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- The Effect of *Pandanus conoideus* Lamik Extract to the Serum Level of TNF- α , IL-10 and Parasitemia of *Plasmodium berghei* Infected in Mice
- Comparison of Immunochromatography Method and Immunocytochemistry Method in Rapid Detection of NS-1 Antigen in Dengue Infection
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Evaluation of the Performance of Malaria Microscopist in Primary Health Center and Cross Checker in Belu East Nusa Tenggara

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ABSTRACT

Introduction: It was reported that error rate of malaria microscopic examination of microscopist at Primary Health Center and District Health office of Belu, Nusa Tenggara Timur (NTT) was high (45-100%). These high level of error rate might be caused by several factors such as lack of qualified personnel, lack of technical skills in the microscopic slide preparation (blood smear) or incompetency in parasite identification on blood smear preparation.

Objectives: To evaluate the performance of malaria microscopists at primary health centers and cross checker in Belu District and determining the factors that contribute to those condition.

Methods: The study was an observational and exploratory research with cross sectional approach. The competency of the malaria microscopist at the primary health center and district health office in examining malaria blood smears were evaluated using standard blood smears and the one from Passive Case Detection (PCD) activities. Performance in preparing blood smear were evaluated by observation and filling the checklist. The study was conducted in December 2012-March 2013 in Belu District, NTT.

Results: The competency of the microscopists to identify malaria infection was low. The error rate in examining PCD blood smears ranged from 20 -100%. The error rate of the microscopic reading at district level reached 52%, with a kappa value between 0.41 to 0.60. There was different levels of the error rate using standard and PCD blood smears. Error rate in diagnosing malaria on PCD blood smears was higher than those on gold standard blood smears. Accuracy to identify *P. falciparum* was high (100%) than those to identify *P. vivax* (0-75%). False-positive diagnosis is as high as 90% when examining PCD blood smears. Missed diagnosis of malaria parasite also occure in around 60% of personnel when examining blood smears with low density of less than 1000 parasites / il of blood)

Conclusion: The accuracy of malaria diagnosis by microscopist in the Health Center in District of Belu is low. The factor that might contribute is the ability of the microscopist to produce a good quality of malaria blood smears. Working experience of the health center microscopist is statistically significant to correlate with accuracy of malaria diagnosis.

Keywords: Error rate, the performance evaluation of microscopists, microscopic examination, blood smear Belu

INTISARI

Pendahuluan: *Error rate* pemeriksaan mikroskopis sediaan darah (SD) malaria oleh petugas mikroskopis Puskesmas dan Dinas Kesehatan di wilayah Kabupaten Belu dilaporkan berkisar antara 45-100%. Tingginya *error rate* ini bisa disebabkan oleh beberapa faktor antara lain kualitas peralatan dan bahan kimia yang kurang baik, kurangnya kemampuan petugas mikroskopis dalam teknis pembuatan sediaan darah maupun kemampuan mikroskopis dalam mengidentifikasi parasit malaria dalam sediaan darah.

Tujuan: Mengevaluasi kemampuan petugas mikroskopis Puskesmas dan cross checker di Dinas Kesehatan Kabupaten Belu dalam mendiagnosis malaria secara mikroskopis serta faktor-faktor yang mempengaruhi.

Metode: Penelitian ini adalah penelitian observasi dan eksplorasi dengan pendekatan *cross sectional*. Kemampuan diagnosa malaria petugas mikroskopis puskesmas dan dinas kesehatan kabupaten dievaluasi dengan menggunakan sediaan darah baku maupun sediaan darah yang didapat dari *Passive Case Detection* (PCD). Evaluasi kinerja dilakukan dengan pengamatan dan pengisian checklist oleh peneliti. Penelitian dilakukan pada bulan Desember 2012-Maret 2013 di Kabupaten Belu Propinsi Nusa Tenggara Timur.

Hasil: Kemampuan tenaga mikroskopis dalam menyiapkan sediaan darah untuk diagnosa mikroskopis masih rendah. *Error rate* tenaga mikroskopis malaria di puskesmas menggunakan sediaan darah baku berkisar antara 0-30% dengan kekuatan kesepakan jelek (K) 0,00-0,20. *Error rate* diagnosa malaria terhadap sediaan darah dari PCD berkisar antara 40 -100%. *Error rate* petugas mikroskopis tingkat kabupaten sebagai *cross checker* dengan slide malaria standar dari Bagian Parasitologi FK UGM mencapai 52% dengan kekuatan kesepakan sedang (K) 0,41-0,60. *Error rate* pada pemeriksaan sediaan darah dari PCD lebih tinggi daripada *error rate* pada pemeriksaan sediaan darah baku. Ketepatan identifikasi spesies *Pfalciparum* mencapai 100% sedangkan spesies *P.vivax* berkisar antara 0 - 75%. Diagnosa positif palsu banyak ditemukan pada pemeriksaan sediaan darah PCD (90%). Sebanyak 58,8% tenaga mikroskopis puskesmas melakukan kesalahan diagnosa pada sediaan darah standar pada kepadatan rendah (<1000 parasit/ μ ldarah)

Simpulan: Kemampuan tenaga mikroskopis puskesmas dalam menyiapkan sediaan darah untuk diagnosa mikroskopis malaria di Kabupaten Belu masih rendah dan *error rate* pemeriksaan mikroskopis malaria masih tinggi. Pengalaman kerja mempunyai hubungan yang bermakna dengan ketepatan diagnosa mikroskopis malaria.

Kata Kunci: *Error rate*, evaluasi kinerja mikroskopis, cross checker, sediaan darah baku, Belu

INTRODUCTION

Malaria is a disease caused by blood protozoa parasite which has a very complex life cycle and therefore difficult to be eradicated. This disease is still endemic in many tropical countries in the world, including Indonesia. Malaria is also known to cause of more than 250-660 million cases with more than one million deaths (mostly African children) every year¹. In Indonesia Malaria spreads in almost all province with various level of endemicity and currently, more than 110 million Indonesian people are living in the high risk areas of malaria². An integrated and comprehensive approach are required to control malaria, including early diagnosis and appropriate treatment with an effective anti-malarial regimen³.

The gold standard for malaria diagnosis is to conduct microscopic examination of prepared blood smear but in remote area, this methods often faces

some difficulties such as unavailability of qualified microscopist personel, the lack of technical skills in preparing and staining of the blood smear as well as in identifying the parasite⁴. According to the result of cross checking on microscopic examination of Puskesmas microscopist by cross checker at Belu District Health Office, the ability and skills of microscopic officers at the local clinic and Primary Health Center level is still low. The error rate of mikroskopis diagnosis in some local clinic are ranged from 45-100%. District of Belu represents high endemic malaria area with Annual Parasite Incidence (API) in the year of 2007 until 2010 are: 431.8 ‰, 22.9 ‰, 28.43 ‰ and 30.07 ‰, respectively⁵. According to the national data at The Indonesian Health Department (2010), District of Belu are classified as a high malaria endemic area with "Annual Parasite Incidence" (API) of more than 5 ‰. NTT is also one of the 6 province in Indonesia that still have

a high malaria transmission level and need special attention from the government to control the diseases. High error rate on the microscopic malaria diagnosis in Belu constitute a significant obstacle in controlling malaria in the area. The standart quality of microscopist in diagnosing malaria infection should has an error rate of less than 5%⁶.

High level of error rate in microscopic malaria diagnosis in a particular clinic or primary health care may be due to a number of factor such as 1) the inability to prepare a good quality of thick and thin blood smear, 2) the inaccuracy of the staining procedure and 3) the inability to characterized the specific microscopic morphology of the parasite in the thick and thin blood smear and identify the Plasmodium species. The World Health Organization (WHO) in 2005 has recomended that the competency of malaria microscopist to identify malaria parasites should always be checked and be improved with routine microscopic training⁷. The accuracy of microscopic diagnosis may also be affected by the quality of laboratory equipment and chemicals as well as the laboratory infra structure. Other factor such as a good supervision, routine laboratory maintenance, workload and working environment are also essential to maintain the performance of malaria microscopist⁷.

In Belu district, the error rate of most of malaria microscopist is still considered to be high, however, it is not been evaluated whether the error is happened in the local/primary health center clinic level or at the district level (cross checker officers). Therefore, it is necessary to evaluate the performance of the microscopist by assessing the ability of the local or primary health center microscopist in preparing and staining thin and thick blood smear for malaria diagnosis and assessing both local clinic and district level microscopist ability in identifying malaria parasites and also to evaluate other possible contributing factors to the accuracy of malaria diagnosis in District of Belu.

MATERIALS AND METHODS

This research was an explorative and observational analytic study using cross-sectional approach. Independent variables consisted of the microscopist competencies: such as education level, training experience, and length of work experience/ years of service; the microscopist performance: such as: the ability to prepare good quality of thin and thick blood smears and Giemsa staining, routine supervision and cross checking routine, the quality of equipment and reagents, work-environment and workload) while the dependent variable are error rate (number of failed diagnosis).

Equipment and materials for microscopic examination comprised of glass slides/object glass, blood lancet, alcohol swab (70% ethanol), dry cotton/tissue, 2B pencils, slide books, Giemsa stain solution, absolute methanol, distilled water, measuring cup, binocular microscope, *emersion* oil.

The study was conducted by evaluating the performance of the Health Center microscopist in preparing thick and thin blood smears, methanol fixation and Giemsa staining of the thin and thick blood smears, and the ability to identify malaria parasite on the blood smears. Evaluation of the factor that contribute to the microscopist performance was done by interviews or discussion with Head of the Health Center or microscopist using prepared questionair which gathering information about the availability of routine supervision, training and cross checking, the availability of good quality of microscope, Giemsa stain and absolute methanol, and also the work-environment condition and the workload of the malaria microscopist. Other factor that contribute to the microscopist competency such as education level, malaria microscopic training experience, length of work as malaria microscopist are also being evaluated. The accuracy in malaria parasite identification was assessed using 2 types of blood smears, firstly, using standard malaria blood slides from Universitas Gadjah Mada and secondly,

using blood slides prepared by their own (the microscopist). Statistical analysis was conducted by using Bivariate Analysis test to find out the relationship between the mastery/ability of the microscopist in preparing blood smear and the diagnosis accuracy by using Chi-Square test. The error rate in malaria microscopic diagnosis was calculated using formula recommended by the Indonesian Department of Health (2003):

$$\text{Error rate} = \frac{\text{number of reading error}}{\text{number of slide read}} \times 100\%$$

RESULTS AND DISCUSSIONS

1. Geographical situation

District of Belu is situated on the east part of the East Nusa Tenggara Province, it has 2,445.57 square kilometer wide, located in between 124° to 126° longitude and minus 9° to minus 10° latitude. The north border is the Ombai Strait, the south border is Timor sea, and to the west is the same border with the District of Timor Tengah Utara (TTU) and Timor Tengah Selatan (TTS). While to the east is to have the same border with the Republic of Timor Leste. The District of Belu administrative territory is consisted of 12 sub-district (Kecamatan), 24 Kelurahan and 196 villages (desa).

The majority of the territory is a low landscape of the coastal plain with some hilly area in other part of the district. The average temperature is between 24° to 34° C with tropical climate and has two different season of dry (around April-August) and rainy (October-March) season.

Based on the data from the Central Statistic Agency of Belu District, the number of population in 2011 is consisted of 174,899 male and 179,777 female, inhabited in approximately 83,275 household. The average population density is 150 people per square kilometer.

2. Public Health Facility

Public health facilities available in the District of Belu consisted of 25 unit of Health Center, 2 District General Hospital (*Rumah Sakit Umum Daerah*) and 12 clinic. The Total number of Medical Doctor is 19, consisted of 13 Government Civil Officer (Pegawai Negeri Sipil) and 6 medical doctor as contract officer (*Pegawai Tidak Tetap atau PTT*). Another health personal in the District of Belu consisted of 11 master in health science, 63 Diploma in health science, 121 nurses, 89 government midwives and another 96 contract midwives.

3. Evaluation of the Microscopist Performance

Several aspect related to the ability of the Health Center microscopist in conducting malaria diagnosis were assessed including the completeness in preparing tools and chemicals, the quality of the glass slide used in the clinic, the quality of the thick blood smear and thin blood smears they produced and the result of the Giemsa staining and finally the capability in microscopically identifying malaria parasite in the blood slide they prepared as well as in standard malaria blood smear preparation. Their diagnosis were then compared to the diagnosis of the microscopist from Universitas Gadjah Mada as the reference and the kappa value were calculated. The results of the evaluation of the microscopist performance is presented in Table 1.

Table 1. The distribution and Bivariate Analysis of the performance of the microscopist in local Health Center clinic (n = 16) in preparing bloodsmear for microscopic malaria diagnosis in Belu

Variabel	Criteria	Frekuensi		Kappa value				Total		P Value
				Poor		Good				
		N	%	N	%	N	%	N	%	
Tool and Material Pereparation Total	Complete	9	56,3	1	6,25	8	50,0	9	56,25	0,192
	Not	7	43,8	4	25,0	3	18,75	7	43,8	
		16	100							
Objek glass preparation Total	Used	6	37,5	6	37,5	0	0	6	37,5	0,08
	New	10	62,25	6	37,5	4	25	10	62,5	
		16	100							
Creating thick blood smear Total	Good	8	50,0	5	31,25	3	18,75	8	50,0	0,51
	Poor	8	50,0	7	43,7	1	6,25	8	50,0	
		16	100							
Creating thin blood smear Total	Good	3	18,75	1	6,25	2	12,5	3	18,75	0,04*
	Poor	13	81,25	11	68,75	2	12,5	13	81,25	
		16	100							
Staining blood smear Total	Good	6	37,5	1	6,25	5	31,25	6	37,5	0,02*
	Poor	10	62,5	17	43,75	3	18,75	10	62,5	
		16	100							
Examining blood smear Total	Capable	11	68,75	8	50,0	3	18,75	11	68,75	0,63
	Incapable	5	31,25	4	25,0	1	6,25	5	31,25	
		16	100							

As shows in Table 1, from 16 Health Center microscopist tested, 9 microscopist prepared material and equipment completely and 7 microscopist prepared uncompletely. From 9 microscopist prepared material completely 1 have poor kappa score (below 0,6) and 8 have good kappa score, while from 7 microscopist prepared material uncompletely 4 microscopist have poor kappa score and 3 have good kappa score. These seem that the more complete they prepared material higher kappa score they get, however, these correlation is statistically not significant $P=0,192$). The correlation between the usage of used glass slide in preparing blood smear with the accuracy of malaria diagnosis was also assessed statistically, however these correlation is also not significant ($P=0,08$). Similar

result was also acquired when the corelation of ability to produced good thick blood smears and the capability in examining bloodsmear to the accuracy of malaria diagnosis. In general, the performace of the local Health Center microscopist are generally still low as evaluated from the completeness of material and reagen during preparation, the quality of material used, the ability to produce good quality of thick and thin blood smears, until the ability to identify malaria parasite by examining blood smear by using microscope. Statistically, some stages of performance had no significant correlation with the accuracy of diagnosis, P value > 0.05 . However, the ability to produce good thin blood smear and Giemsa staining showed statistically significant corelation between good thin smear preparation and staining quality with

the diagnostic accuracy value of $P = 0,04$ and 0.02 respectively.

The ability of local Health Center microscopist and the cross checker at District Health office to identify malaria parasite was assessed using standard malaria blood slide produced by Department of

Parasitology Faculty of Medicine, Universitas Gadjah Mada. The error rate was assessed using reference of the result of the standard blood slide reading of the certified microscopist from Department of Parasitology Faculty of Medicine, Universitas Gadjah Mada, and the result are shown in Table 2.

Table 2. Comparison of diagnosis result of local Health Center and District Health Office (cross checker) microscopist and compared to Reference Diagnosis of Microscopist from Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada.

Name of local Health Center	Microscopist Code	Diagnosis by local/cross checker microscopist		Comparison to Reference Diagnosis from UGM				
		(+)	(-)	True Positive	False Positive	True Negative	False Negative	Error Rate(%)
Biudufoho	A	15	5	15	0	2	3	15
Weoe	B	17	3	17	0	2	1	5
	C	13	7	13	0	2	5	25
	D	12	8	12	0	2	6	30
Weliman	E	16	4	16	0	2	2	10
	F	20	0	18	2	2	0	10
	G	14	6	14	0	2	4	20
Betun	H	18	2	18	0	2	0	0
	I	15	5	15	0	2	3	15
Kota	J	20	0	18	2	2	0	10
Seon	K	20	0	18	2	2	0	10
Atapupu	L	19	1	18	1	1	0	5
Nurobo	M	16	4	16	0	2	2	10
Umanaen	N	18	2	18	0	2	0	0
	O	17	3	17	0	2	1	5
Atambua Selatan	P	15	5	15	0	2	3	15
Cross checker	Q	16	4	16	0	2	2	10

From Table 2 it can be seen that the error rate of malaria diagnosis by microscopist from Belu Health Centers and district Health Office varies between 5-30% using standard malaria blood smears prepared from Universitas Gadjah Mada, with exception of the

microscopist from Health Center of Betun and Umanen which have error rate of 0%.

Assessment of the accuracy of malaria diagnosis of malaria patient by local microscopist in the Health Center was also done by comparing the reading of 5

blood slides/Health Center prepared from blood of 5 malaria patient visiting Health Center. The blood smears were processed and Giemsa stained and read microscopically by local microscopist, and the

result was compared to the reading result of the same slide by certified microscopist from reference Laboratory of Universitas Gadjah Mada. The result can be seen in Table 3.

Table 3. Error rate and accuracy of species identification upon 5 blood slides prepared and processed from 5 patient/Health Center by local microscopist as compared to reading result of certified microscopist from Universitas Gadjah Mada.

No	Local Health Center	Diagnosis by Local microscopist		No. of Blood smear	Diagnosis by Reference Laboratory			Species Accuracy (%)		Error Rate (%)
		Pf	Pv		Pf	Pv	Negative	Pf	Pv	
1	Biudukfoho	1	4	5	0	3	2	0	75	40
2	Weoe	1	4	5	1	4	0	100	100	0
3	Weliman	1	4	5	1	0	4	100	0	80
4	Betun	2	3	5	2	3	0	100	100	0
5	Puskot	1	4	5	0	0	5	0	0	100
6	Seon	1	4	5	0	0	5	0	0	100
7	Atapupu	1	4	5	0	0	5	0	0	100
8	Nurobo	1	4	5	0	0	5	0	0	100
9	Umanen	2	3	5	2	0	3	100	0	60
10	Atambua Selatan	1	4	5	1	1	3	100	25	60
Total		12	38	50	7	11	32	58,3	28,9	

Table 3 showed that the error rate of malaria diagnosis of 8 local/Health Center microscopist in diagnosing malaria patient ranged between 40-100%. However, there were 2 local clinics, Weoe and Betun, which did not do misdiagnosis with 0% error rate. The accuracy of identification of the species of *P. falciparum* reached up to 100% in five local clinics/Health Center, ie. Biudukfoho, Weoe, Betun, Seon and Umanen. On the other hand, there were five local clinics did misidentification of species *P. falciparum* 0%, whereas for *P. vivax* species identification ranged between 0-75%.

From a total of 50 malaria patient blood from 10 Health Center (5 sample from each Health Center), bloodsmear were prepared and Giemsa stained by trained staf from the Department of Parasitology. These blood slides were then used to assessed the competency of the cross checker from the Belu District Health Office and cpmpared to the refference result read by microscopist of the Department of Parasitology Faculty of Medicine, Universitas Gadjah Mada. The result can be seen in table 4.

Table 4. The accuracy of species identification and error rate of Cross-checker of the Belu District Health Office using 50 PCD blood slides prepared by trained staf of the Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada.

50 PCD blood slides		Diagnosis by Cross Checker			Refference Diagnosis of UGM			Species Accuracy (%)		Error Rate (%)
Positive	Negative	Pf	Pv	Negative	Pf	Pv	Negative	Pf	Pv	
15	35	8	33	9	7	8	35	46,7	53,33	52

Table 4 showed that the error rate of the cross checker at the Belu District Health Office in reading 50 PCD blood smear was as high as 52% as compared to the refference reading of the Department of Parasitology, Faculty of Medicine, Gadjah Mada University. On the otherhand the accuracy in species identification of both *P. faciparum* and *P. vivax* were also not so high, it was only 46.7% for *P. falciparum*, and 53,3% for *P. vivax*.

The high error rate in malaria diagnosis in many Health Center as well as the cross checker in the

District of Belu might be due to several factors that directly or indirectly related to the performance of the microscopist. Further study were also conducted to explore the possible factor that might affecting the performance of the microscopist. Several factors which were considered to directly influence the accuracy of microscopic diagnosis of the Health Center Microscopist in the District of Belu are seen in Table 5.

Table 5. The Bivariate analysis of the factors that might affected the Kappa value of the microscopist of the Health Center in the District of Belu.

No	Variable		Kappa value						P Value
			Poor		Good		Total		
			N	%	N	%	N	%	
1	Education	Low	1	5,8	2	11,76	3	17,6	0,19
		High	11	64,7	3	17,64	14	82,4	
2	Training	No	11	64,7	1	5,88	12	70,6	0,10
		Yes	1	5,8	4	23,52	5	29,4	
3	Number of Training	1 x training	0	0	3	60	3	60	0,080
		2 x training	1	20	0	0	1	20	
		3 x training	1	0	1	20	1	20	
4	Working Experience	≤ 3 tahun	8	47,0	0	0	8	47,1	0,029
		≥ 3 tahun	4	23,5	1	29,41	9	52,9	
5	Supervision	No	1	6,25	0	0	1	6,25	1,00
		Yes	11	68,7	4	25,0	15	93,75	
6	Availability of tools and materials	not available	9	56,2	4	25	3	18,8	0,52
		Availabel	3	18,7	0	0	13	81,3	
7	Cross Check	No	6	37,0	4	25	6	57,5	0,23
		Yes	6	37,0	0	0	10	62,5	
8	Working environment	No	5	31,25	1	6,25	6	57,7	1,00
		Yes	7	43,75	3	18,75	10	62,5	
9	Work load	No	1	6,25	0	0	1	6,3	1,00
		Yes	11	68,7	4	25,0	15	93,8	

In Table 5, most of the variables studied such as education, training, number of training, supervision, availability of tools and materials, working environment and workload showed no statistically significant relationship with the accuracy of diagnosis. However, variables working experience, has shown to contribute significantly on to the accuracy of malaria diagnosis of the Health Center microscopist in the District of Belu $P = 0.029$.

Microscopy is at the moment still represent the gold standard for malaria diagnosis. This method is based on the characteristic morphology of each stages of the parasite which inhabited intracellularly within the red blood cells. All of these specific morphology are easily been identified in the well prepared thick and thin blood smears which have been stained properly using Giemsa solution with range of pH between 7,2-7,5. The accuracy of malaria diagnosis using microscopy conducted by well trained microscopist is considerably high. This methode is also not only cheap but also have high specificity and can be conducted at any laboratory condition, even in laboratory without electricity. However, there are some weakness such as the processing in time consuming, and the limmitation on the number of blood slide examined in a given time. The accuracy will be decreasing when the microscopist already exhausted due to too many blood slide to be examined. In order to have accurate reading, the average number of slide to be examined dayly is about 50-60.

The accuracy of malaria diagnosis is influenced by many factor that contribute to the physiological and psychological condition of the microscopist. The microscopist competency in preparing blood smears and conducting staining procedure are also very important since this proces will directly contribute to the quality of the blood smear and the clearness of parasite morphology in the thick as well as in the thin blood smears³.

As can be seen in the table 1 that most of the microscopist in Belu Heath Center was incapable in preparing tools and materials prior to the proces of blood smear production, such as they did not prepare alcohol swab, glove and clean glass slide. Alcohol swab is very important in preparing malaria blood smear because in addition to as for disinfectantion, alcohol swab can also be used to clean the finger of the patient from dirt and grease that might stucked on the glass slide and resulted in a bad quality of blood smears⁹.

During Giemsa staining proces, majority of microscopist use ordinary water as as dilution buffer that produce pH level of below 7.2. This condition resulted in the quality of the staining become, usually the color of the blood smear become too redish or too blueish. In addition to the pH level of the water, staining quality was also influenced by the staining time. If it was too fast or too slow, the result might had less colour¹⁰. The condition of the microscope is also very important. Some of microscope do not have appropriate (100x) magnification lenses, or if it have sometimes the lense is not so clear and difficult to identifiythe parasite. Althoughthe statistical analysis showed not significant, but one of the key for accurate microscopic reading is the usage of good quality and proper microscopic lens magnification¹¹.

To assess the accuracy of the malaria microscopic diagnosis by local/Health Center microscopist, 2 types of blood smear were used. As shown in table 2 when standard malaria blood smears were used the error rate of malaria diagnosis was ranged between 5-30% as compared when blood smear from the PCD were used, the error rates were ranged between 40-100%. Similar result were also shown when cross checker from Belu District Health Office was tested. Using standard malaria blood smear showed error rate of the cross checker is only 10%, and while when using blood slide prepared by Health Center microscopist from patient during PCD, as shown in Table 4, the error rates of

the cross checker was as high as 52%. These data indicated that the quality of malaria blood smear they produced were not so good, and contributed to the high error rate of malaria diagnosis in those area. However, two Health Center i.e. Betun and Umanen have a good quality of microscopist. Training or refreshing of malaria microscopy as well as regular supervision from the provincial or district levels may also important to maintain the performance of microscopist in each Health Center.

The result of the error rate of malaria diagnosis using blood smear prepared by microscopist from patient during PCD tend to be higher, this may be caused due to an error on the beginning of blood slide processing and preparing the blood smear, the procedure of fixation and drying and eventually the procedure of Giemsa staining. The proper procedure for malaria blood smear preparation and staining should be followed in order to have high quality of malaria blood smears and high accuracy of malaria diagnosis¹¹.

The accuracy of microscopic species identification of health center microscopist and district cross checker was reached up to 100% for *P. falciparum* in 5 Health Center and 0% in other 5 Health Center, while for *P. vivax* the accuracy of 2 Health Center were 100%, 1 Health Center 75% and the other 7 Health Center were 0%. Other similar result was found also in Kalimantan and Sumatra where the error rate of microscopist in identifying species of *P. vivax* tended to be higher. These might because by the morphology of *P. vivax* is more diverse, and sometimes microscopist often misdiagnosed artifacts as positives of *P. vivax*. Several artifacts are always found such as blood components (leucocytes, erythrocytes, platelets), bacteria, spores, plant cell, and Giemsa crystal¹⁰.

Beside the competency of health center microscopist in species identification of the parasite, several other factors may also directly or indirectly influenced the accuracy of diagnosis. The level of

education, number of training attended, working experience, frequency of supervision, quality of tools and materials, routine cross checking of the blood smear examined, working environment, and working load were considered to have some contribution in the accuracy of malaria diagnosis. However, when the data was statistically, as shown in Table 5, the influence of most of those factor was statistically not significant except for the working experience. Similarly, studies in Kalimantan also reported the statistically non-significant influenced of some factor on the malaria microscopic diagnostic accuracy carried-out by local health Center microscopist in Kalimantan and also in Jepara¹².

The observed data showed that most of Health Center microscopist have education level of D1-DIV of health analysts. This level of education is in accordance with the WHO recommendation for the selection process for malaria inspectors¹⁰.

As shown in Table 5, most of the Health Center microscopist in District of Belu have not been receiving any microscopy training. Only one out of 12 microscopist have been sent for training and the other 11 never received any microscopic training. Furthermore, there is no statistically significant correlation between training and the accuracy of malaria diagnosis ($P > 0,05$). Similar finding was also reported from study in Ogan Komiring Ulu, South of Sumatra, which also indicating of non-significant correlation between training experience and accuracy of malaria diagnosis by the local Health Center microscopist¹². In the reality in the field the competency of health center microscopist is varies, and this seem to be due to multi factor. Similar condition also seen in the cross checker at the District levels. However, higher performance or competency or in other words lower error rates in cross checker was also noticed as compared to Health Center microscopist. This condition is easily been accepted since the cross checker positions are usually allocated for the more experienced personal¹⁰.

Working experience/working period as microscopist in this study were classified into two, ie, below 3 years (≤ 3 years) and over 3 years (≥ 3 years) of working experience. There was a microscopist that had been working for ≥ 3 years but he had poor Kappa agreement value. However, when all data were statistically analysed, working period had a statistically significant correlation with the accuracy of malaria diagnosis by health center microscopist in the district of Belu. This data is in the contrary with the condition reported from Kalimantan and Jepara where there was no significant correlation between working period and the accuracy of diagnosis. The difference may reflect the variation of the microscopist status, competency, education status, social-economic condition and working environment that might influenced their daily activities and the accuracy of malaria diagnosis. However, Level of education and years of service were represent two of the basic considerations in determining need for training activities which would be given to each microscopist^{10,13}.

Supervision or inspection by cross checker at the district or provinsial level are intended to evaluate and to control the quality of the local microscopist performance. Routine supervision from higher outhority personel was supposed to give better guidance and motivation to keep-up and enhanced their performance. The data in this study showed that fifteen (15) out of 16 (93,75%) of health center microscopist in this study stated to have routine supervision from the district or provinsial levels, however, the accuracy of malaria diagnosis still low and the kappa value of 11 microscopist (68,7%) is poor as compared against refference of the certified microscopist from Department of Parasitology Faculty of Medicine, Gadjah Mada University. Therefore, it could be concluded that supervision or inspection that was conducted by the district officers did not give a significant changes to the microscopist performance in the local health center level⁸.

Availability of tools and reagents (stock of tools and materials) was consider as complete for malaria examination in each local health center, but 85% of them had not been adequate in terms of quality and quantity. This parameter was no statistically significant relation to the accuracy of blood smear reading. Similar result was also showed from study in Ogan Komiring Ulu, South of Sumatra which also indicated that the quality of the tools and reagent hane no significant correlation with the accuracy of malaria diagnosis¹². Availability of tools and materials such as binocular microscope, a clean glass slide, sterile lancet, immersion oil, drier basin, slide box, alcohol, and Giemsa are a pre-requisit for good malaria diagnostic laboratory, and should meet the international standards^{8,1}.

Cross Check of the malaria blood slide reading is an alternative method to monitor the ability of the local microscopist personel. The standard procedure is by re-examining or checking all the positive slide read by local/health center microscopist plus 10% of negative slides¹. The purpose of this cross-examination is beside to monitor the quality of first diagnosis, but hopefully also as a method to stimulate the local microscopist to maintain and keep-up their performance¹. Evaluating the microscopist competency can also be done by re-freshing to examine malaria positive blood smears with various parasite density as recomended by WHO (2005). It was expected that excersize by repeated reading of blood smear with low parasite density would be able to stimulate the sensitivity of the microscopist in identifying parasites at the low density.

Working environment in this study is also had no statistically significant relationship with the accuracy of malaria diagnosis. However, working environment are known to have effect physiologically and psychologically on the power and capacity of individuals in executing their task. A good working environment include adequate lighting (natural sun lighting during day time, orelectric light),

the availability of clean water ergonomic tables and chairs to ensure the comfortable working environment^{1,8}.

Workload and work status of the microscopist personels is a common problem in the public health services especially in East part of Indonesia. Multi-tasking personel in a certain ublic health services are usually as a result of the general shortage of man power. These condition is still very common in many part of the country, especially in very remote areas with limited social and economic resouces. Therefore a large number of task must be excecuted by a limited number of personel, and consequently one task might be excecuted by non-profecional personel and this will eventually resulted in the low or sub-optimal output. Other simillar condition is the job-mutation for the microscopist personal. Sometimes a welltrained microscopist from certain health center in the rural areas are mutated to other more urban places which the job has no relation et all to microscopy. This common problem will resulted in the empty personal in the microscopist position in one place and improper competency personel in other places, and these problem should be a high priority in malaria endemic area⁸.

CONCLUSIONS

The accuracy of malaria diagnosis by microscopist in the Health Center in District of Belu is low. The factor that might contribute is the ability of the microscopist to produce a good quality of malaria blood smears. Error rate of the Health Center microscopist in identifying malaria parasite in standard malaria bloodsmear varies from 20-80%, while the cross checker is 0-10%. Working experience of the health center microscopist is statistically significant to correlate with accuracy of malaria diagnosis.

RECOMMENDATION

The competency of malaria microscopist in the health center in the District of Belu to identify malaria parasite in the blood need to be improved. Training activities need to be conducted periodically to improve the ability of of the microscopist to prepare thick and thin bloodsmears and properly stained with Giemsa solution and to improve the ability to identify malaria parasite in bloodsmear preparation. A high quality of microscope and reagent are pre-requisit for accurate malaria diagnosis in the District of Belu.

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