isolates, five possible molecular markers analysis involved in resistance *P. vivax* showed that none of 3 markers *Pvmdr1*, *Pvcrt1* and *Plasmepsine 4* (>1.5 copy) in these isolates. Only two *Pvcrt-o* and V552 *Pvk12* marker of 6 case (6.67%) and 9 case (10%).

Table 3.12. Single or multiple molecular markers in *P. vivax* isolates

#	Molecular markers	Number	Proportion (%)
1	Single marker	11	*12,2*
2	Multiple marker	2	*2,22*

In all samples with marker detected, most of *Pvcrt-o* and *Pvk12* markers with single marker 12.2% (11/90), and two isolates with double markers (2.22%).

3.2.4. Efficacy of CQ regimen in the treatment for *P. vivax* malaria

Table 3.13. Efficacy of the CQ in treatment for *P. vivax*

CQ Efficacy evaluation parameters		Number (%)	95%CI
Efficacy	Early Treatment failure (ETF)	0	0
	Late Clinical Failure (LCF)	1 *(1.1)*	0,0 - 6,0
	Late Parasitological Failure (LPF)	0	0
	Adequate Clinical and Parasitological Response (ACPR)	89 *(98.9)*	94 - 100
	Total of analysed case	90	

	Withdrawn	0	*0*
	Loss of follow up during 42 days	0	***0***
	Total of trial cases	**90**	

In total of 90 *P. vivax* cases, proportion of adequate clinical and parasitological respone 98.9% and only one case (1.1%) late clinical failure (GLCQ41 case on day D_{42} with patient's temperature 38.6^0C and *P. vivax* parasite density was 1.030/µL.

Table 3.14. The CQ and active metabolite (desethylchloroquin DCQ) in whole blood of recurrent of *P. vivax*

Patient code	**Day for drug concentration measure**	**CQ + DCQ concentration**	**WHO criteria**
GLCQ41	D_7	467.3 ng/mL	> 100 ng/mL
GLCQ41	D28	52.5 ng/mL	< 100 ng/mL
GLCQ41	D_{42} (Recurrent date)	27 ng/mL	< 100 ng/mL

According to WHO classifiation, GLCQ41 patient was classified by late clinical failure (LCF) with recurrent *P. vivax* parasite on day $D_{42.}$ Due to elimination half life of CQ+DCQ is 55 days suitable for full dose of CQ 25 mg per kg and CQ+DCQ concentration was 27ng/ml at that time (<100ng/mL), hence this case was not resistance, possible reinfection.

3.2.5. Some adverse events on study patients after CQ using

Table 3.15. Some of adverse events of CQ on study patients

#	**Adverse events of CQ (n=90)**	**Number**	**Proportion (%)**	**At time (day….)**
1	Headache, dizziness	9	*10*	D_1 - D_3
2	Nausea, vomitting, anorexia	2	*2.2*	D_1 - D_3
3	Abdomen pain, digestive disorder	2	*2.2*	D_1 - D_3
4	Skin rash, itching	1	*1.1*	D_3 - D_7
5	Abnormal alopeia	1	*1.1*	D_3 - D_7

After using of CQ, on the day D_0 showed some adverse events as headache, dizziness (10%), abdomen pain, nausea, vomitting, digestive discomforts, loss of appetite (2.2%), skin rash, itching 1 case (1.1%), alopecia (1.1%).

Chapter 4. DISCUSSIONS

4.1. The malarial parasite formula in Krong Pa district (2010-2019)

4.1.1. Distribution of the malaria parasites at the study site

Analysis of the parasite composition in 10,100 malaria patients identified in Krong Pa district during a 10-year period (2010-2019) showed that *P. falciparum* accounted for 57.05%, *P. vivax* accounted for 40.58%, *P. malariae* accounted for 0.03%, mixed infection of *P. falciparum* with *P. vivax* or *P. malariae* accounted for 2.34%, while no cases of *P. ovale* or *P. knowlesi* were detected. Males (82.22%) were more affected than females (17.78%). The age group over 15 accounted for 90.45%, the age group of ≥ 5-< 15 accounted for 7.33%, and the age group under 5 accounted for 2.23%. Most of the malaria patients belonged to the local Ja Rai ethnic group (91.50%), while the remaining were migrants from mountainous provinces in the North such as Tay (1.49%), Nung (0.64%), and Kinh (6.37%). The distribution of malaria patients according to their residence in the studied areas showed that malaria was present in all 14 communes, with varying infection rates. The communes with the highest infection rates were Chư R'Căm (12.81%), Ia Drech (11.07%), Chu Gu (10.80%), Ia M'Lah (7.67%), Phu Can (7.5%), Dat Bang (7.46%), Ia R'mok (7.44%), while the remaining communes had lower infection rates below 10%.

4.1.2. The malarial parasite composition in Krong Pa (2010-2019)

The development of *Plasmodium* spp. composition according to the aforementioned interpretation showed differences in each period; specifically in the first 3 years (2010-2012), the ratio of *P. falciparum* was dominant over *P. vivax* in 2010 (86.34%/13.66%), 2011 (76.11%/23.89%), and 2012 (64.45%/35.55%). In the following 4-year period (2013-2016), the ratio of *P. vivax* tended to be equal or even superior to falciparum, with 2013 (49.95%/50.05%), 2014 (48.35%/51.65%), 2015 (31.11%/68.71%), and 2016 (21.71%/78.29%). In the most recent 3-year period (2017-2019), the ratio of *P. falciparum* returned to dominate over *P. vivax*. Comparison of this study with data on parasite composition in the entire Gia Lai province and other Central Highlands provinces in 2010 also showed no reversal, with a total of 1869 cases, of which *P. falciparum* was more than 5.42 times higher than *P. vivax* (1566/289) in Gia Lai and similarly in other provinces such as Dak Lak with *P. falciparum* more than 7.16 times higher than *P. vivax* (1038/145), Kon Tum with *P. falciparum* more than 4.75 times higher than *P. vivax* (418/88), and Dak Nong with *P.*

falciparum more than 2.47 times higher than *P. vivax* (465/188). Similarly, comparison with data in coastal provinces of the Central region also showed no change in composition in 2010, specifically in Quang Binh, where *P. falciparum* was 7.6 times higher than *P. vivax* (266/35), or this ratio was 10.9 times higher (774/71) in Quang Tri; 2.56 times (46/18) in Thua Thien Hue; 2.28 times (1957/860) in Quang Nam; 1.75 times (131/75) in Quang Ngai; 2.15 times (181/84) in Binh Dinh; 4.29 times (300/70) in Phu Yen; 3.1 times (921/299) in Khanh Hoa; 4.16 times (891/214) in Ninh Thuan; and 1.14 times (389/253) in Binh Thuan. The data above shows that the Central Highlands region have higher rates of *P. falciparum* compared to *P. vivax*, ranging from 1.14 to 10.9 times higher. Some provinces in the South, such as Binh Phuoc and Lam Dong, also have similar trends. In Binh Phuoc, *P. falciparum* is 2.14 times more prevalent than *P. vivax* (1869/873), while in Lam Dong, *P. falciparum* is 2.27 times more prevalent than *P. vivax* (397/175). However, in the 28 northern provinces, *P. vivax* has always been more prevalent for many years, and in 2010 there were a total of 870 confirmed cases of malaria, with a similar proportion of *P. falciparum* and *P. vivax* (372/387).

Compared to some data worldwide, over the past decade, in many areas with severe malaria, the proportion of *P. falciparum* and *P. vivax* has reversed or even balanced. Prior to 2010, most malaria-endemic areas around the world had a higher proportion of *P. falciparum* compared to *P. vivax*, especially in key malaria-endemic areas (with the exception of North Korea, South Korea, Indonesia, East Timor, Papua New Guinea, and some South American countries where the number of *P. falciparum* cases is lower or equal to *P. vivax*). However, recently, the number of cases of *P. vivax* has tended to shift to almost equal or even higher than *P. falciparum* in countries such as Thailand, Bhutan, and Myanmar. Nevertheless, countries that have long had a high proportion of *P. vivax* cases have not seen significant changes in their composition, such as North Korea, Indonesia, East Timor, Bangladesh, and India (WHO, 2015).

4.2. The CQ efficacy in treating *P. vivax* malaria in Krong Pa

4.2.1. The overal characteristics of the patients in the study

The analysis data of 90 patients participating in this clinical trial showed that the average body temperature before taking the experimental drug CQ (D_0) was 39.0 ± 0.6^0C, and the weight was 60.3 ± 7.6 kg. Most patients had a fever when they came for a medical examination (63.3%) or

had a history of fever within 48 hours (86.7%). The proportion of patients with splenomegaly of level 2 or higher was 17.7%. The average asexual parasite density of the patient group was 7151/µl, and 81.1% of the patients had *P. vivax* gametocytes in their blood at the same time as the asexual forms. The average Hb concentration was 11.7 (g/dL) and the average Hct ratio was 36.8%. There were no cases of severe anemia, and these parameters satisfied the inclusion criteria for CQ treatment and the 42-day follow-up process for each patient.

4.2.2. The CQ regimen efficacy in treating for *P. vivax* malaria

Regarding the efficacy of the CQ regime in the treatment of 90 study participants, the adequate clinical and parasitological response rates were 98.9%, with only a 1.1% late clinical treatment failure rate. These results are consistent with some previous studies in the same or different study sites of the same province, The monitoring of the progress of clearing *P. vivax* asexual stages showed that after 24 hours, 91.1% were still asexual stages, but after 48 hours, only 11.1% remained asexual, and by 72 hours, there were no more asexual stages of *P. vivax*. The rate of clearing *P. vivax* from D_0 to $\geq D_3$ was only 11.1%, with *P. vivax* asexual stages present at D2, but none remained at D3. When examining individual cases, the density of *P. vivax* at each D_0 time point was not high, not exceeding 100,000/µL, with an average density of 16,397/µL on D_0. After treatment on D1, the average density decreased to 3,293/µL, and by D2, the average was only 110.5/µL, with no asexual stages remaining by D3. This is similar to studies in the Brazilian Amazon region, which also showed rapid parasite clearance due to the sensitivity of the trophozoite stages to CQ. The average time to clear the parasite was 36 hrs, and the average clinical fever clearance time was 24 hours. Detailed analysis of each time point from the use of CQ on the clinical response and the monitoring of the progress of clearing *P. vivax* asexual stages showed a K value of 0.1979, with a time to clear 50% of *P. vivax* load of 3.5024 hrs (<5.2 hrs). Importantly, no cases took longer than 60 hours to clear *P. vivax* asexual stages with CQ, indicating that the *P. vivax* pop. in Krong Pa is still sensitive to the recommended dose of CQ by WHO (2015). The late clinical treatment failure rate was 1.1% (41GLCQ), with the reappearance of *P. vivax* on day 42 with a body temperature of 38.6°C and a *P. vivax* density of 1,030/µL. To confirm whether this case was drug-resistant or

not, the drug and metabolite concentrations (CQ+DCQ) were measured on D_7, D_{28}, and D_{42} to evaluate the drug resistance status.

The CQ drug can distribute extensively into the bloodstream and bind to tissues after ingestion, so elimination of the drug from the body is slow, with a mean final elimination half-life of 45-55 days for CQ and 59-67 days for its metabolite DCQ. Therefore, similarly to previous studies, measuring the concentration of CQ+DCQ in blood using filter paper at the time of *P. vivax* reappearance can help assess the resistance of *P. vivax* to CQ. However, for case 41GLCQ, the reappearance of *P. vivax* occurred on day D_{42}, while the elimination half-life of CQ+DCQ was 55 days, which was appropriate after completing a CQ treatment regimen with a total dose of 25 mg/kg and measuring the drug and metabolite concentration (CQ+DCQ = 27 ng/ml) at the time of *P. vivax* reappearance, which did not exceed 100 ng/ml, indicating that this case did not have CQ resistance (WHO, 2017) but may have had relapse or reinfection requiring further molecular analyses for evaluation.

Compared to the collected data on the efficacy of CQ against *P. vivax* in Vietnam, a reduced response to CQ has been observed in *P. vivax* in various regions, including Binh Thuan, Quang Nam, Gia Lai from previous years, as well as in different areas worldwide according to the WHO's collection of data from 198 TES studies conducted between 2010-2019 with 12,372 participants. The region with the most research was Southeast Asia (41.9% of total studies). Although CQ is still effective in treating *P. vivax* in many countries, the phenomenon of CQ resistance has been identified in all regions with varying degrees and late treatment failure case has been reported in this district, indicating the need to continue monitoring the efficacy of CQ and develop appropriate drug substitution strategies.

4.2.3. Some adverse effects of CQ medication

After taking CQ medication, adverse events were reported such as headache, dizziness in 9 (10%), abdominal pain, nausea, gastrointestinal disorders, loss of appetite in 2 cases (2.2%), skin rash, itching in 1 case (1.1%), and notably, 1 case had abnormal hair loss (1.1%). In addition, the hematological parameters at baseline (D_0) and on day D_7 showed no significant changes in red blood cell count, white blood cell count, Hb, Hct, and platelets, which is similar to some multicenter evaluation results conducted worldwide.

CONCLUSIONS

1. The malarial parasite formula in Krong Pa district, Gia Lai province (2010-2019)

- In the Krong Pa district: *P. falciparum* (57.05%), *P.vivax* (40.58%), *P.malariae* (0.03%), mixed *P. falciparum* plus *P. vivax* infection (2.34%), none of *P.ovale, P.knowlesi*;
- In the period (2010-2012): The *P. falciparum* predominently occupied *P.vivax* (86.34%/13.66%; 76.11%/23.89%, and 64.45%/35.55%); 2013-2016: *P. vivax* was equal or than *P. falciparum* (49.95%/50.05%; 48.35%/51.65%; 31.11%/68.71% and 21.71%/78.29%); 2017-2019: *P. falciparum* was predominent occupied in *vice-versa P. vivax.*

2. The efficacy of CQ regimen to *P. vivax* malaria

- Adequate clinical and parasitological responses (ACPR) was 98.9%, LCF of 1.1% in treatemnt for *P. vivax* malaria;
- None of case with day 3 positive, median PCT was 36 hours, and median FCT was 24 hours;
- Biomass *P. vivax* parasite clearance time at 50%; 75%; 90%; 95% and 99% after using CQ were 8.86 hours; 12.61 hrs, 16.39 hrs, 18.82 hrs and 23.59 hrs;
- Well tolerance with oral tolerability, advert events included of headache, dizziness 9 cases (10%), nausea, abdomen pain 2 cases (2.2%), skin rash, itching 1 case (1.1%), alopecia 1 case (1.1%), none of significantly changed in haematological parameters.

THE NEW FINDINGS AND SCIENTIFIC SIGNIFICANCE

1. Research on the CQ resistance due to *P. vivax* was using the CQ plus desethylchloroquin metabolite (DCQ) by intergrated HPLC/MS system (Agilent 1200) with analyis columme in Zorbax Ecllpse XDB-C18 convert phage, active isocratic phage contained de-ionized water, acetonitrile, triethylamine for detaild resistance evaluation followed by WHO guidelines;
2. Applying the PCR and gene sequencing into potential marker that possibility relating to antimalarial drugs in *P. vivax* population in global malaria endemic zones of *P. vivax.*

PhD THESIS RELATED SCIENTIFIC PUBLICATIONS

1. Ly Chanh Ty, Hoang Dinh Canh, Tran Thanh Duong, Trieu Nguyen Trung (2022). Evaluation of changing in malarial parasite formula of *Plasmodium* spp. at Krong Pa district, Gia Lai in ten years (2010-2019). *Vietnam Journal of Community Medicine*, N0.6/2022.
2. Ly Chanh Ty, Hoang Dinh Canh, Tran Thanh Duong, Trieu Nguyen Trung (2022). Efficacy of cloroquin regimen in the treatment for *Plasmodium vivax* malaria in Krong Pa district, Gia Lai (2018-2020). *Vietnam Journal of Community Medicine*, special issues, the 49th National Parasitology Conference.
3. Ly Chanh Ty, Hoang Dinh Canh, Tran Thanh Duong, Trieu Nguyen Trung (2022). Evaluation of chloroquin resistance *Plasmodium vivax* by chloroquine plus desethylchloroquine concentration via the intergrated HPLC/MS system. *Vietnam Journal of Community Medicine*, special issues, the 49th National Parasitology Conference.