V. Methods

Data collection: Two village volunteers per village were selected and trained/refreshed for malaria diagnosis by using the rapid diagnosis test to the fever patients consulted and provide ACT for all positive cases tested. All examined cases performed by the volunteers were recorded in the data sheet provided. The collection of blood samples by filter paper from the infected villagers were also conducted and transferred to NIID, Japan for examined by genetic methods. The data and blood sample collection from each village was gathered every month through the monthly monitoring of the project supervisors for analysis.

Picture 1 : Training activities of the village malaria data collectors

VI. Results

A)- Monitoring of the malaria cases in the study villages in Stung Keo

- Data collection

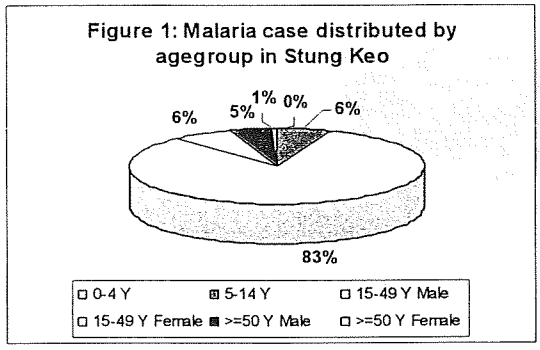
During the project implementation, a total of 624 patients in Stung Keo were tested and 66.99% (418 patients) of them were positive and treated by antimalaria drug. 82.06% of all positive cases were Plasmodium Falciparum and the remaining 9.33% and 8.61% belonged to Plasmodium Vivax and Plasmodium Mix respectively. The data collected from the villages could be summarized data in the table below:

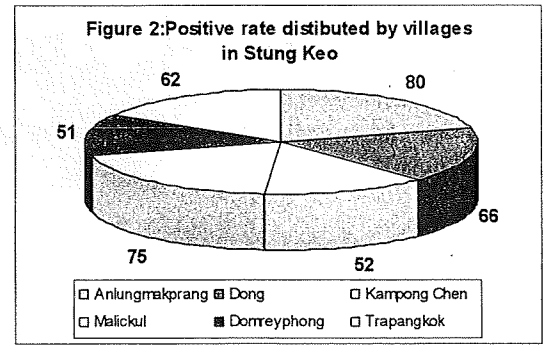
Table 1: Malaria data collected from the project villages in Stung Keo

Village	RDT Results					Age 0-4			5-14 Y			Age 15-49						Age >=50					
	Total tested	Total RDT +				RDTP+			RDTVE +			PF		PV		MX		PF		PV		MX	
		Total+	PF	PV	MX	PF	PV	MX	PF	PV	MX	M	F	M	F	M	F	M	F	M	F	M	F
Anlunmakprang	163	130	108	16	6	0	0	0	6	4	0	93	5	9	3	6	0	4	0	0	0	0	0
Dong	64	42	36	2	4	0	0	0	2	0	0	29	3	2	0	4	0	2	0	0	0	0	0
Kampongchen	77	40	31	2	7	0	0	0	2	0	0	25	2	2	0	3	0	2	0	0	0	4	0
Malickul	134	100	74	11	15	0	0	0	2	1	0	66	2	10	0	13	0	3	1	0	0	2	0
Domreyphong	88	45	41	3	1	0	0	0	5	0	0	33	2	2	1	1	0	1	0	0	0	0	0
Trapangkok	98	61	53	5	3	0	0	0	2	0	0	41	7	4	1	2	1	2	1	0	0	0	0
Total case	**624**	**418**	**343**	**39**	**36**	**0**	**0**	**0**	**19**	**5**	**0**	**287**	**21**	**29**	**5**	**29**	**1**	**14**	**2**	**0**	**0**	**6**	**0**

- **Data analysis**

- No any malaria case was found in the age group 0-4y in Stung Keo.
- In the age group 5-14y, there are only 6% malaria positive cases compare to the total positive cases. However, among the 32 patients tested in this age group, 75% (24 cases) them were positive.
- Nearly 90% (372 cases) of the malaria cases detected in Stung Keo was predominant by the age group 15-49y and 83% of them was male. Among 565 patients tested in age group 15-49y, 65.84% (372 cases) were positive and only 7.26% of them were females (27 cases). Among 34 Plasmodium Vivax infected patients, 14.70% (5 cases) were females.
- Only 11% of the malaria case distributed in the age group >=50y in Stung Keo and there was only 1% different between male and female patients. Among the total of 27 patients tested, 81.48 % (22 cases) were positive and among them 72.73% is Plasmodium Falciparum and only 10% of them were females (2 cases). No Plasmodium Vivax infected was found in this age group.





B) Monitoring of the malaria cases in the study villages in Koh Sla

- **Data collection**

There were 214 patients were positive(57.53%) among 372 patients tested in Koh Sla and 79.44% (170 patients) of them were mainly Plasmodium Falciparum and the remaining 18.22% and 2.34% belonged to Plasmodium Vivax and Plasmodium Mix correspondingly. The summary data was shown in the table below:

Table 2: Malaria data collected from the project villages in Koh Sla

Village	RDT Results					Age 0-4			5-14 Y			Age 15-49						Age >=50					
	Total tested	Total RDT +				RDTP+			RDTVE +			PF		PV		MX		PF		PV		MX	
		Total+	PF	PV	MX	PF	PV	MX	PF	PV	MX	M	F	M	F	M	F	M	F	M	F	M	F
Kbal Domrey	98	46	42	4	0	2	0	0	19	0	0	13	6	2	2	0	0	2	0	0	0	0	0
Stungankanh	64	55	44	10	1	1	0	0	3	3	0	25	12	3	4	1	0	3	0	0	0	0	0
Anlungkrom	87	46	27	17	2	1	0	0	9	5	1	8	5	6	5	1	0	3	1	0	1	0	0
317	123	67	57	8	2	0	0	0	9	0	0	32	13	3	5	1	0	3	0	0	0	1	0
Total case	**372**	**214**	**170**	**39**	**5**	**4**	**0**	**0**	**40**	**8**	**1**	**78**	**36**	**14**	**16**	**3**	**0**	**11**	**1**	**0**	**1**	**1**	**0**

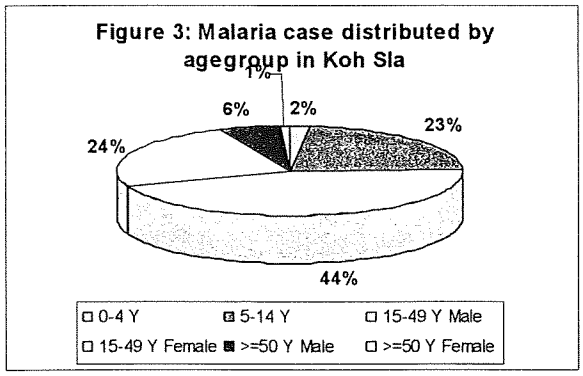
- **Data analysis in Koh Sla**

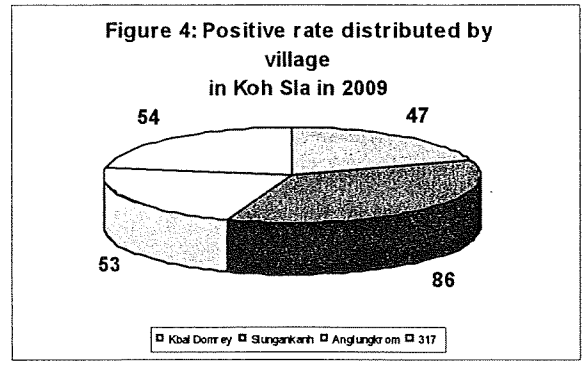
- ❖ Only 2% of malaria case distributed in age group 0-4y compare to the total positive cases in all age group. However, the positive rate among age group 0-4y is 57.14% a total of 4 Plasmodium Falciparum positive were recorded among 7 patients tested.

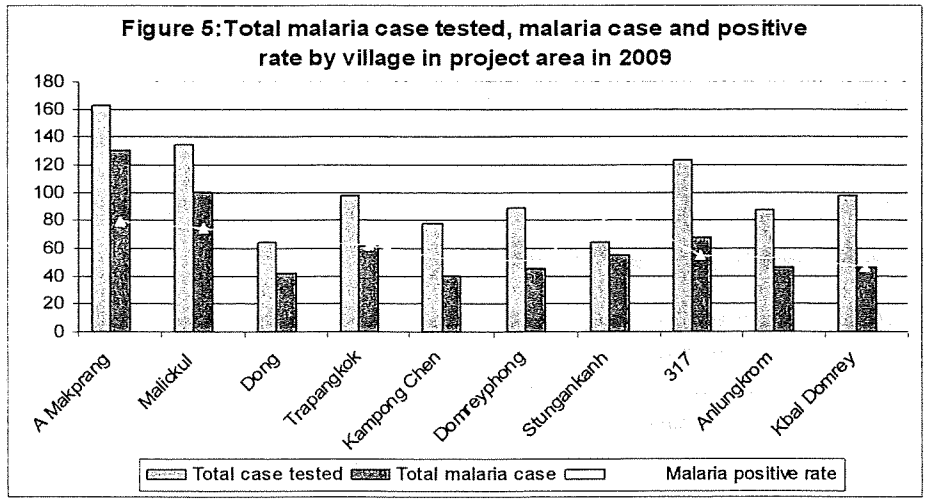
- ❖ There were 23% of malaria cases in the age group 5-14y and a total of 81 patients were tested and 60.49% of them (49 cases) were positive and Vivax infection was 9.88%.

- ❖ 68% of the total malaria cases in Koh Sla took over by the age group 15-49y and nearly two third is male patients. Among 262 patients tested, 56.11% (147 cases) were positive and 35.37% of them were females (52 cases). Among 30 Plasmodium Vivax infected patients, 53.33% of them (16 cases) were females.

- ❖ There were only 7% of malaria cases in the age group >=50y in Koh Sla and 6% was male. Among a total of 22 patients were tested and 63.64% (14 cases) were positive and among them 85.71% is Plasmodium Falciparum. Among 14 positive cases only 17% of them were females (2 cases). Plasmodium Vivax and Mix infected are 7.14% each in this age group.







C) Spleen survey in school students and household children near by school

The spleen survey of the children aged from 2-9 years old was conducted in order to detect and assess the local transmission and the degree of endemicity among the school students and children who live around the school campus. A total of 148 children were screened for the spleen enlarged but no any splenomegaly and the positive RDTs were found among all of them.

Picture 2: Spleen Survey activities for the children aged 2-9 years old

D) Collection of blood samples by filter paper from the infected village people

During the project period, 45 blood samples by filter paper were collected from the malaria patients who confirmed by RDT test. Among those blood samples, we observed that 87% (39 samples) of them were males and 13% were females. The age of the patients was ranked from 12 to 49 years old. The result of the filter paper analysis has been under process and will be reported later.

Table 3: The result of the blood filter papers collection during the project period

Sl	Villages	Number of Blood filter papers collected	Age rank of patient	Female patient	Male patient	% of female patient infected	% of male patient infected
1	Trapangkok	12	16-26	1	11	8%	92%
2	Kampong Chen	4	18-23	0	4	0%	100%
3	Anglungmakprang	5	17-25	1	4	20%	80%
4	Malickul	16	16-42	0	16	0%	100%
5	Dong	2	18-25	0	2	0%	100%
6	317	3	23-49	2	1	67%	33%
7	Anlungkrom	3	12-38	2	1	67%	33%
Total		**45**		**6**	**39**	**13%**	**87%**

E) Student blood survey in Stung Keo school

The blood survey was conducted in the primary and secondary school in project area in Stung Keo commune and all students come from those 6 near by villages that situated around the school. All 560 students in Stung Keo School were screened for malaria by using RDT that could detect Plasmodium Falciparum, Plasmodium Vivax and mix infection. 55% of the students screened are female and their ages rank from 2 to 17 years old.

The collection of 560 blood samples by filter paper was carried out during the blood survey through the finger prick blood sample taken for gene analysis. Another 560 samples of the

blood slide were also collected for the microscopic examination of Giemsa stained blood smears to examine the presence of malaria parasites in school student in study villages.

Table 4: Result of student blood survey in Koh Sla School

Age	Total number of slides examined	Male	Female	Number of slides positive	Number of RDTs positive	*Pf*	*P.v*	*Mix*
2-7y	94	37	57	0	0	0	0	0
8-12y	291	133	158	0	0	0	0	0
13-17y	175	83	92	1	0	0	1	0
Grand Total	**560**	**253**	**307**	**1**	**0**	**0**	**1**	**0**

Picture 3: Activities of the student blood survey in Koh Sla School

F) Number of bed nets distributed and re-impregnated

As one of the personal protection strategy to prevent from mosquito bites, the distribution and retreatment of the insecticide treated nets have been distributed and reimpregnated every year. In 2009, there are the total number of 9,331 insecticide treated nets are existing in the study villages with the rank of ITN coverage varies from 100 to 164%.

Table 4: Result of insecticide bed nets distributed and re-impregnated in 2009

Village	No.of family	Pop. at risk	Total number of the distributed/ retreated nets	% of ITN coverage
Stung Kbal Damrei	1141	3903	1945	100
Stung Angkanh	304	1043	700	134
317	340	1529	1132	148
Anlung Krom	429	1953	1125	115
Kampong Chen	307	1542	1150	149
Trapeang Kak	171	758	571	151
Doung	163	778	637	164
Malich Kul	122	651	446	137
Anlong Meakprang	304	1372	1039	151
Damrei Phong	175	841	586	139
Total	**3456**	**14370**	**9331**	**130**

G)- Student survey on malaria knowledge, malaria prevention and treatment seeking

- The survey team also randomly selected 40% (224 students) among the 560 students screened and administered the questionnaire to them. The questions were mainly asked related to the malaria prevention, sign and symptom of malaria and treatment seeking when gets malaria. 100% of the students recognized that malaria causes by mosquito bites. More than 90% of them knew the common malaria signs such as fever, chill and sweat. When asking where to go if they get malaria all most all said that they will go to health center and only few of them mentioned that they go to private provider. Regarding the question how to prevention from malaria, all of them stated that bed net use could protect them from malaria, especially the treated insecticide net. According to the interview with the school teachers and school director, the malaria topic was introduced in the study program and regularly educates them by their teachers and through the integrated health activities for instance vaccination campaign, malaria campaign, malaria education during the bed net distribution, especially the message and malaria information were disseminated by the village health volunteer etc....

H- Health education's activities at the project areas

The health education has played a key role in malaria prevention for the remote and poor communities, particularly to strengthen and enhance the communities' knowledge and responsiveness for participating as the malaria control activity at the village level. That is why the intensive health educations' activities were provided to the communities through various approaches in order to obtain the community awareness for a better malaria prevention purpose. Many IEC/BCC materials such as posters, leaflets, flipcharts, T-shirts, calendar etc... were distributed to the villagers during the implementation of the project. Weekly health educations have been conducted to the villagers throughout the personal contact at home, group education at school, in the filed or during the special events organized at the community.

Picture 4: Health education activities at the community

VII. Discussions

- The overall malaria positive rate in Stung Keo villages is higher than those in Koh Sla 9.46%. Among the positive cases in Stung Keo, Pf. covered 82.06% which is higher than Koh Sla 2.62% but the percentage of Vivax in Koh Sla is more compare to that of Stung Keo.
- Due to the data collected, there is no any malaria case was detected in age group 0-4 years old in Stung Keo 0-4 years old. In Koh Sla, there are 2% of malaria case distributed in age group 0-4 years old if compare to other age group but its positive rate is more than 50% (57.14%) within the same group. No any Pv was found in this agegroup in two study areas.
- The malaria case distributed in the age group 5-14 years old in Stung Keo(6%) is 17% less than Koh Sla(23%). The malaria positive rate among the same age group tested in Stung Keo is 75% which is higher than Koh Sla(60.49%) around 15% however the malaria cases collected were less than Koh Sla around 50%(24 cases vs. 49 cases). The Pv positive rate of Pv in Stung Keo(26%) among the total positive case in this agegroup is 10% higher than in Koh Sla (16%) but the Vivax cases collected in Stung Keo is 60% less than Koh Sla (5 cases vs. 8 cases). Even the proportion of malaria cases in Stung Keo is higher than Koh Sla but the malaria cases reported in Stung Keo are less than Koh Sla.
- The agegroup 15-49 years old is the most malaria affected groups among other groups in both Stung Keo (90%) and Koh Sla (69%), especially 83% and 65% of that agegroup were predominant by male. The positive rate among all 15-49 years old tested in Stung Keo and Koh Sla are 65.84% and 56.11% consecutively. A round 50% (16 cases) of all Vivax infection (30 cases) in Koh Sla is female but in Stung Keo only 14.70% (5 cases) of the total Vivax case (34 cases) is female.
- In two study areas in Stung Keo and Koh Sla, the malaria case distributed by agegroups, the agegroup >=50y is responsible for 11% and 7% accordingly. The malaria positive rate among the same age group tested in Stung Keo is 81.48% which is higher than Koh Sla(63.64%) around 18%. Both areas in this agegroup have equal number of female infected. No any Vivax infection was found in Stung Keo but 7.14% of Vivax infection was detected in Koh Sla.

VIII. Conclusions:

- ✓ Even the malaria mortality is prevented but the malaria morbidity is still a public health problem for these two study areas. In Stung Keo almost all of cases are the forest malaria related with higher both malaria cases and malaria positive rate than those in Koh Sla but its malaria cases in agegroup 5-14 years old has only 50% of Koh Sla's malaria cases.
- ✓ The agegroup 5-14 years old in Koh Sla is still rank as the secondary affected group(26%) which is different from Stung Keo that the agegroup 15-49 years old is the only main group for the malaria infection.

- ✓ The agegroup 15-49 remains the major affected group among others in both areas and Stung Keo has higher proportion than Koh Sla. The malaria in female patients among the above mentioned agegroup is only 7% in Stung Keo whereas in Koh Sla is 35% and its number of malaria cases reported have only 50% of Koh Sla's malaria cases. More Vivax infected cases reported in the Koh Sla than in Stung Keo in all agegroup.
- ✓ There are no any spleen enlarged were found during the survey and only one Vivax case was detected among all school students in Koh Sla.
- ✓ There are many new families moved to the study areas due to the census of the ITN distribution. The total increasing of the number of families and population at risk in the study areas in 2009 is 35% and 17% respectively compare to 2008.

IX. Recommendations

- o Since the population movement to the newly developed areas is increasing from year to year, the monitoring of the epidemiological trend and the intensification of malaria control activities will provide a clear picture of the malaria situation in those areas for an effective intervention.
- o Due to the epidemiological results in the study areas, the other prevention measures and health education messages should be mainly focused in the group of people that often go to the forest and the insecticide treated hammock nets are the best prevention means for the above mentioned group.
- o The seroepidemiological results collected from the study areas will provide further specific information for comparing the trend of the malaria parasites' genes among previous and new targeted villages as well as with other endemic areas in the country. For this reason the continuation of the support for the study project in the newly developed areas is very important for analyzing and interpreting the information gathered by the grant support for these few years of the project implementation.

X. Acknowledgements

- ➢ The National Center for Parasitology, Entomology and Malaria Control would like express the profound thanks to the Ministry of Health, Welfare and Labor, Japan, for their generous support and constant assistance to the Center.
- ➢ A special thank also to National Institute of Infectious Disease, Japan for their technical support and grant application.
- ➢ A great appreciation to all people who have involved in the project and made it success.

Annual Report for April 2009- February 2010

Proposal title: Screening of transmission blocking efficacy of antibodies produced against *Plasmodium vivax* and *P. falciparum* vaccine candidates.

Objectives:

1. Identify *Plasmodium falciparum* and *P. vivax* transmission-blocking vaccine candidates.

2. Screening for transmission blocking efficacy of animal antibodies produced against the vaccine candidates by membrane feeding assay

Project Principal Investigator

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Introduction:

Transmission-blocking vaccines (TBVs) prevent the transmission of malaria by inducing antibodies against antigens specifically expressed on the sexual stage parasites. Since well-characterized TBV candidates are only four (Pfs25, Pfs28, Pfs48/45, Pfs230), it would be necessary to prepare as many novel TBV candidates as possible for making the TBV development successful. In order to

identify the novel TBV candidates, Dr. Tsuboi and his group has established a post-genome approach using wheat germ cell-free protein expression system. Based on searching the combined datasets between genome and transcriptome databases, genes that are expected to be expressed in gametocyte stage of *P. falciparum*, have been selected, templates for transcription through PCR-based procedures have been prepared and followed by high throughput recombinant protein synthesis by wheat germ cell-free system. For *P. vivax* the *P. falciparum* orthologs genes will be selected. Many proteins will be produced and used to immunized mice and rabbits. Antibodies raised against these proteins will be screened for blocking of *P. falciparum* or *P. vivax* development in the mosquitoes.

Progress for this reporting period:

1. Transmission blocking efficacy of new candidates against gametocyte and ookinete stages of *P. vivax* were evaluated using blood collected from patients at malaria clinics in Mae Sod district, Tak province, Thailand. Membrane feeding assay was performed following the established SOP.
2. Total of 17 batches of mosquitoes (23,200 females of *An. dirus*) were used for evaluation of the vaccine candidates. Summary of the vaccine efficacy will be reported by Dr. Tsuboi when study is completed.
3. *Plasmodium vivax* gametocyte and ookinete antigens were prepared from patients' blood and infected mosquitoes to be used for western blot and immunofluorescent screening of *P. vivax* vaccine candidates.

Plan for 2010:

1. Evaluate more vaccine candidates produced by cell-free system from Ehime University.
2. *Plasmodium falciparum* and *P. vivax* antigens for immunofluorescent assay and western blotting will be prepared from blood stage (gametocytes) and mosquito stages (ookinetes and sporozoites) parasites.
3. Survey for field site in Mae Sod, Tak province to collect serum/plasma from population with naturally-acquired immunity for screening of new transmission blocking vaccine candidate.

FINAL REPORT

Evaluation of Malaria Diagnostic

In North Sumatera Province, Indonesia

MAIN INVESTIGATOR

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BACKGROUND

Malaria remains an important public health concern in countries where transmission occurs regularly, as well as in areas where transmission has been largely controlled or eliminated. Malaria is a complex disease that varies widely in epidemiology and clinical manifestation in different parts of the world. The key strategies has been the promotion of early diagnosis and treatment with effective antimalarials.

In Indonesia malaria is still a major public health problem, especially out of Java and Bali. In North Sumatera Province, malaria is not affecting the entire population, because cases are concentrated in some specific districts. Cases seen in other districts are imported cases, who have visited malaria areas. Since 1997 until 2001, the Department of Health has done a survey of malaria in North Sumatera Province and found that there was two species of plasmodium,

Plasmodium falciparum and *Plasmodium vivax*. But in another study, there was also found *Plasmodium malariae*.

One of the priorities of a malaria elimination programme is to identify and treat malaria patients and all people carrying parasites, including those carrying gametocytes, ensuring that they become non-infectious as soon as possible. Microscopy of Giemsa-stained thick and thin films by a skilled microscopist has remained the standard laboratory method for the diagnosis of malaria both in regions where malaria is endemic and in regions where malaria is nonendemic. In North Sumatera Province, there are problems with microscopic diagnosis, particularly at the periphery of the health care system, Public Health Services (PHC). These include lack of skilled microscopists, maintenance of microscopes, delays in results, and inadequate quality control. Therefore, Department of health in North Sumatera Province usually report Annual Malaria Incidence (AMI) rather than Annual Parasitology Incidences (API). Actually, the API always lower than AMI.

In North Sumatera Province, the Annual Malariae Incidence (AMI) in the year 2000 is 6.03%, and in the year 2001 is 3.42%. Although reliable diagnosis cannot be made on the basis of signs and symptoms alone because of the non-specific nature of clinical malaria, clinical diagnosis of malaria is common in many malarious areas. In much of the malaria-endemic world, resources and trained health personnel are so scarce that presumptive clinical diagnosis is the only realistic option. Clinical diagnosis offers the advantages of ease, speed, and low cost. In areas where malaria is prevalent, clinical diagnosis usually results in all patients with fever and no apparent other cause being treated for malaria. This approach can identify most patients who truly need antimalarial treatment, but it is also likely to misclassify many who do not. Over-diagnosis can be considerable and contributes to misuse of antimalarial drugs. Considerable overlap exists

between the signs and symptoms of malaria and other frequent diseases, especially acute lower respiratory tract infection and can greatly increase the frequency of misdiagnosis and mistreatment. Attempts to improve the specificity of clinical diagnosis for malaria by including signs and symptoms other than fever or history of fever have met with only minimal success. Therefore, diagnostic by Rapid Diagnostic Test, nowadays is used in North Sumatera Province.

Accurate information about the burden of malaria infection at the district or provincial level is required both to plan local malaria control efforts and to measure the impact of such efforts. Although several studies of malaria epidemiology and drug resistance have been conducted at many sites in North Sumatera Province, there is only little published literature describing malaria prevalence at the district and province level. Therefore, prevalence surveys for malaria, designed to estimate malaria prevalence in North Sumatera Province, Indonesia, will be conducted.

OBJECTIVES

This study is cross sectional design and does in 6 districts in North Sumatera Province, such as : Asahan, Serdang Bedagai, Toba, Samosir, Tapanuli Utara and Tapanuli Selatan district. The increasing of the Annual Malaria Incidence in each district is the reason to conduct this study in this area. Eventhough have low malaria incidence than other endemic malaria in North Sumatera Province, but the development and spread of anti-malarial drug resistance, will make it difficult to control malaria.

The objectives of this study are :

1. To evaluate malaria diagnostic tests in North Sumatera Province.

2. To investigate prevalence of malaria cases from wet and dry season in North Sumatera Province.
3. To analyse genes of malaria parasites by PCR
4. To refresh medical worker about malaria diagnostic
5. To cooperate with Department of Health in malaria control in North Sumatera Province
6. As a beginning step for the next study in malaria control, especially to find gene marker for antimalarial resistance in North Sumatera Province.

METHODS

This study conducts in several districts in North Sumatera Province. Once in the wet season and once in the dry season, in order to obtain, district-wide estimates of malaria prevalence. All individuals with fever or history of fever, will be included, except those unwilling to provide informed consent or have a severe or acute illness that require immediate medical attention. Blood from each patient will be taken for diagnostic by Rapid Diagnostic Test (One Step Mal), Microscopic examination and PCR diagnostic. Especially for Microscopic examination and PCR diagnostic, the test will use blood spot on object glass and filter paper. Blood sampling will be collected over the period of the study in the Public Health Center (PHC).

The diagnostic methods that use in this study are :

1. Rapid Diagnostic Test

The diagnostic approach involves the rapid detection of parasite antigens using rapid immunochromatographic techniques. Rapid Diagnostic Tests are able to distinguish between falciparum (detection of the histidine-rich protein

2 (HRP-II) of *P. falciparum*) and non-falciparum infections (detection of a specific parasite enzyme, lactate dehydrogenase or pLDH). Advantages to this technology are that no special equipment is required, minimal training is needed, the test and reagents are stable at ambient temperatures, and no electricity is needed. The principal disadvantages are a currently high per-test cost and an inability to quantify the density of infection. Furthermore, detectable antigen can persist for days after adequate treatment and cure; therefore, the test cannot adequately distinguish a resolving infection from treatment failure due to drug resistance, especially early after treatment.

2. Microscopic examination

Simple light microscopic examination of Giemsa-stained blood films is the most widely practised and useful method for definitive malaria diagnosis. Advantages include differentiation between species, quantification of the parasite density, and ability to distinguish clinically important asexual parasite stages from gametocytes which may persist without causing symptoms. These advantages can be critical for proper case-management and evaluating parasitological response to treatment. Specific disadvantages are that slide collection, staining, and reading can be time-consuming and microscopists need to be trained and supervised to ensure consistent reliability.

3. PCR diagnostic

Detection of parasite genetic material through polymerase-chain reaction (PCR) techniques is becoming a more frequently used tool in the diagnosis of malaria, as well as the diagnosis and surveillance of drug resistance in malaria. Specific primers have been developed for each of the four species of human malaria. One important use of this new technology is in detecting mixed infections or differentiating between infecting species when microscopic

examination is inconclusive. Primary disadvantages to these methods are overall high cost, high degree of training required, need for special equipment, absolute requirement for electricity, and potential for cross-contamination between samples.

All blood samples collected from PHC, will be analysed in each center and also confirm to the Parasitology Department, Medical Faculty, North Sumatera University. All malaria-positive samples (by RDT or microscopy), either as a single or co-infection of another *Plasmodium* species will be assayed by PCR to determine specific species.

RESULT

The *accessible population* in this study is people who joined with *the Active Case Detection*, namely Mass Fever Survey (MFS) and Mass Blood Survey (MBS) as mush as 289 participants. The positive samples in Rapid Diagnostic Test (One Step Mal) are fivity six participants, which eighteen are infected with *Plasmodium falciparum* and thirty eight are mixed infected.

Table 1. Distribution of Accesible Population

	District	Acessible Population	RDT (+)
1.	Serdang Bedagai	34	5
2.	Asahan	52	9
3.	Toba	79	15
4.	Tapanuli Utara	51	10
5.	Tapanuli Selatan	26	8
6.	Samosir	47	9
	T O T A L	289	56

But after evaluated with Microscopic examination, apparently it turned out that plasmodium spp was only found in twelve samples that contain about 1000 /µl.of parasites.

Table 2. Distribution of Microscopic Examination

	Diagnosa	Sample	Mean (parasites/µl)	Range (parasites/µl)
1.	P.falciparum	3	373	120 – 560
2.	P.vivax	2	580	320 – 840
3.	P.malariae	1	440	440
4.	P.falciparum + P.vivax (mix)	5	256	120 – 440
5.	P.vivax + P.malariae (mix)	1	240	240
	T O T A L	12		

Where as, in the evaluation of PCR found only five positive samples. Two of them are Plasmodium falciparum, which both among them had been resisted to antimalarial Choloroquine, that moleculer marker *pfcrt* is not broken by APO I enzyme.

Table 3. Distribution of PCR Diagnostic

	Diagnosa	Sample
1.	P.falciparum	1
2.	P.vivax	1
3.	P.malariae	1
4.	P.falciparum + P.vivax (mixed)	1
5.	P.vivax + P.malariae (mixed)	1
	T O T A L	5

DISCUSSION

The sampling was started on May until December 2009. There were not many samples obtained in this study. It since the sampling was equal with Ramadhan and Aidil Fitry that's August and September, so more people were not ready if their bloods taken when they were fasting.

Because of lacking skilled microscopist staff, so for diagnosing malaria still used the Rapid Diagnostic Test (RDT). The RDT basic principle is finding out the existence of antigen as the product of Plasmodium, such as *Plasmodium falciparum* histidine-rich Protein-2 (pfHRP2) that produced by *Plasmodium falciparum* and lactose dehydrogenase (pLDH) produced by *Plasmodium* spp. The Antigen can be survived for a long period in human body,

eventhough the Plasmodium had been died. It may because the RDT will be kept positive while at the microscopic examination no plasmodium found.

The differences between Rapid Diagnostic Test and microcopic examination is a must noticed. The low sensitivity of RDT in this study may be happened because the RDT is out of order as the effect of the uncorret way in storing, in the using procedure and in the reading of the result. The other case RDT is used for patient who had consumed antimalarial and also not recommended for follow-up patients. The problem that's rising up of course will be the matery in re-extension and training for the health staff in that district. But the training of microcopic examination is still be the main priority. The training have been done.

In the meantime, the different result of microscopic examination and PCR also must be inquired. Are the differences of this result have relationship with the different source of DNA from the same patiens with low parasite density? According to the opportunity, it is possible there are different amount of Plasmodium in blood at the glass object (for Microscopic Examinaton) and Whatman paper (for PCR), eventhough come from the same sample. To decrease that imbalance, perhaps it is better the isolated DNA for PCR, are also from the same blood, that's the blood at the glass object for Microscopic Examination.

From this study can be concluded it's needed improvement in Malaria Diagnose. As standard diagnose, the Microscopic Examination still must be done. So that Microscopic training keep hold continously. Where as, it's needed to re-evaluate the sensitivity of RDT used. And also the exact way for storing, the RDT using procedure, and the result reading. Beside that, also needed to activate the cross-check mechanism toward the result of examination of one level to the upper level. So it decreases the error diagnose.

Where as, DNA that will be isolated for PCR, it should taken the blood at that object glass of microscopic examination. It is for avoiding different result, especially in patient with low level of parasites.

CONCLUSION

1. RDT using at Malaria Regular Investigation is not accurate yet. There are some important noticed in using RDT, namely the way in storing, the using procedure and the reading of the result. RDT also may not used for re-checking for patients had been treated before (follow-up patient). The usage of Microscopic Examination is still the first choice in Malaria Diagnose.
2. *Plasmodium falciparum* obtained had been resisted toward anti malarial Chloroquine.
3. It's important to cross check again the result of examination in district area to province, as a quality control of malaria diagnostic.
4. It needs advanced study for obtained *genotyping* from Plasmodium, especially Plasmodium vivax in endemic area, such as Madina and Nias district in North Sumatera Province.