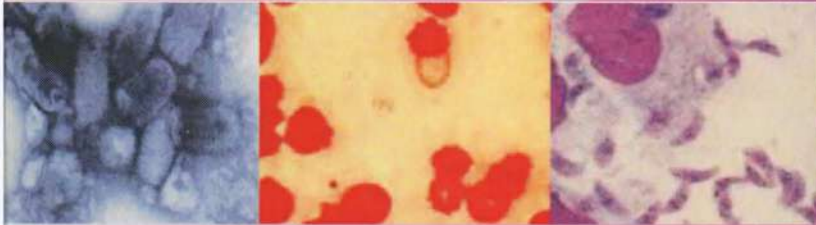




PROCEEDING

International seminar

STRATEGIES FOR THE CONTROL & PREVENTION OF ZOOONOTIC DISEASES



Surabaya, 22-23 June 2010

Tandjung Adiwinata Room

Faculty of Veterinary Medicine Airlangga University

Editor:

Pudji Srianto (Indonesia)
Mustopha B. Abubakar (Nigeria)
M. Yunus (Indonesia)
Suzanita Utama (Indonesia)
Suwarno (Indonesia)



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MEMBER OF IKAPI: 001/JTI/95



REPORT OF ORGANIZING COMMITTEE

Dr. Anwar Ma'ruf, M.Kes., DVM
Chairman

Indonesia is the one of countries which has many varieties of plants and animals that spread in all over Indonesia region. The kinds of Indonesian animals are domestic, aquatic, exotic, wild animal. There is possibility of that animals to spread of zoonotic diseases if the relationship between man and animal is not in a good control.

During the last decades zoonotic diseases threat has been increasing sharply, therefore control of those contagious diseases is definitely needed to prevent their outbreaks. Various zoonotic diseases spread all over the world such as influenza, rabies, brucellosis, anthrax and toxoplasmosis. Global warming has a big chance to change the characteristic of infectious agent that subsequently causes mutation. Mutation will emerge new strains and variants that many threaten mankind health.

Related to those instances, the aim of International Seminar "New Strategies for the Control and Prevention of Zoonotic Diseases" is organized to anticipate the prevention of zoonotic diseases by production of vaccine candidate and antigen diagnostic preventing infectious diseases.



WELCOME SPEECH

Prof. Hj. Romziah Sidik, Ph.D., DVM
Dean Faculty of Veterinary Medicine
Airlangga University

Assalamu'alaikum wr. wb.

The honorable Rector of Airlangga University,
and our Distinguish Guest Prof. Zainudin (Executive Director of IMHERE),
Prof. Zamri-Saad, Ph.D., DVM. (University Putra Malaysia),
Prof. Dr. Yoes Prijatna Dachlan, MD., MSc. (Triopical Disease Center Unair),
Mrs. Laksmi Wulandari, MD., Sp.P (K), FCCP (Dr. Soetomo Public Hospital),
Mr. Regis Vialle (Asia-Pacific Area Manager of Ajuvant Vaccine for Zoonotic),
Mr. Bora Gokylmaz (Bioengineering – PT. Global Haditech, Jakarta),
Mr. Soegiarto, DVM., MSc., Ph.D (Disease Investigation Center, Maros),
Dr. Mustapha Bala Abubakar (University of Maiduguri, Nigeria),
Mr. Anton Soekoco, ST. (PT. Fajar Mas Surabaya),
All Head and Secretary Department of Faculty of Veterinary Medicine Unair, all participants, and students.

Ladies and Gentlemen,

On behalf International Seminar Organizing Committee and Faculty of Veterinary Medicine, Airlangga University, I would like to say thank you for your coming to attend this International Seminar on “New Strategies for the Control and Prevention of Zoonotic Diseases”. I am very appreciate and pleased to welcome you in Airlangga University, Surabaya.

It is very grateful that Faculty of Veterinary Medicine, Unair hold IMHERE- Project B.2.c component for three years implementations that starting from year 2009 to 2012. The Project Implementation Programs include three activities: (1) Research Development on Rabies, *Avian Influenza* (AI), and *Brucella abortus*; (2) Development of Veterinary Diagnostic Services and Training; (3) Development of Frozen Semen Unit Production. As we know, zoonotic diseases need some extra appointed to do any specific researches to obtain a good strategy for prevention and eradicated it that to protect any transmitted diseases from animals to human. The other way, based on Unair as the Autonomous University, Faculty of Veterinary Medicine must to be have sharing to develop Revenue Generating Activity by Development of Veterinary Diagnostic Services and Training as well as Frozen Semen Unit Production, which are proposing the sustainability and achievement the goals of our faculty vision and mission.

In this event be expressed of increasing information and publication of research result that focus on producing vaccine and diagnostic kit of strategic animal diseases. Again, welcome to the International Seminar and it is great to see so many enthusiastic researchers here to get and bring some innovation and new information about these zoonotic diseases. Please have a nice and enjoy this International Seminar with a great beneficial for us.

In this moment, let me to say a thousand thank you to all speakers, participants, our special guest, and students for your dedicated and patient during follow this International Seminar. I am understand that many of you have traveled from quite a distance and I would like to acknowledge all our distinguish guest.

Billahi Taufiq wal Hidayah,
Wassalammu'alaikum wr. wb.



WELCOME MESSAGE

Prof. Dr. H. Fasich, Apt.
Rector of Airlangga University

Assalamua'alaikum warahmatullahi wabarakatuh

Let us be grateful for God's blessings to us that we all can meet here today. Today we are here to talk and discuss about how to control and prevent zoonotic disease.

As we can gather from the media, infectious disease have not been fully controlled in Indonesia and some other countries. As a consequence, this condition brings some disadvantages to people living in those countries.

For example, failures in controlling such diseases in a country can lead to travel ban and travel warning, which in turn will close various opportunities. This alone is a big disadvantage for the development of the country, including human resource development.

Base on those condition, as well as taking into account Airlangga University role in controlling infectious diseases such as Avian Influenza, this forum is an important step in controlling other infectious diseases.

This is also supported by the fact that many modern diseases, including epidemic ones, started from zoonotic diseases. Even in the modern world, in which people have got sufficient immunity, we should still be aware of zoonotic diseases.

Therefore, in thin very special moment, I would like to invite researchers from all over the world to conduct collaborative research, as specially in tropical diseases, at the hospital of Tropical Infectious Diseases, Located at Airlangga University.

By the end of the year 2010, the hospital is ready to operate and conduct various research on diseases caused by specific microorganism of tropical area. Such research, I believe, will be of beneficial for the development of science and technology.

I certainly hope that collaboration among us will be beneficial in finding a better method for the prevention can control of diseases. This, in turn, will bring a better life for sake of humanity. Thank you very much.

Wassalamu'alaikum warahmatullahi wabarakatuh.

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FACULTY OF VETERINARY MEDICINE AIRLANGGA UNIVERSITY INTERNATIONAL SEMINAR 22-23 JUNE 2010

'New Strategies for the Control and Prevention of Zoonotic Diseases'

PROGRAM SCHEDULE FIRST DAY: TUESDAY 22 JUNE 2010

TIME	PROGRAM	
07.30 – 08.30	Registration	
08.30 – 09.00	Opening Ceremony <ul style="list-style-type: none"> • Traditional Dance • Report: Chairman of the Organizing Committee • Welcome: Dean, Faculty of Veterinary Medicine Airlangga University • Opening: Rector of Airlangga University 	
09.00 – 09.30	Refreshment Break	
PLENARY LECTURE-1		
09.30 – 10.00	Keynote Speaker 1: Dr. Suwarno, M.Si., DVM (Microbiology Department Faculty of Veterinary Medicine, Airlangga Univ.: Characteristic Rabies Virus in Indonesia).	
10.00 – 10.30	Keynote Speaker 2: Laksmi Wulandari, dr., Sp.P(K), FCCP. (RS. Dr. Soetomo, Surabaya: Avian and Swine Flu Outbreak: Surabaya Experience)	
10.30 – 11.00	Keynote Speaker 3: Prof. Dr. Fedik A. Rantam, DVM. (Vice President of Indonesia Society for Microbiology: Biology and Characterization of Borna Disease Virus Infection)	
	(Moderator: Dr. Soelih Estoepangesti, DVM.)	
SYMPOSIUM-1		
11.00 – 11.45	Keynote Speaker 4: Regis Vialle (Seppic, SA, Perancis: Adjuvant for zoonotic diseases, model specificity?)	
11.45 – 12.45	Keynote Speaker 5: Bora Gokylmaz (Bioengineering Switzerland – PT. Global Haditech Jakarta: (Bioequipment for Vaccine Production and Design Engineering Consultant for Pharmaceutical Industry)	
12.45 – 13.15	Discussion (Moderator: Prof. Dr. Rahayu Ernawati, M.Sc., DVM.)	
13.15 – 14.15	Lunch and Prayer Break	
FREE PAPER -1/PARALLEL SESSION		
	Small Class Room Lv 1	Tanjung Adiwinata Room
14.15 – 14.25	Presentation AOP 1	Presentation BOP 1
14.25 – 14.35	Presentation AOP 2	Presentation BOP 2
14.35 – 14.45	Presentation AOP 14	Presentation BOP 3
14.45 – 15.00	Discussion: Dr. NMR. Widjaja	Duiscussion: Dr. Garry Cores
15.00 – 15.15	Refreshment Break	
15.15 – 15.25	Presentation AOP 4	Presentation BOP 4
15.25 – 15.35	Presentation AOP 5	Presentation BOP 5
15.35 – 15.45	Presentation AOP 6	Presentation BOP 6
15.45 – 16.00	Discussion: Dr. NMR. Widjaja	Discussion: Dr. Garry Cores

SECOND DAY: WEDNESDAY 23 JUNI 2010

TIME	PROGRAM	
SYMPOSIUM-3		
08.00 – 08.30	Keynote Speaker 6: Soegiarto, DVM, M.Sc, Ph.D. (Disease Investigation Center, Maros: Epidemiology of Rabies in Indonesia 2005–2009)	
08.30 – 09.00	Keynote Speaker 7: Dr. Mustapha Bala Abubakar (University of Maiduguri, Nigeria: Avian Influenza in Nigeria)	
09.00 – 09.30	Discussion (Moderator: Dr. Mustofa Helmi Efendi, DVM.)	
09.30 – 09.45	Refreshment Break	
SYMPOSIUM-4		
09.45 – 10.15	Keynote Speaker 8: Prof. Zamri Saad, DVM., Ph.D. (UPM Malaysia: Brucellosis in Malaysia)	
10.15 – 10.45	Keynote Speaker 9: Prof. Dr.Yoes Prijatna Dachlan, M.Sc., dr. (Faculty of Medicine, Unair: Parasitic Zoonosis)	
10.45 – 11.15	Discussion (Moderator: M. Yunus, Ph.D., M.Kes., DVM.)	
SYMPOSIUM-5		
11.15 – 11.45	Keynote Speaker 10: Anton Sukoco, ST. (PT. Fajar Mas Surabaya, Bio Imaging Navigator: Compound Microscopes in One Box)	
11.45 – 12.00	Discussion (Moderator: Dr. Dady Soegiarto Nazar, M.Sc., DVM.)	
12.00 – 13.00	Lunch and Prayer Break	
FREE PAPER-2/PARALLEL SESSION		
	Small Class Room Lv 1	Tanjung Adiwinata Room
13.00 – 13.10	Presentation AOP 7	Presentation BOP 7
13.10 – 13.20	Presentation AOP 8	Presentation BOP 8
13.20 – 13.30	Presentation AOP 9	Presentation BOP 9
13.30 – 13.40	Presentation AOP 10	Presentation BOP 10
13.40 – 13.50	Presentation AOP 3	Presentation BOP 14
13.50 – 14.15	Discussion: Dr. Suzanita Utama	Discussion: Boedi S., DVM
14.15 – 14.30	Refreshment Break	
14.30 – 14.40	Presentation AOP 11	Presentation BOP 11
14.40 – 14.50	Presentation AOP 12	Presentation BOP 12
14.50 – 15.00	Presentation AOP 13	Presentation BOP 13
15.00 – 15.10	Presentation AOP 15	Presentation BOP 15
15.10 – 15.25	Discussion: Dr. Suzanita Utama	Discussion: Boedi S., DVM
15.25 – 16.00	Door Prize/Closing Ceremony	

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MOLECULAR CHARACTERIZATION OF *pfprt* GENE FOR DETECTION CHLOROQUINE ANTIMALARIAL DRUG RESISTANCE AT CLINIC ISOLATES *PLASMODIUM FALCIPARUM*

Lilik Maslachah,¹ Eddy Soewandojo,² Mufasirin,³ Yuni Priyandani,¹ Loeki Enggar Fitri,⁴ Didi Candradikusuma⁵

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ABSTRACT

Research of molecular characterization *pfprt* gene to detection chloroquine antimalarial drugs resistance at clinic isolates *Plasmodium falciparum*, aims to prove and lay open mechanism and cause antimalarial drugs resistance at local clinic isolat of *Plasmodium falciparum* through determination of *pfmdr1* gene. Method of the research: Making thin and thick blood films from malaria patient for microscopic inspection to determine density and identify parasite. In vitro culture to get *Plasmodium falciparum* parasite Isolate. Drug susceptibility testing to determine minimum inhibiting concentration (MIC) to antimalarial drugs. DNA extraction. Amplification *pfprt*, gene with PCR technique. Result of research sensitivity test by in vitro Chloroquine 500 ng/ml for the *Plasmodium falciparum* isolates from malaria patient used micro test method modify from WHO by using flat bottom well 96 (WHO, 1990) Result of IC50 test got Chloroquine drug can only be pursued with big dose 500 ng/ml. Result of PCR by using specific primary for the amplification gene region coding *Pfprt* gen at 479- 630 bp genes *Plasmodium falciparum* can be expressed so that result of this can be used as marker for the detection anti malarial drugs resistance. The concluded: Minimum concentration to pursue growth of parasite equal to 50%. Chloroquine require big cosentration to pursue growth parasite 500ng/ml. *Pfprt* gene *Plasmodium falciparum* can be used as marker to detect chloroquine antimalarial drugs resistance. Suggestion: Result of gene from PCR have to be continued with squencing to know mutation gene. Furthermore gene marker to make diagnostic kit as rapid test so that can give input and benefit to government evaluating and updating antimalarial drugs treatment policies, and give input to doctor in therapy with correct antimalarial drugs to malarial patients.

Key words: chloroquine, resistance, *pfprt* gene

INTRODUCTION

Malaria was still a public health problem in many countries around the world, especially in the tropic countries. Three hundred million people were attacked each year and die 2-4 million. Various efforts have been made to eradicate malaria, but malaria was still high. Indonesia was malaria endemic area, although it has been doing the program and the eradication of malaria since 1959, but until now the morbidity and mortality is still high, because of problems in the eradication of malaria including vector resistance to insecticides and parasite resistance to antimalarial drugs (Simanjutak and Arbani, 1999).

Malaria resistance. parasite to antimalarial drugs, especially to chloroquine first appeared in Thailand in 1961 and in the United States in 1962. Then the resistance spread throughout the world. In Indonesia, the resistance of *Plasmodium falciparum* to chloroquine was reported for the first time in Samarinda in 1974, then resistance continues to expand and in 1996 of chloroquine-resistant malaria cases found in all provinces in Indonesia. Six of chloroquine-resistant malaria cases were recorded in Sumatra province during the period January 2001 until April 2001. Apparently also found six cases resistant *Plasmodium*

falciparum sulfadoxin-pyrimethamine in Irian Jaya (Tarigan J, 2003).

The emergence of resistance *Plasmodium falciparum* parasite to carried neither increasing malaria eradication chloroquine more complex while the mechanisms of resistance is not known with certainty, the results of investigations conducted by the Institute of Eijman biomolecules stated that almost 100% in Indonesia malaria parasites have been object of genetic mutation and resistance to chloroquine medicines *Plasmodium* resistance mechanisms very complex process was safe and detail. Results Lopes et al., (2002) suggests that resistance to chloroquine and the MDR caused by mutations in the gene *pfprt* and *pfmdr1*. Although *pfprt* gene and mutation *pfmdr1*, related to the emergence of resistance to anti-malarial drug chloroquine, but not much research, especially in Indonesia, which describe the relation is clear, especially at the local clinical isolates of *Plasmodium falciparum*. This was still a need for further research to identify the causes and mechanisms of resistance that can be used to perform precise treatment.

The aims of this study knowing the characteristics of *pfprt* gene local clinic isolates of *Plasmodium falciparum*. Knowing the in vitro sensitivity of antimalarial drug chloroquine local clinic isolates

of *Plasmodium falciparum*. Advantages of the first study. *Plasmodium falciparum* *pfprt* gene can be used as a marker of chloroquine antimalarial drug resistance detection. Marker that gene can be used as a diagnostic kit for quick test, precise and accurate detection of antimalarial drug resistance to chloroquine. Contribute to the Government policy for the treatment of malaria, as well as consults a doctor in determining appropriate anti-malarial drugs for malaria.

METHODS

Research procedure conducted in this study consisted of collecting blood samples from patients suspected falciparum malaria. Microscopic examination of thick and thin blood preparation methods for the examination of thick blood look asexual plasmodium parasites. Blood of patients placed on a glass object and allowed to dry, after which blood washed with water, stained with Giemsa 10% in pH 7 buffer solution for 20-30 minutes. After that blood flow carefully washed for 1-2 seconds and allowed to dry and ready for inspection. How to thin the blood tests are useful for identifying species of malaria parasites. How to paint with thick blood test but before the painted blood preparations were fixed with pure methanol.

Culture procedures (Pusarawati S *et al.*, 1997) Blood malaria is carried through the blood vessels of about 10 ml, then the blood is transferred from sterile syringes to tube containing 200 IU of heparin plus a transport medium (Medium added without serum) with the same volume and well mixed. If not done immediately stored at 4 ° C. This storage can be done for 24-36 hours. Then blood centrifugation with speed 1500 rpm for 5 minutes at a temperature of 4 ° C. Plasma, buffy coat and supernatant removed by using ficoll hypaque isolation until live erythrocyte sediment (packed cells), and then washed three times the average plus. Made each 1 ml solution of suspensions of erythrocytes and mix with 9 ml medium plus with 15% human serum and put into the bottle culture (Culture flasks) and incubated at temperature of 37 ° c 5% CO₂, 5% O₂ and 90% N₂. The average replacement every 24 hours using sterile Pipets Pasteur added 9 ml culture medium on every bottle and incubated again. Parasite growth regularly every 24 hours or 48 hours. If you would like to make a sub culture spreads and frozen storage can be used again.

Examination in vitro drug sensitivity tests on the drug (Chloroquine 500 ng/ml) for *Plasmodium falciparum* isolates from malaria patients using the WHO micro-modification experiments using flat

bottom 96 well (WHO 1990). Extraction of genomic DNA of *Plasmodium falciparum* parasite using a DNA isolation kit protocol Macherey-Nagel. DNA yang been isolated stored at 4 ° C until ready for further use.

Primers for amplification *pfprt* gene (Schonfeld, 2007), which used to amplify the gene region code (codon) genes as follows: *pfprt76* (sense: CAAGAAG GAAGTAAGTATCCAAAAATGG; antisense: GTAG TTCTTGTAAGACCTATGAAGGC). PCR reaction mixture for the process be done in a volume of 25 µl containing 25 pmol primers, 5 µl template solution, 1.25 units of Taq DNA polymerase, 1X PCR buffer (10 mM Tris HCl pH 9, 1.5 mM MgCl₂, 50 mM KCl) and dNTPs (dGTP, dATP, dCTP and dTTP) with respective concentrations of 100 µl. The process will be done by DNA PCR Thermal cycler machine as much as 25 cycles, with each cycle consisting of denaturation step at a temperature of 94 ° C for 30 seconds, annealing step at an optimal temperature of 56-59 ° C for 30 seconds and extensions temperature the phase polymerization 60-68 ° c for 60 seconds. The results will be analyzed further amplification with an agarose gel 1% (w/v), containing 0.6 ethidium bromide. Display of the electrophoresis was performed with ultraviolet light at a wavelength of 205 nm and documented with digital cameras.

RESULTS AND DISCUSSION

Blood samples collected from Saiful Anwar hospital Malang. Four samples was identified with the thick and thin blood smears for determination of *Plasmodium falciparum* just not mixed infections performed in the laboratory of biomedical Brawijaya University School of medicine.

Microscopic examination results of microscopic examination of thin blood preparations with a thin blood smear examination is important for proper identification of the parasite. In this research to determine that patients infected by *Plasmodium falciparum* very visible with the obvious difference with plasmodium infection by the others as can be seen that the characteristic red blood cells infected by *Plasmodium falciparum* not grow but experienced shrinkage (distortion) with Crenation. Reddish with a dark side and there are rough spots (Mauer's nipple). So that the sample was infected with *Plasmodium falciparum*

Results from in vitro cultured *Plasmodium falciparum* various stages of development, found that young trophozoite stages that are often found by drawing a thin ring of pale blue cytoplasm and 1 or 2 small red dots chromatin. Mature trophozoite was also often found in the cytoplasm of thin blue ring-shaped or comma an exclamation point. medium-sized red chromatin

numbered 1 or 2. Schizont with dark brownish-black pigment with approximately 18-23 merozoites and gametocytes like a banana.

Results IC50 in vitro sensitivity on the drug Chloroquine 500 ng/ml for *Plasmodium falciparum* isolates from malaria patients using the WHO micro test method modification experiments using flat bottom 96 well (WHO 1990) with the following results IC50 of test results obtained from the growth of parasites in the blood of infected control compared with infected blood given some type of drug therapy during the 24 hours after incubation with several dilutions of the drug dose was found in parasite drug. Chloroquine can be inhibited only by concentrating on large doses of Chloroquine 500 ng/ml. These results may have indicate that this parasite is resistant to chloroquine. The drug therefore require higher doses to inhibit its growth. As a result of research carried out by Tuti (1992) testing in vivo and in vitro chloroquine resistance found almost in all provinces in Indonesia.

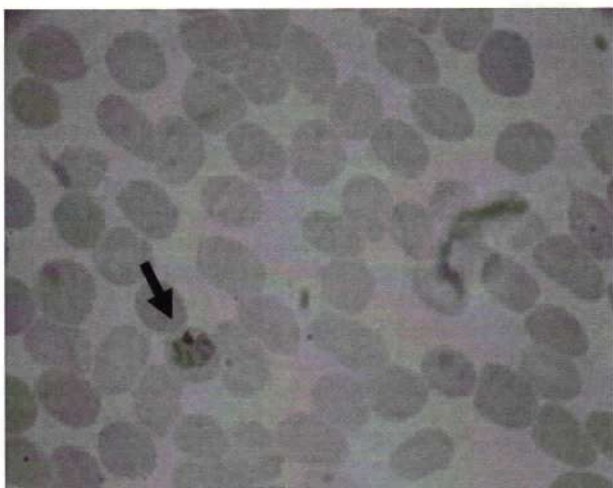


Figure 1. Schizont containing merozoites

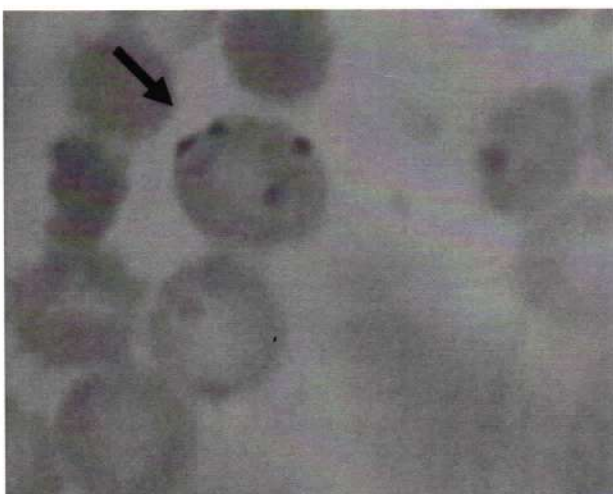


Figure 2. Young trophozoite

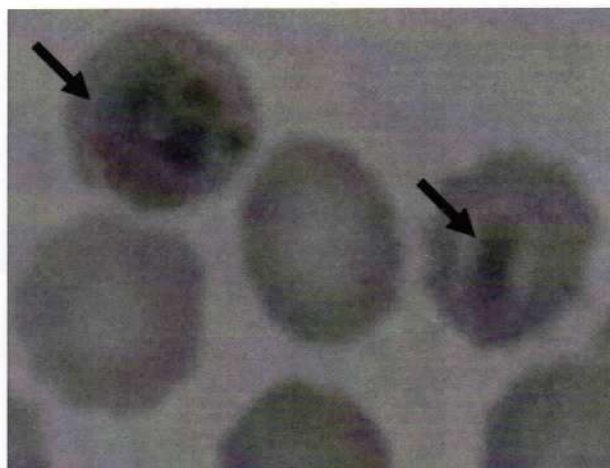


Figure 3. Gametocytes. Schizont

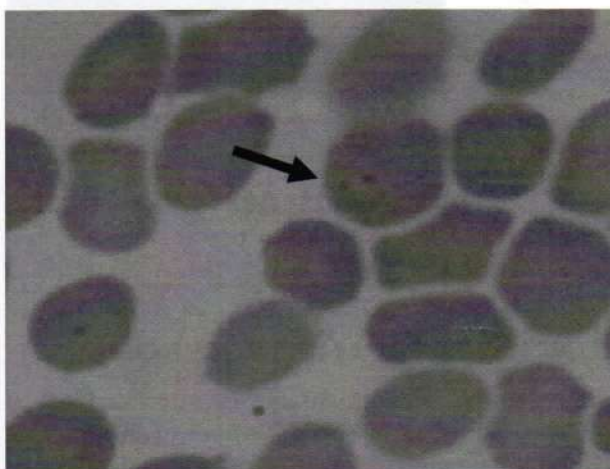


Figure 4. Trophozoite mature rings 2dot

Extraction DNA samples of *Plasmodium falciparum* culture from malaria patients made Macherey-Nagel Kit. Results drawn from cultures parasite 3D7, B011 A021, samples, and then performed using electrophoresis marker ladder with bands high of around 10 000 bp, while the lowest around of 250 bp. Results of the bands was displayed in the B011, A021, Y and 3D7 showed higher than the marker used performance.

Results for amplification *pfcr* gene by PCR shown in the figure 5 the band emerges with a certain size by using specific primers to strengthen the gene coding region (codon) as desired. *pfcr* gene appeared in the 479-630 bp. Genes can be expressed in the *plasmodium falciparum* so these results can be used as a marker to determine the detection of resistance to anti-malarial drugs. Chloroquine antimalarial resistance associated with changes in the amino acid lysin Threonine at codon 76 of chloroquine resistance transporter gene (*pfcr*) *Plasmodium falciparum* (Djimde, A., et al.2001). Detection of the gene can be used to predict resistance

pfcr76 chloroquine in epidemiological studies with a high sensitivity (Jelinek, et al., 2002).

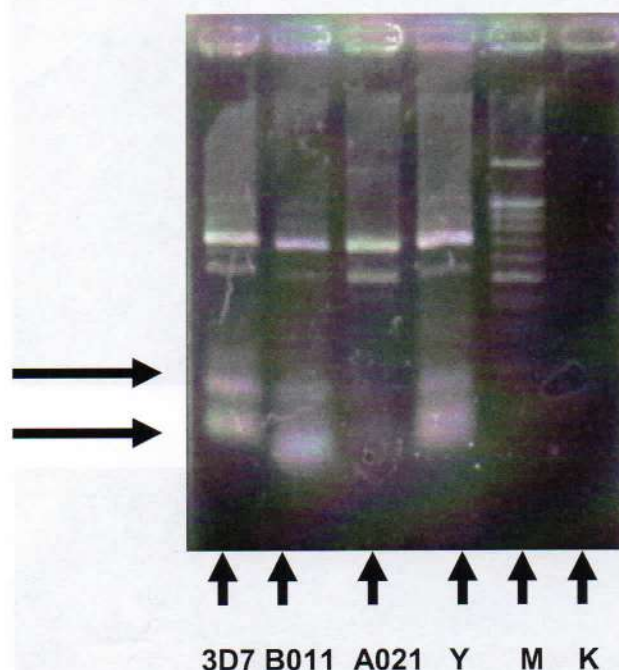


Figure 5. *pfcr76* gene by PCR

CONCLUSIONS AND SUGGESTION

The results of this study can be summarized as follows: Require large concentrations of chloroquine to inhibit parasite growth 50% that is equal to 500ng/ml. By using specific primers *pfcr76* gene with PCR amplification was found at 479-630 bp. *Pfcr76* genes in *Plasmodium falciparum* can be used as markers to detect resistance to antimalarial drugs. Suggestions

from this studied were the PCR products should be continued with sequencing to identify mutations in the gene in each sample.

REFERENCES

- Djimde, A., Doumbo, OK, Cortese, JF, Kayentao, K., Doumbo. S., Diorte, Y., Dicko, A., Su, XZ, Names, T., Fidock, DA Wellem TE, CV Plwe., Coulibly, D. (2001). A molecular marker for malaria *P. chloroquine*-resistant *falciparum*. *N Engl j* 344: 257-263.
- Jelinek, T., Aida, AO. Hoffmann Peyerl, G., S., older, S., Heuschkel, C., El Valy, AO, Von Sonnenburg, F., Christophel, MS. (2002) Diagnostic Molecular markers in *Falcifarum* chloroquine-resistant value Mauritania malaria in South. *Am J Trop Med Hyg.* 67: 449-453.
- Lopes, D., Rungsihirunrat, K., Nogueira, F., Seugorn, A., Gil, JP, Rosario, VE and Pedro Cravo. (2002) Molecular characterization of *Plasmodium falciparum* resistant from Thailand. *The malaria Journal.* 1: 12.
- Pusarawati S, Tantular I.S, Hidayati S, Prijatna Dachlan Y, (1997) Biakan Berkesinambungan *Plasmodium falciparum* Stadium Eritrosit. *Biologi Molekuler Kedokteran.* 95-108.
- Schonfeld, M. Miranda, I.B., Schunk, Manduhu, I., Mboko, L., Hoelsher, M., Riha, NB.
- Kitua, A and Loscher, T. (2007). Molecular Surveillance of Drug Resistance associated Mutation of *Plasmodium falciparum* in Southwest Tanzania. *Malaria Journal* 6: 2.
- Simanjutk, C.H., Arbani, P.R. (1999). Status Malaria Di Indonesia. *Cermin Dunia Kedokteran.* 55: 3-11.
- Tarigan, J (2003). Kombinasi Kina Tetrasiklin Pada Pengobatan Malaria *Falciparum* Tanpa Komplikasi di Daerah Resisten Multidrug Malaria. *Bagian Ilmu Penyakit Dalam Fakultas Kedokteran Universitas Sumatera Utara.* Usu digital Library. 1-20.
- Tuti, S (1992). Resistensi *Plasmodium falciparum* Terhadap Beberapa Obat Anti Malaria Di Indonesia. *Cermin Dunia Kedokteran* 76. 49-52.