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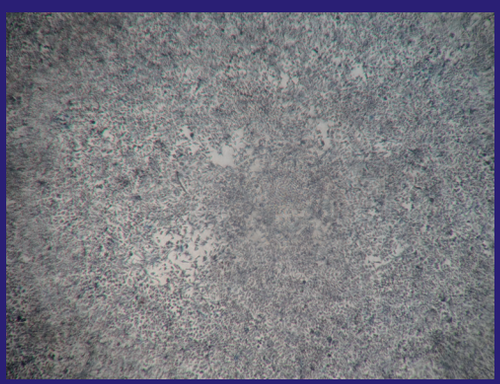

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Research Report

DIVERSITY, DOMINANCY, AND PERIODICITY OF MOSQUITOES IN FILARIASIS ENDEMIC AREAS IN SAMBOREJO VILLAGE TIRTO DISTRICT PEKALONGAN REGENCY

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ABSTRACT

Vector-borne mosquito diseases are still as a public health problem in the world, including in Indonesia. Many of mosquitoes species are significantly as vectors of patogen, such as virus, bacteria, protozoan, and helminths due to human health. Samborejo Village is one of filariasis endemic areas and it is still in a high microfilaria rate Each of mosquito species has a differential of distribution, bioactivities pattern, and type of habitat of their breeding sites with others. The objective of this study was to determine the diversity, dominancy, and periodicity pattern of mosquitoes during night time in Samborejo Village Tirto District Pekalongan Regency. Mosquitoes collections were done by landing method, from 6 pm - 6 am of in an hour period of collection, for biting and resting activities and also for indoor and outdoor collection respectively. Mosquitoes were then identified and the diversity was analized by Shannon-Wienner Index. The total number of each species was served in percent. Totally there were 339 collected mosquitoes, consisting of 165 (48.67%) females and 174 (51.33%) males. Of all, there were 4 species identified which were Culex quinquefasciatus *(92.1%),* Culex tritaeniorhynchus *(0.6%),* Culex vishnui *(1.8%), and* Aedes aegypti *(5.5%). Samborejo Village showed in low diversity with the index of 0.338, and Cx.* quinquefasciatus *to be the dominant species in this area.* Culex quinquefasciatus *also became the frequent species in each period of collection for indoor and outdoor, and it showed the indoor active biting at 9 pm, 01 am, and 03 am; furthermore, the outdoor active biting was at midnight (00) and at 03 am. However,* Aedes *mosquito was showed active biting in earlier, it was at 6 pm, 7 pm, and at 02 am.*

Keywords: *diversity, dominancy, mosquito, periodicity, Samborejo Village.*

ABSTRAK

Penyakit tular vektor oleh nyamuk masih menjadi masalah kesehatan masyarakat di dunia termasuk di Indonesia. Berbagai jenis nyamuk memiliki peran sebagai inang perantara (vektor) dalam penularan agen penyakit baik dari jenis virus, bakteri, protozoa, hingga cacing, dan Kalurahan Samborejo adalah salah satu daerah endemik filariasis di Kabupaten Pekalongan dengan tingkat mikrofilaria yang masih cukup tinggi. Pola distribusi, keragaman, perilaku setiap nyamuk beserta lingkungannya merupakan kajian yang masih sangat terbatas informasinya. Tujuan penelitian ini adalah menjelaskan keragaman, dominansi, dan pola aktivitas nyamuk pada malam hari di Kalurahan Samborejo Kecamatan Tirto Kabupaten Pekalongan. Koleksi nyamuk dilakukan dari pukul 18.00 - 06.00WIB pada periode setiap satu jam, dengan metoda landing biting, dan nyamuk dikoleksi pada saat biting dan resting baik indoor maupun outdoor. Nyamuk hasil koleksi diidentifikasi dan data keragaman dianalisis dengan Shannon-Wienner Index, data jenis dan jumlah nyamuk ditampilkan dalam persen. Sejumlah 339 ekor nyamuk dikoleksi terdiri atas 165 ekor (48.67%) adalah nyamuk betina dan 174 ekor (51.33%) adalah nyamuk jantan, dengan jenis Culex quinquefasciatus *(92.1%),* Culex tritaeniorhynchus *(0.6%),* Culex vishnui *(1.8%), dan* Aedes aegypti *(5,5%). Kelurahan Samborejo menunjukkan keragaman nyamuk yang rendah, dengan nilai index 0,338, dan nyamuk*

Cx. quinquefasciatus *menunjukkan dominansi dan dapat ditemukan pada setiap periode koleksi baik indoor maupun outdoor. Pada periode waktu pukul 21.00, 01.00 dan 03.00 WIB nyamuk* Cx. quinquefasciatus *menunjukkan puncak periode aktif indoor, sedangkan puncak aktivitas outdoor adalah pada pukul 24.00 dan 03.00 WIB. Untuk nyamuk* Ae. aegypti *dapat ditemukan pada periode lebih awal yaitu pukul 18.00, 19.00, dan pukul 02.00 WIB.*

Kata kunci: *Diversitas, dominansi, periodisitas, nyamuk, Kalurahan Samborejo.*

INTRODUCTION

Mosquitoes have been known as one of haematophagus insects and some of them can serve as vector for transmitting the protozoan, worms, or virus, agent of human diseases in many parts of the world, including in Indonesia.[1,2] Lymphatic filariasis (LF) is one of public health problems, caused by one of 3 species of filarial nematodes, namely, *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori.* The microfilariae are found in the human blood only around midnight.[3-4] It is transmitted by mosquitoes and globally about 120 million people in 73 countries have been affected.[1,2,4,5] In Indonesia, there is an increasing number of lymphatic filariasis endemic areas in which filariasis cases are increased significantly. It was about 8,000 cases in the year 2004 and to more than 14,900 cases in 2016.[6-8] Study by Febrianto[9] found that there was 7.6% from 76 of respondents showed the positive microfilaremia in Samborejo Village.

Some mosquitoes are cosmopolitan and live nearby human habitations.[10] In Indonesia, 20 mosquitoes species have been known as filariasis vectors, 8 species of *Anopheles* spp., 3 species of *Culex* spp., 6 species of *Mansonia* spp., one species of *Aedes*, *Armigeres,* and *Coquillettidea*,[1] and some of them have biting behaviour in the night time.[11] The nocturnal active biting of vector could be considered as an intermediary for filariasis transmission. Studies on the distribution, behavior, and ecology of mosquitoes in filariasis endemic areas are significant studies in studying parasitic-vector interactions and are studies of novelty that are still very limited in information.[12]

Samborejo Village is located in Pekalongan Regency Central Java and the ecotype is a semiurban areas. This village was an endemic area for urban filariasis caused by *Wuchereria bancrofti*.[9] However, there is unwell known in the distribution, species variation, dominancy and periodicity of mosquito population in Samborejo Village Pekalongan Regency. The purpose of this study was to examine the diversity, dominancy, and active period of mosquitoes during night time in Samborejo Village.

MATERIAL AND METHOD

Materials

Mosquitoes were the subjects of this study. The materials for mosquitoes collection were aspirator, hand net, paper cup/coffee cup with gauze covered, and cotton with sugar solution. Microscope, cover glass petridish, and small brush were provided for mosquitoes identification.

Methods

The study was under consideration with the Ethical Commission of Faculty Medicine Gadjah Mada University, number REF: KE/FK/0612/EC/2017. *Study area*. Samborejo Village in Tirto District Pekalongan Regency was selected as the study area. It was semiurban types for the settlement.

Mosquitoes Collection. Mosquitoes collections were done every hour from 6 pm - 6 am by Landing Bite method and mosquito's net in the selected house of the area. Mosquitoes were put in paper cup covered by gauze. Then, mosquitoes were identified by using identification key book, Mosquito Pictorial Key of Indonesia.[13] Data were tabulated and analyzed. The environmental parameter, air temperature (ºC) and humidity (%), of selected location of settlement were measured.

Data analysis. The number of mosquitoes from the study area was performed in percent. The diversity of mosquito species was analyzed by Shannon Weinner Diversity Index.[14] Correlation analysis of ecological parameters with the active period of the mosquitoes was done by Correlation Test (SPSS 20. version).

RESULT AND DISCUSSION

Diversity

From Samborejo Village, totally there have been 340 collected mosquitoes that consisted of 175 males and 165 females (Table 1).

Based on the Shannon-Wienner Index Samborejo Village was low in diversity (0,338). It was understood that there were only 2 genera, *Culex* and *Aedes* has collected. This finding was similar to our previous study in other the endemic areas, in Pasirsari Villages (Pekalongan City) and Simbangkulon Villages (Buaran Distric Pekalongan Regency). In the both areas the total number of 323 adult female mosquitoes had been collected and consisted of 8 species of the genus *Culex* and 1 species of *Aedes aegypti.*[15] However, in Jenggot Village (Pekalongan City) it is showed moderate diversity and more various the mosquitoes genus, which were 5 of mosquito genera, which were genera of *Culex*, *Aedes*, *Armigeres*, *Anopheles*, and the genus of *Lutzia.* This finding is different from the results of the research conducted by Arimurti (2008) which concluded that the *Cx. quiqueefas ciatus* mosquito from Pekalongan

Table 1. Diversity and total number of female mosquitoes in Samborejo Village Tirto District Pekalongan Regency.

Species	Number of Mosquitoes				Total	%
	Indoor Biting	Outdoor Biting	Indoor Resting	Outdoor Resting		
Culex quinquefasciatus	61	49	7	35	152	92.12 %
Culex tritaeniorhynchus	0	1	0	0	1	0,60 %
Culex vishnui	1	1	0	1	3	1,83 %
Aedes aegypti	8	0	0	1	9	5,45 %
Total Number of Mosquito					165	100 %

city and Regency showed a high level of diversity with polymorphism reaching 100% based on the RAPD-PCR method.[16]

The *Culex* and *Aedes* are *culicinae* mosquito, they were belong to *Culicidae* family and the order of Diptera. Morphologically the adult female mosquito in the genera of *Culex* and *Aedes* may distinguished mostly by the head and thorax character. In both genera, at the head the *palpus* were shorter than the proboscis, however in the *scutum* of thorax's *Culex* mosquito covered pale brown scales and in *Aedes* the scales usually dark and sometimes with contrast white scales.[13] The adult female *Cx. quinquefasciatus* may be recognized easily with other *Culex* species (*Cx. tritaeniorhynchus* or *Cx. vishnui*) by the dark brown without pale band of the proboscis and the terga of abdomen was dark with broad white basal bands.[13]

Geographically, mosquito is cosmopolite insect, and it widespreads in the tropic and subtropic region,[10] and the changing of the environment will affect the insect activity in which will have an impact on diversity and distribution.[1,4,10]

Dominancy and Periodicity

Culex quinquefasciatus to be the dominant in the area of study (92.12%). The dominancy this mosquito also is showed in the different endemic areas in Pekalongan, such as 66.67% in Pekalongan Selatan District[17], 97.3% in Buaran District[18], and in Samborejo the density was 5.25 mosquito/human/ hour.[9] As in Table 1, *Cx. quinquefasciatus* was abundant and it was the most frequent active in each of period of collection for indoor and outdoor collection. Every mosquito species has its own distribution, behaviour pattern and character of its habitat different from others. The daily behaviour pattern of mosquito activities will occur at day or night time depending on the species.[19] There was limited information in the periodicity of biting and resting behaviour of *Cx. quinquefasciatus,* especially in filariasis endemic areas. This study will improved the collecting data of its bionomic.

In this study, the period of collection from 6 pm-6 am showed that only *Cx. quinquefasciatus* was the most frequent active, and the indoor activities was rather higher than the outdoor ones (Figure 1). Resting activities was showed the earlier period of collection, it was around 9 pm for indoor and 7 pm for outdoor (Figure 2). This phenomenon may be due to the moving of this mosquitoes from the breeding sites to the houses before they get for the source of blood.

This mosquito was mostly active in outdoor biting and the period of active was at 01 and 4 am[18], while in Pasir Sari Village Pekalongan Selatan District the peak of active biting period was at midnight and around 02 pm.[15] This peak timing of the mosquito periodicity may synchronous with the abundant of mf in the peripheral blood.

As Sack[20] has said that the secretion of overnight host melatonin may introduce the release of *W. bancrofti* mf in the blood circulation. The host's melatonin profile study is revealed that there was significantly increased and the peak of the concentration was at 0 - 4 am. Besides that, the total mf also significantly increased from about 82% at 22 pm and reached out 100 % at 1 am. At the next period the total number of mf was still high, it was around 98% and 80% at 2 am and 3 am respectively, then it was drastically reduced, from 62% at 4 am to 2% at 10 am.[20]

The study in the some filariasis endemic areas in Pekalongan is showed that *Cx. quinquefasciatus* caughted more frequently during the time of collection rather than other species. As in Pasirsari Village, in Pekalongan Selatan District Pekalongan City and in Paweden Village Pekalongan Regency is showed that 76%, 57.7% and 98.9% respectively were *Cx. quinquefasciatus*.[15,18,19]

The mosquito existence was important for transmitting the parasite. Some studies was reported that the environment around settlement and human activities in the night time had significant effect for increasing the risk factor of people to get the infection.[9,19] Windiastuti *et al*.[17] has said that the existence of breeding places, resting places around houses will increased the risk of infection 8.7 times and 2.17 times respectively, even it would be 9.03 times to get infection for people who has active in the night time. This study is revealed that on the period of midnight to 4 am was the active peak of *Cx. quinquefasciatus* (Figure 1B).

There was new finding that *Ae. aegypti* mosquito was active indoor in the early night, it was about at 6 pm and at 2 am in early morning (Figure 1A). Other species, *Cx. vishnui* and *Cx. tritaeniorhyncus* were known as Japanese Encephalitis (JE) virus.[2,4] In this area the occurrence proportion of the two species was low. This may because of they more like the animal rather than human as their source of blood, such as chicken and domestic birds.

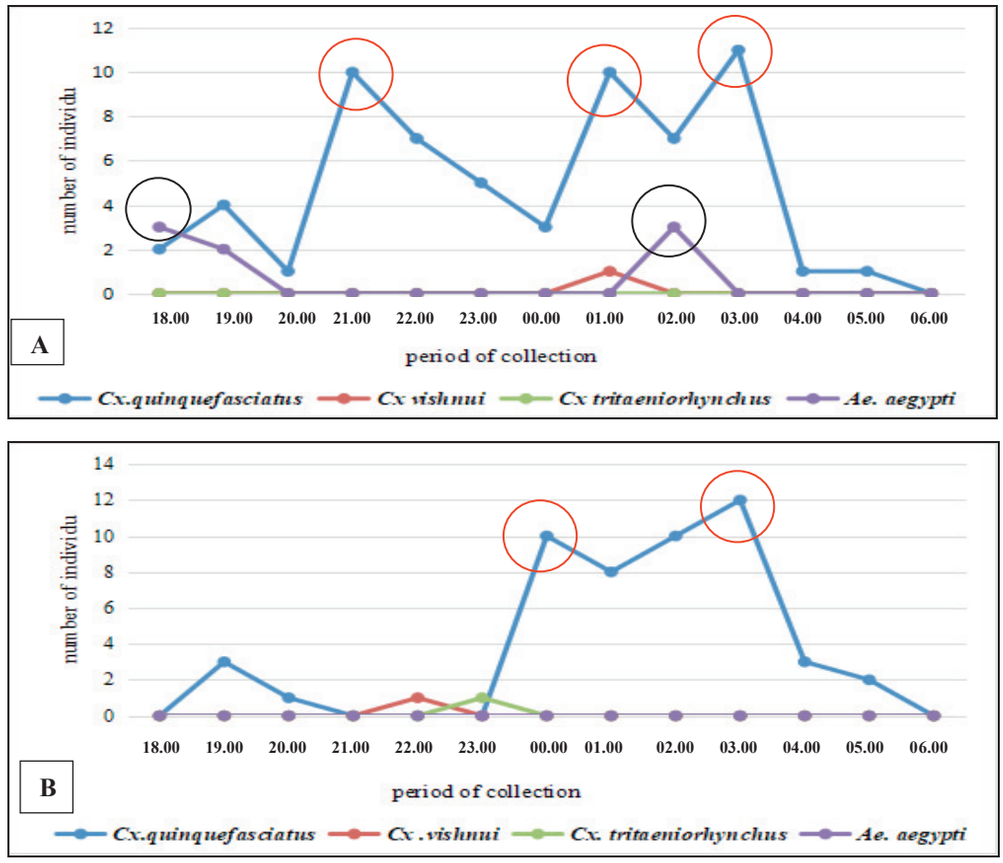

Figure 1. Mosquitoes diversity and periodicity of indoor (A) and outdoor (B) biting activities in Samborejo Village.

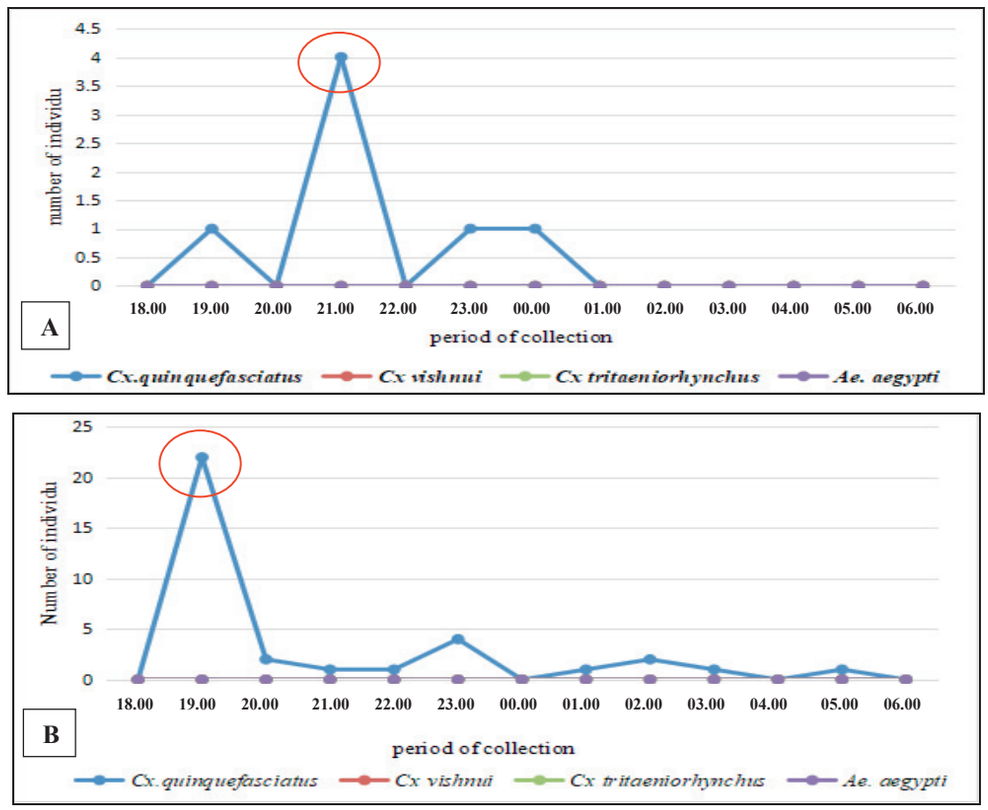

Figure 2. Mosquitoes diversity and periodicity of indoor (A) and outdoor (B) resting activities in Samborejo Village.

Ecological Parameter

In this study the ecological parameter affected in various indoor and outdoor activities of mosquitoes, especially for *Cx. quinquefasciatus* both for the biting and for the resting activities (Table 2).

Table 2. Correlation of ecological parameter to the activities of mosquito in Samborejo Village Tirto District Pekalongan Regency**.**

Species	Level of coefisien Correlation			
	Indoor Biting	Outdoor Biting	Indoor Resting	Outdoor Resting
Temperature (°C)	0,0663	-0,484	0,414	0,384
Humidity (%)	-0,513	0,398	0,150	-0,437
Wind Velocity	-0,977*	-0,107	0,017	0,801

Each mosquito species needs specific ecological requirement for their survival, distribution, and abundance.[4,21] There was limited information of the effects of temperature and humidity to the mosquito activities in such filariasis endemic areas. Moise *et al.*[21] has stated that *Cx. quinquefasciatus* abundance increased significantly in May and June annually as their observed in 2006 to 2010, and temperature was positively affected for the mosquito abundance.

As shown in Table 2, there was no correlation among the temperature to the indoor biting, humidity to the indoor resting, and wind velocity to outdoor biting and indoor resting. Low correlation in positive and negative was showed by the temperature, humidity to the indoor or outdoor, and to biting and resting activities. However, there was a strongly negative correlation of wind velocity to the indoor biting activities and strong positive correlation to the resting activities. It is said that by increasing the wind velocity will significantly reduce the indoor biting activities. However, it significantly will increase the number of mosquitoes to rest.

For comparison, the study in Paweden Village Pekalongan Regency is showed that there was moderate negative correlation (-0.50 and -0.64) between temperature and the *Cx. quinquefasciatus* mosquito for indoor and outdoor biting activities. It was mean that as increasing temperature will reduce the indoor and outdoor biting activities, however the humidity has strong positive correlation (0.75) to the activities.[18]

Based on the results, the abundance and periodicity were likely match with the microfilaria in the blood of patient. This condition will maintain the high risk of filariasis transmission. For that purpose, vector surveillance should be considered to gain the filariasis elimination program together with Mass Drug Administration (MDA).

CONCLUSION

This study is revealed that Samborejo Village showed low diversity (0,338) of mosquitoes fauna, and *Culex quinquefasciatus* to be the dominant species in this area. The indoor biting activities of *Cx. quinquefasciatus* were at 9 pm, 01 am, and 03 am; furthermore, the outdoor biting activities were at midnight (00) and at 03 am. However, *Aedes* mosquito was showed active biting in earlier periods, which were at 6 pm, 7 pm, and at 02 am.

CONFLICT OF INTEREST

There was no conflict of interest for this paper.

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REFERENCES

1. Sutanto I, Ismid IS, Sjarifuddin PK, and Sungkar S. Parasitologi Kedokteran. Edisi ke 4. Badan Penerbit FK UI. p: 245-279. 2013
2. Vythilingam I. *Mosquitoes of Public Health Important:* Mosquitoes and Public Health. Labert Academic Publisher. pp: 80. 2016.
3. Manguin S, Mouchet J, and Carnevale P. Main Topics in Entomology: Insects and Disease Vector, Green Trends in Insect Control, Chapter 1. ed: Lopez, O and Fernandez-Balanos J. RSC Green Chemistry No. 11. Royal Society of Chemistry. www.rsc.org. p: 1-16. 2011
4. Fenwick A. Accelerating progress towards elimination of some Neglected Topical Diseases (NTDs). International Congress on Parasitology (ICOPA). 2018.
5. WHO. Elimination of Lymphatic Filariasis in the South-East Asia Region. Reports of the 8th meeting of the Regional Programme Review Group, Srilanka, 28-29 April, 2011.
6. Aditama TY. Control Policy NTD’S in Indonesia. Asean Seminar on Neglected Tropical Diseases Committee Report. Jakarta 28-29 September. 2012.
7. Kemenkes RI. Filariasis di Indonesia. Buletin Jendela Epidemiologi. Pusat Data dan Surveilans Epidemiologi Kemenkes RI., vol 1. ISSN 2087-1546. Juli 2010.
8. Kemenkes RI. Peraturan Menteri Kesehatan RI no. 29 tahun 2014 tentang Penanggulangan Filariasis. 190 hal. 2015.
9. Febrianto B, Maharani AIP, dan Widiarti. Faktor Resiko Filariasis di Desa Samborejo Kecamatan Tirto Kabupaten Pekalongan Jawa Tengah. Bul. Penel Kesehatan, Vol.2036, No. 2, pp: 48-58. 2008.
10. Rahman GMS. Manholes as an important breeding sites for *Culex* mosquitoes in Gazipur City Corporation, Bangladesh. *Bangladesh J. Zool*. 45(2): 139-148. 2017.

11. Astuti UNW, Hadisusanto S, dan Sari IY. Periodisitas Nyamuk *Culex quinquefasciatus* Say, 1823 dalam Hubungannya dengan Potensi Transmisi Filariasis Di Kelurahan Ngampilan dan Notoprajan Kecamatan Ngampilan Yogyakarta. Seminar Nasional Biologi. Yogyakarta. pp: 1108-1116. 2010.
12. Takken W and Koenraadt CJM. Ecology of Parasite-Vector Interaction, Ecology and Control of Vector-Borne Diseases 3, DOI: 10.3920/978-90-8686-744-8_1. Wageningen Acad.Publ. 2013.
13. DepKes RI. Buku Identifikasi Nyamuk di Indonesia. 2015.
14. Ismaini LM, Lailati, Rustandi D, Sunandar. Analisis komposisi da keanekaragaman tumbuhan di gunung Dempo, Sumatera Selatan. Pros Sem Nas Masy Biodiv Indon (1):1397-1402. 2015.
15. Astuti UNW, Poerwanto SH, Handayani NS, and Hadisusanto S. Abundance and periodicity of *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) as early indicator for filariasis transmision in Pekalongan Central Java Indonesia. AIP Conference Proceedings.1744.020045.doi:10.1063/1.4953519. 2016.
16. Arimurti ARR. Keanekaragaman Genetik Nyamuk Vektor Filariasis *Culex quinquefasciatus* Say. 1823 (Diptera: Culicidae) di Kota dan Kabupaten Pekalongan dengan Metode PCR-RAPD. The Journal of Muhammadiyah Medical Laboratory Technologist. 2008. 1(2): 42-61.
17. Windiastuti IA, Suhartono, and Nurjazuli. Hubungan Kondisi Lingkungan Rumah, Sosial Ekonomi, dan Perilaku Masyarakat dengan Kejadian Filariasis di Kecamatan Pekalongan Selatan Kota Pekalongan. Jurnal Kesehatan Lingkungan Indonesia. Vol 12 (1): 51-57. 2013.
18. Astuti UNW and Jasmi RA. Periodisitas Nyamuk Nokturnal di daerah endemik filariasis di Pekalongan Jawa Tengah. Laporan Penelitian, Hibah Penelitian Biodiversitas Tropika Dosen Untuk Pengembangan Materi Pembelajaran. Fakultas Biologi UGM. 2015.
19. Price PW, Denno RF, Eubanks ND, Finke DI, and Kaplan I. Insect Ecology: Behaviour, Populations and Communities. Cambridge University Press. 2011.
20. Sack RL. Host Melatonin secretion is a timing signal for the release of W. bancrofti microfilaria into the circulation. Medical Hypotheses, Elsevier, 73: 147-149. 2009.
21. Moise IK, Riegel C, and Muturi EJ. Environmental and social-demographic predictors of the southern house mosquito *Culex quinquefasciatus* in New Orleans, Louisiana. Parasites & Vectors, 11:249.2018.

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Research Report

PREVALENCE, INTENSITY AND RISK FACTORS OF SOIL TRANSMITTED HELMINTHS INFECTIONS AMONG ELEMENTARY SCHOOL STUDENTS IN NGIS VILLAGE, KARANGASEM DISTRICT, BALI

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ABSTRACT

Soil Transmitted Helminths (STH) infection is one of health issues in Indonesia based on social and environment problems. It is classified as neglected disease. The Indonesian government already has STH eradication program, but it is not supported by evaluation and monitoring program. The purpose of this study is to determine the prevalence and relation of each risk factors related to STH infections in elementary school in Ngis village, Karangasem regency, Bali. The study was done by analytical description using cross sectional study. Samples were selected from population based on inclusion and exclusion criteria. Primary data about suspected risk factors were collected using questionnaire. Diagnosis was established using Kato-Katz modification method. Data were analyzed using chi-square with confidence interval 95% or p value ≤ 0.05 categorized as significant. 138 students was enrolled in this study, the median age is 9 (6-13) years. The prevalence of STH infections is 10.1% with 78.6% is single infection of Trichuris trichiura *and 21.4% mixed infections. The proportion of STH infections in males (64.3%) is higher than female (35.7%) but it is statistically non significantly different. STH infections have significant relationship with some risk factors such as unwashed hand after defecation, unwashed hand after playing with soil, barefoot, uncut nails and dewormed. The highest risk factor of STH infections in Ngis village is not having available and proper latrine. (OR=33.9; 95%CI=5.749-199.769). The prevalence of STH infection is quite high with mild to moderate intensity and risk factors namely low hygiene and limited latrines. The implementation of monitoring and evaluation can be an effort to control risk factors and stop the STH transmission chain.*

Keywords: *Elementary School, Intensity, Kato-Katz Modification, Risk Factors, Soil Transmitted Helminths (STH).*

ABSTRAK

Soil Transmitted Helminths (STH) adalah salah satu masalah kesehatan di Indonesia berdasarkan aspek sosial dan lingkungan yang digolongkan sebagai penyakit terabaikan (neglected disease). Pemerintah saat ini telah melaksanakan program eradikasi, namun tidak didukung dengan tahap evaluasi dan monitoring (monev). Penelitian ini bertujuan untuk menentukan prevalensi dan hubungan antara faktor risiko dengan kejadian infeksi STH pada siswa sekolah dasar di Desa Ngis, Karangasem, Bali. Penelitian ini dilakukan dengan metode deskriptif analitik menggunakan studi cross-sectional. Sampel dipilih dari populasi berdasarkan kriteria inklusi dan eksklusi. Data primer mengenai faktor-faktor risiko yang dicurigai dikumpulkan dengan menggunakan kuesioner tervalidasi. Diagnosis ditegakkan menggunakan metode Kato-Katz modifikasi. Analisis data menggunakan chi-square dengan tingkat kepercayaan 95% atau dikategorikan sebagai signifikan apabila nilai p ≤0,05. Sebanyak 138 siswa berpartisipasi dengan median usia 9 (6-13) tahun. Prevalensi kejadian infeksi STH yakni 10,1%, dengan 78,6% infeksi Trichuris trichiura *dan 21,4% infeksi campuran. Infeksi STH dominan terjadi pada laki-laki (64,3%) daripada perempuan (35,7%) tetapi secara statistik tidak bermakna. Kejadian infeksi STH memiliki hubungan yang signifikan dengan faktor risiko seperti; tidak mencuci tangan setelah buang air besar, tidak mencuci tangan setelah bermain tanah, tidak memakai alas kaki, tidak memotong kuku dan minum obat cacing secara rutin. Faktor risiko tertinggi kejadian infeksi STH pada*

siswa di Desa Ngis adalah tidak adanya ketersediaan jamban (OR=33,9; IK%95=5,749-199,769). Prevalensi infeksi STH tergolong cukup tinggi dengan intensitas ringan – sedang dan faktor risiko yaitu rendahnya higienitas dan keterbatasan jamban. Pelaksanaan monev dapat menjadi upaya untuk mengontrol faktor risiko dan menghentikan rantai transmisi STH.

Kata kunci: *Faktor Risiko, Infeksi STH, Intensitas, Kato-Katz Modifikasi, Sekolah Dasar.*

INTRODUCTION

Soil Transmitted Helminths (STH) infection is one of the health issues in Indonesia that has environment and social basis.[1] Inadequate sanitation, poor economic conditions, and suitable climatic conditions for worm growth support the high prevalence of helminthiasis in Indonesia. STH infection is classified as neglected disease which is defined as an infection that is rarely noticed and chronic without causing obvious clinical symptoms. The impact of the infection is usually noticeable in long term such as malnutrition, growth and developmental disorder, and cognitive impairment in children.[2]

More than two billion of the world's population is estimated to be infected with STH. Approximately 300 million of them are people with severe infections with 150 thousand cases of death due to STH infection occur every year. Most infections were caused by *Ascaris lumbricoides* of 1.2 billion, *Trichuris trichiura* of 795 million, and *Necator americanus* and *Ancylostoma duodenale* as many as 740 million cases.[3]

The prevalence of STH infection, especially in Indonesia, is still high with most infections caused by *Ascaris lumbricoides*.[4] As many as 60% to 80% of Indonesia's population is infected by STH,[1] the prevalence is even higher in certain regions.[1,3,5] Primary school age is a high-risk group to be infected with STH.[5] This is due to poor immunity and lack of awareness to live clean and healthily.[6] Especially in Bali, the prevalence of STH infections in rural areas is still high. In the village of Telaga, the prevalence of intestinal worm infections was reached 68.41% of 93 public elementary school students of Telaga I and 83.87% of 72 elementary school students in Telaga II. The most prevalent infection was *Ascaris lumbricoides* (49.65%).[7]

Factors which cause high STH infection are poor sanitation, such as the habit of unwashed hand before eating and after defecation (defecation), uncut nails, snacking in unhygienic places, not having a decent toilet and difficult to access clean water.[1-3]

The impact of STH infection is quite serious, therefore an effective and efficient control strategy is needed. The World Health Organization (WHO)[8] was recommended routine deworming as a major morbidity control strategy in countries with a high prevalence of helminthiasis.[8] The program has already been implemented in Indonesia, especially in the province of Bali which still has a high prevalence of STH infection. Some areas in Karangasem Regency still have a high incidence of STH infection even though the government has implemented a worm eradication program in the form of routine deworming in every elementary school. Therefore, to increase the effectiveness of helminthiasis, valid data is needed regarding the incidence of helminthiasis, and education on the prevention of intestinal worm infections and administration of helminthic drugs to infected students is necessary.[5-8]

Based on the exposition above, this study is to find out the prevalence and risk factors which contribute to the incidence of STH infection in elementary school children in Ngis Village, Karangasem District, Bali.

MATERIAL AND METHODS

This research is a descriptive analysis study with cross-sectional study design. This research was conducted in three elementary schools in Ngis Village, Manggis Subdistrict, Karangasem Regency, Bali, namely SDN 1 Ngis, SDN 2 Ngis, and SDN 3 Ngis. The study was conducted on July 28 to August 27, 2017. The target population of this study is school-age children (6-12 years). Reachable population of this study were all elementary school students in Ngis Village totaling 157 people. Reachable populations are selected in the period of July 2017. The sampling process was not done randomly (non probability sampling) with total sampling technique. Samples were selected from the population based on the inclusion criteria and exclusion criteria as follows. Inclusion criteria were students who were willing to become respondents, aged 6 to 12 years, filled out validated questionnaires and collected feces. Exclusion criteria were students who moved school or did not approve inform consents.

Research Instrument

Research instruments in the form of tools and materials used in the study are distilled water, glycerin solution, 3% malachite green solution, physiological NaCl or 2% eosin, object glass, Kato-Katz modification kit, 10-15 ml plastic pot (faecal pot), filter wire, toothpicks, plastic sticks, cellophane tape, wipes, waterproof markers, scissors, rubber gloves, microscopes, and questionnaires.

Kato-Katz Modification Method

Stool pots are distributed a day before feces collection. Before being given stool pots, students were given a

validated questionnaire regarding worm disease risk factors. Personal data on validated questionnaires were adjusted according to data on faecal pots. The amount of stool that is put into a pot is about 100 mg (as big as a marble or thumb).

The procedure used in this research is the Kato-Katz modification method (see Figure 1). This method is used to assess the degree of infection. The degree of infection established by WHO is defined as the number of worm eggs per gram of feces (epg). The degree category of *Ascaris lumbricoides* infection is mild (1-4,999 epg), moderate (5000-49,999 epg) and severe (> 50,000 epg). Category of degree of *Trichuris trichiura* infection is mild (1-999 epg), moderate (1000-9.999 epg) and severe (> 10,000 epg).[8]

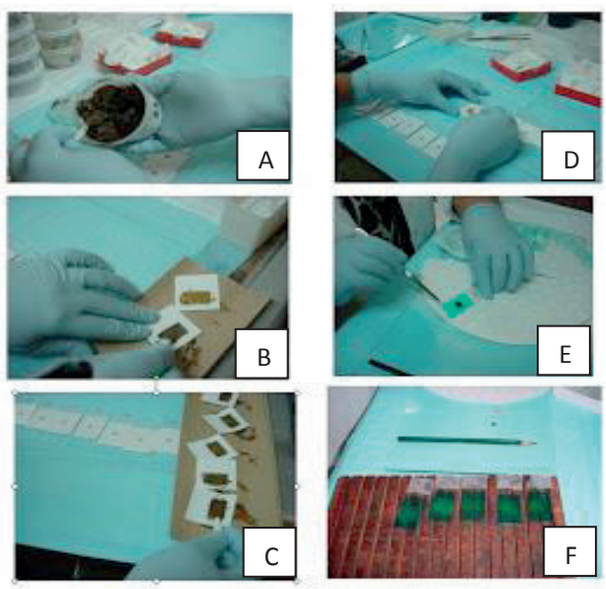

Figure 1. Kato-Katz modification method.[9]

A) Label the glass slide with the sample number and take the faecal sample from container B) Place a small amount of the faecal sample on a paper C) Press the faecal with wirenet to filter the debris D) Place a hollow carton on slide glass then fill with faecal E) Replace the cartoon with cellophane which has been soaked overnight in methylene blue glycerol solution and press the top slide firmly to spread the stool F) let the slide dries about 20-30 minutes before reading on microscope.

Data analysis techniques were carried out using SPSS software. The data that has been obtained is analyzed descriptively and analytically. First, univariate analysis is carried out, is shows data in proportion in the form of respondent characteristics presented in tables and graphs. Second, continued bivariate analysis was carried out to determine the relationship between STH-infected students and risk factors analyzed using chi-square test in crosstab. Odd ratio (OR) analysis was carried out to determine risk factors that affect the occurrence of STH infection in elementary students in Ngis Village with a confidence interval (CI) of 95%. The results of bivariate analysis are presented through tables and graphs.

RESULT AND DISCUSSION

Based on the data obtained (Table 1), there were 138 students who participated in this study with 14 people (10.1%) having STH infection. Male students (64.3%) have a higher proportion of infections compared to female students (35.7%). Third grade students (19%) and five (13.8%) had the highest proportion of students who had STH infection. There were eleven students experiencing a single type of *Trichuris trichiura* infection (78.6%) and the other three experienced mixed infections with *Ascaris lumbricoides* and *Trichuris trichiura* (21.4%). Of the eleven students who had a single *Trichuris trichiura* infection, ten students had a mild infection (90.9%) and one student had a moderate infection (9.1%). Of the three people who had mixed infections with *Ascaris lumbricoides* and *Trichuris trichiura*, two students had a mild infection (66.7%) and one person had a moderate infection (33.3%). Of all students who were infected with STH, there were no students who had severe infections.

Table 1. The Characteristics of Respondents.

Characteristics	STH Infection (n=138)		
	Positive (n=14)	**Negative (n=124)**	**Total (n=138)**
Age (Median)	9 (6-13)	9 (6-13)	138
Gender, n (%)			
Male	9 (12,5)	63 (87,5)	72 (100,0)
Female	5 (7,6)	61 (92,4)	66 (100,0)
Grade Level, n (%)			
Grade 1	1 (5,0)	19 (95,0)	20 (100,0)
Grade 2	2 (9,1)	20 (90,9)	22 (100,0)
Grade 3	4 (19,0)	17 (81,0)	21 (100,0)
Grade 4	2 (9,1)	20 (90,9)	22 (100,0)
Grade 5	4 (13,8)	25 (86,2)	29 (100,0)
Grade 6	1 (4,2)	23 (95,8)	24 (100,0)
Type of Infection, n (%)			
Single Infection *T. trichiura*	11 (78,6)	-	
Mixed Infections *A. lumbricoides* and *T. trichiura*	3 (21,4)	-	
STH Infection Intensity, n (%)			
Single Infection *Trichuris trichiura*			
- mild	10 (90,9)	-	
- moderate	1 (9,1)	-	
- severe	-	-	
Mixed Infections *Ascaris lumbricoides* and *Trichuris trichiura*			
- mild	2 (66,7)	-	
- moderate	1 (33,3)	-	
- severe	-	-	

Children are one of the groups that is vulnerable to infection due to poor self-protection efforts, both in terms of the body's immunity and adequate knowledge about hygiene.[10] Village children in Bali often spend their time playing with the soil and this causes them to be easily infected by bacteria or parasites.[11-14] The incidence of STH infection in children has brought the attention of the government. The Indonesian government program currently wants to reduce the incidence of helminthiasis in children, both through counseling and deworming for six months on a regular basis, targeting primary school children in remote villages.

In this study, researchers were found the prevalence of STH infection in elementary school children is 10.1%. This result is lower than the results obtained by Siregar[11] which is 25.7% and Damayanti[12] in Baturiti, Tabanan is 38.57%. In 2017, the nation-wide prevalence is in the range of 28.9%. This difference is caused by several factors such as time of study, geographical location, culture, social and economic conditions.[11,14-16]

Based on the data in Table 2, the results of statistical tests found that the proportion of male sex is higher than that of women experiencing STH infection. Gender has no risk for the incidence of STH infection. There was a low proportion of elementary school students who were infected with STH with frequent playing behavior (8.1%). Students who often play with soil are not at risk for the incidence of STH infection. There were 66.7% of students with STH infection who did not wash their hands after defecation. Unwashed hands after defecation has a significant relationship to the incidence of STH infection ($p < 0.05$). Furthermore, unwashed hands after defecation can increase the risk of STH infection by twenty times with a confidence interval of 1,730-242,98. It was also found that the proportion of students infected with STH who did not wash their hands after playing with soil was 42.6%. Unwashed hands after playing the soil can cause STH infection ($p < 0.05$). This can put the student at risk for by twelve times higher. STH infection is also caused by being barefoot. The proportion of students who are barefoot with the incidence of STH infection is greater than those who have are not barefoot. This is supported by a significant relationship between being barefoot with the incidence of STH infection ($p < 0.05$). Students who are barefoot is

Table 2. STH Infection Risk Factors in Elementary students in Ngis Village, Karangasem, Bali.

STH Infection					
	Positive n (%)	**Negative n (%)**	**p**	**PR**	**95%CI**
Gender					
Male	9 (12,5)	63 (87,5)	0,339	0,57	0,182-1,809
Female	5 (7,6)	61 (92,4)			
Often Playing with Soil					
Yes	3 (8,1)	34 (91,9)	0,631	1,38	0,364-5,270
No	11 (10,9)	90 (89,1)			
Dewormed					
Yes	9 (7,6)	110 (92,4)	0,012*	0,23	0,067-0,781
No	5 (26,3)	14 (73,7)			
Unwashed Hands after defecation					
Yes	2 (66,7)	1 (33,3)	0,001**	20,50	1,730-242,98
No	12 (8,9)	123 (91,1)			
Unwashed Hands after Playing with Soil					
Yes	6 (42,6)	7 (53,8)	0,000**	12,54	3,401-46,210
No	8 (6,4)	117 (93,6)			
Barefoot					
Yes	3 (37,5)	5 (62,5)	0,008**	6,491	1,365-30,856
No	11 (8,5)	119 (91,5)			
Uncut Nails Regularly					
Yes	3 (17,6)	14 (82,4)	0,274	2,143	0,538-8,625
No	11 (9,1)	110 (90,1)			
Unavailable Latrine					
Yes	5 (71,4)	2 (28,6)	0,000**	33,89	5,749-199,77
No	9 (6,9)	122 (93,1)			

Explanation: * $p < 0,05$, ** $p < 0,01$

at risk of STH infection by six times. The proportion of students infected with STH by uncut nails is greater than those that cut nails regularly. Uncut nails is not a risk factor that affects the incidence of STH infection in elementary students in Ngis Village. There were 5 (71.4%) students who were infected with STH with unavailable latrine. The availability of toilet is one of the efforts made to protect against STH infection. This is proven through the research results which states the absence of latrines can put students at risk of STH infection by 33 times higher. A significant relationship between STH infection and unavailable latrines in elementary students in Ngis Village ($p < 0.05$) was also found. There was a low proportion of STH infected elementary school students who took deworming drugs regularly. Dewormed regularly is a protective effort against the occurrence of STH infection. This is proven by a significant relationship between taking deworming drugs regularly with no STH infection ($p < 0.05$).

Based on some of these risk factors, there are risk factors that influence the incidence of STH infection in students in Ngis Village, including unwashed their hands after defecation, unwashed their hands after playing with soil, being barefoot, unavailable latrines, and dewormed regularly.

In this study, researchers were found that elementary school children had previously gone through dewormed as part of government program in eradicating the incidence of STH infection in the area. This was conveyed by the Head of Community Health Center (Puskesmas) II Manggis during the interview session, who stated the administration of deworming drugs was implemented as a district government program to eradicate the incidence of STH infection in elementary school children. As for the program implementation, the administration of worm medicine (Albendasol dose 1x400mg) once in six months was done as an effort to eradicate or reduce the incidence of STH infection in children. The administration of these drugs is carried out routinely and is carried out directly, meaning the administration of deworming drugs was done in the classroom to avoid students who do not take deworming drugs (program in 2016). This program has been carried out twice in December 2016 and in July 2017. However, the implementation of this program only provides deworming drugs directly to children, without educating them on basic health hygiene practices, such as washing hands with soap after playing.

In addition, there is a limitation to this program, namely the lack of monitoring and evaluation during program implementation. It also becomes an obstacle in determining whether the program is implemented properly or not. Based on the results of our study, it was found that only 10.1% of children infected with STH. It is below the nation-wide prevalence of STH infection in Indonesia by 25.7%, thus this program had an effect on decreasing the incidence of STH infection in children.[17,18] Furthermore, the intensity or degree of infection of children who have STH infection is mild to moderate according to the results of calculations with the Kato-Katz modification method.[15] This program can be continued to reduce the incidence of STH infection in children especially in Karangasem district, Bali.

In this study, it was found that children who had STH infection lacks knowledge about self hygiene. It was proven that the hygiene of children from the habit of hand washing and playing outside the house was known to be significant in causing the incidence of STH infection. Another risk factor which caused the occurrence of STH infection is hygiene after defecation, because children often forget to wash their hands after defecation. Infection is transmitted through dirty hand nails which mediate the entry of worm eggs into the body. In addition, the habit of unwashed hands after defecation can be a strong risk factor in children having STH infection.

It was found that playing with soil often is not a risk factor for STH infection in elementary students in Ngis Village. These results are differ from the research results by Samad who found contamination of *Ascaris lumbricoides* eggs in children who enjoy playing with soil.[19] It is also inversely proportional to Juhairiyah's findings which was stated that the habit of playing with soil will increase the risk of STH infection.[20] These results may be influenced by children who no longer play with soil as often. It was also found that the proportion of students infected with STH who did not wash their hands after playing the soil was 42.6%. Not washing hands after playing the soil can increase risk of being infected with STH by twelve times in elementary students. These results are in line with the research results obtained by Wiryadana *et al.*[14] In addition, which researcher also was found that children who are barefoot when they are outside the house was also significantly associated with the incidence of STH infection. Being barefoot can cause the occurrence of *Ascaris lumbricoides* infection. In line with Kartini's[21] research which was stated that is a relationship between children who were barefoot against STH infection, but in Wiryadana *et al.*[14] study, no significant results were found.[14,21]

Researcher was also found that children who did not have latrines had a significant association with the incidence of STH infection. However researcher was found the protective value of children who have latrines. Supported by the research results of Wiryadana *et al.*,[14] children who have latrines are not infected with STH due to the inability of worms to develop in the latrine whereas on the ground it can develop and re-infect.[14] This study was found that there were still children who did not have latrines at home. This is the biggest risk factor for the incidence of STH infection. Countermeasures are needed in the form of latrines for families who do not have latrines as an effort to reduce the incidence of STH infection.[14,20,21,22]

In this study, researchers were also found that elementary school children had been dewormed before. Researchers were found that taking deworming drugs had a protective relationship to STH infection. This is supported by the results of research conducted by the researcher, which found that dewormed regularly can prevent students from

getting STH infection.[14] Therefore, regular administration of deworming drugs can be a prophylactic effort to prevent children from having STH infection. However, there were some children who had mild STH infection even though they had been dewormed. It is also part of the WHO program to eradicate STH infections.[21]

The STH eradication program should be implemented as the government's priority program. The development of this program will be good if proper monitoring and evaluation can be carried out. In eradicating STH infection, information and health education is needed for both parents and children as well as all components of society. Collaboration and good performance in this program will make the target of eradicating STH a success.[14,20-22]

The limitations of this study are the distance between the location of the research and the examination laboratory. Due to the unavailability of the epidemiological data from Bali and Indonesia, this study was conducted with a cross-sectional design. At present, it is not possible to carry out studies with a more comprehensive design. Thus, this study was used a cross-sectional design which could not assess the risk factors but limited only to the incidence of STH. Furthermore, the researchers collect only one specimen in each child which may be different from the worm egg count in that child later on.

CONCLUSION

Based on the results of the study and the discussion above, it can be concluded that the prevalence of STH infection in elementary students in Ngis Village is 10.1%. Intensity of STH infection occurs with mild to moderate. Risk factors that cause the incidence of STH infection in students in Ngis Village are unwashed hands after defecation, unwashed hands after playing with soil, being barefoot, unavailable latrines, and not dewormed regularly. Researcher was found a decrease in the prevalence of STH infection compared to the national prevalence rate. The knowledge in prevention and attitude of elementary school students are fairly good but efforts are needed to improve hygiene in order to prevent the onset of infection. Monitoring and evaluation efforts from the Community Health Center (Puskesmas) are also needed to maximize the efforts to eradicate STH infection.

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REFERENCES

1. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasites & vectors. 2014 Dec;7(1):37.
2. Zerdo Z, Yohanes T, Tariku B. Soil-transmitted helminth reinfection and associated risk factors among school-age children in Chencha District, southern Ethiopia: a cross-sectional study. Journal of parasitology research. 2016;2016.
3. Idris OA, Wintola OA, Afolayan AJ. Helminthiases; prevalence, transmission, host-parasite interactions, resistance to common synthetic drugs and treatment. Heliyon. 2019 Jan 1;5(1):e01161.
4. Ganguly S, Barkataki S, Karmakar S, Sanga P, Boopathi K, Kanagasabai K, *et al.* High prevalence of soil-transmitted helminth infections among primary school children, Uttar Pradesh, India, 2015. Infectious Diseases of Poverty. 2017;6(1).
5. Yuwono N, Husada D, Basuki S. Prevalence of Soil-Transmitted Helminthiasis among Elementary Children in Sorong District, West Papua. Indonesian Journal of Tropical and Infectious Disease. 2019 Feb 22;7(4):86-91.
6. Simarmata N, Sembiring T, Ali M. Nutritional status of soil-transmitted helminthiasis-infected and uninfected children. Paediatrica Indonesiana. 2015;55(3):136.
7. Devi NP, Sudarmaja IM, Swastika K. Prevalensi infeksi soil transmitted helmith di Sekolah Dasar Negeri 1 Padangbulia Kecamatan Sukasada Kabupaten Buleleng, Bali-Indonesia. Intisari Sains Medis. 2018;9(3): 59-61.
8. WHO. Soil-transmitted helminthiasis. In: Sustaining the drive to overcome global impact of neglected tropical diseases: Second WHO Report on neglected tropical disease. Geneva: WHO; 2013: 106-111.
9. Miércoles. Técnica de Kato Katz para la cuantificación de huevos de helmintos [online]. Laboratorio de Parasitología 2009 [cited 2018 Jun 28]. Available from: http://parasitosintestinalesguatemala.blogspot.com/2009/09/comentarios.hhtm.
10. Montresor A. Helminth control in school-age children. Geneva: World Health Organization; 2011.
11. Siregar M. Kejadian infeksi STH dan gambaran kepemilikan jamban dan air bersih pada anak usia sekolah dasar di Yayasan Nanda Dian Nusantara 2011 [thesis]. Jakarta: Universitas Islam Negeri Syarif Hidayatullah; 2012.
12. Damayanti PA. Pengobatan dan Penilaian Status Gizi Anak SDN 1 Luwus, Baturiti yang Menderita Cacingan (Soil-transmitted Helminthiasis). Buletin Udayana Mengabdi. 2009;12(1).
13. Diarthini NL, Swastika IK, Ariwati L, Isyaputri R, Hidajati S, Basuki S. Blastocystis and Other Intestinal Parasites Infections in Elementary School Children in Dukuh Village, Karangasem District, Bali. Indonesian Journal of Tropical and Infectious Disease. 2018 Oct 31;7(3):57-61.
14. Wiryadana K, Putra I, Rahayu P, Pradnyana M, Adelaida M, Sudarmaja I. Risk factors of soil-transmitted helminth infection among elementary school students. Paediatrica Indonesiana. 2018;57(6):295.
15. Endris M, Tekeste Z, Lemma W, Kassu A. Comparison of the Kato-Katz, Wet Mount, and formol-ether concentration diagnostic techniques for intestinal helminth infections in Ethiopia. ISRN parasitology. 2012 Oct 22;2013.
16. Ginandjar P, Saraswati L, Martini . Soil-Transmitted Helminth Infection in Elementary School Children: An Integrated Environment and Behavior Case Study in Bandungan Sub-District, Semarang District. Advanced Science Letters. 2017;23(4):3565-3568.

17. Elmi, Sembiring T, Dewiyani BS, Hamid ED, Pasaribu S, Lubis CP. Status gizi dan infestasi cacing usus pada anak sekolah dasar. Bagian Ilmu Kesehatan Anak FK USU. [Cited 2018 June]. Available from:www.repository.usu.ac.id.
18. Hackenberger D, Palijan G, Lončarić Ž, Jovanović Glavaš O, Hackenberger B. Influence of soil temperature and moisture on biochemical biomarkers in earthworm and microbial activity after exposure to propiconazole and chlorantraniliprole. Ecotoxicology and Environmental Safety. 2018;148:480-489.
19. Marlina L, Widjaja J. Hubungan pendidikan formal, pengetahuan ibu dan sosial ekonomi terhadap infeksi soil transmitted helminths pada anak sekolah dasar di Kecamatan Seluma Timur Kabupaten Seluma Bengkulu. Indonesian Journal of Health Ecology. 2012;11(1).
20. Juhairiyah J, Annida A, Indriyati L. Gambaran Faktor Resiko Kecacingan pada Anak Sekolah Dasar di Kota Banjarmasin. Jurnal Vektor Penyakit. 2016;9(1):21-27.
21. Kartini S. Kejadian Kecacingan pada Siswa Sekolah Dasar Negeri Kecamatan Rumbai Pesisir Pekanbaru. Jurnal Kesehatan Komunitas. 2016;3(2):53.
22. Blum AJ, Hotez PJ. Global" worming": Climate change and its projected general impact on human helminth infections. PLoS Neglected Tropical Diseases. 2018 Jul 19;12(7):e0006370-.

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Research Report

INCIDENCE OF DENGUE HEMORRHAGIC FEVER (DHF) IN SEMARANG COASTAL AREA: EPIDEMIOLOGY DESCRIPTIVE CASE AND BIONOMIC VECTOR

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ABSTRACT

Semarang Utara sub-district is located on the coast of the Java Sea. The coastal area is characterized by high salt content on both the ground and the water compare to other areas. The high salt content environment should have limited the breeding of Dengue Hemorrhagic Fever (DHF) vectors; yet, quite high incidents of DHF cases are reported taken place in Semarang coastal area. The aim of this study was to describe the epidemiology of DHF incidence, characteristic of cases, and bionomics vector in the coastal area of Semarang Utara sub-district. This study was applied descriptive observational design to analyze samples consisting of 62 dengue cases and 184 houses. The research variables consisted of coordinate of DHF cases, water salinity, House Index (HI), Container Index (CI), and Aedes species. Data were processed using SPSS in a bivariate manner; while, mapping was analyzed spatially using ArcGIS 10.3. A total of 184 houses were surveyed and 55 cases of DHF were identified. Most cases occurred in 6 -16 year age group (47.3%), water salinity ranged from 2-3%, indicating that the water in the coastal area tended to be brackish water. The results of the Pearson Correlation test showed that there was no relationship between HI and Incidence of DHF in Semarang Utara sub-district. Aedes aegypti was identified in a positive container, otherwise Aedes albopictus was not found. DHF cases mostly occurred in school age groups, and were distributed in all villages near or far from the beach. DHF vector could breed in areas with little brackish water, so that dengue transmission might occur in this area.

Keywords: *DHF, Aedes Aegypti, Aedes Albopictus, Bionomic Vector, Semarang Beach*

ABSTRAK

Kecamatan Semarang Utara terletak di pantai Laut Jawa. Kondisi daerah pantai dicirikan dengan kandungan garam baik di tanah dan air menjadi lebih tinggi dibandingkan area lain. Lingkungan dengan kadar garam yang tinggi dapat membatasi perkembangbiakan dari vektor Demam Berdarah Dengue (DBD), namun laporan kasus DBD di wilayah pantai Semarang selalu ada dengan insiden yang cukup tinggi. Penelitian ini bertujuan untuk mendeskripsikan epidemiologi kejadian DBD di wilayah pesisir Kecamatan Semarang Utara, karakteristik responden serta vektor bionomik. Penelitian ini menggunakan desain deskriptif observasional. Sampel penelitian sebanyak 62 kasus DBD dan 184 rumah sekitar kasus. Variabel penelitian meliputi koordinat kasus DBD, salinitas air, House Index (HI), Container Index (CI) dan spesies Aedes. Data diolah menggunakan SPSS secara bivariat, sedangkan pemetaan dianalisis spasial menggunakan ArcGis 10.3. Sebanyak 55 kasus DBD teridentifikasi dan 184 rumah telah disurvei. Sebagian besar kasus dalam kelompok usia 6-16 tahun (47,3%). Salinitas air berkisar 2-3 ‰, tingkat salinitas ini menunjukkan air di wilayah pantai cenderung dikategorikan air payau. Hasil uji Pearson Correlation menunjukkan tidak ada hubungan antara HI dengan Incidence Rate (IR) DBD di Kecamatan Semarang Utara. Aedes aegypti teridentifikasi dalam kontainer yang positif sebaliknya tidak ditemukan Aedes albopictus. Kasus DBD sebagian besar terjadi pada kelompok usia sekolah, dan terdistribusi di semua kelurahan baik dekat atau jauh dari pantai. Vektor DBD dapat berkembangbiak di wilayah yang airnya sedikit payau, sehingga penularan DBD dapat terjadi di wilayah ini.

Kata kunci: *DBD, Aedes Aegypti, Aedes Albopictus, Bionomik vector, Pantai Semarang*

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a disease caused by dengue virus and can be transmitted through the bite of *Aedes aegypti* or *Aedes albopictus* mosquitoes.[1] Factors that play an important role in the transmission of dengue virus infection are humans, intermediary vectors, and environment.[1] In addition, factors of population density, rainfall, humidity, wind speed, air temperature, and altitude can also affect the rapid spread of dengue transmission.[2]

DHF is a contagious disease that becomes a health problem in the city of Semarang. Based on the health profile data of Semarang City, the incidence of dengue fever in Semarang City tends to be fluctuated with high numbers. The incidence of DHF in Semarang City decreased to 18.14 per 100,000 population[3,4,5,6,7] in 2016 and 2017 due to the changes in the operational definition of dengue cases as of October 1, 2016. Currently, DHF case is defined as a case with dengue fever (DF) symptoms followed by an increase in hematocrit š 20% without taking into account the results of serological examination.[7] However, these conditions do not eliminate the risk of dengue disease occurrence in the city of Semarang because of the population of DHF vector, *Ae. aegypti*, still not fully maintained and controlled.

Ae. aegypti mosquito is the main vector of dengue disease. Theoretically, *Ae. aegypti* mosquitoes reproduce in clear water that does not touch soil. However, the results of recent studies suggest that the *Ae. aegypti* larvae are able to survive in the clear water from precipitated water in the ditch.[8] In addition, the growth of *Ae. aegypti* also depends on the chemical conditions of the environment. *Ae. aegypti mosquitoes* can survive in containers containing water with normal pH ranging from 5.8 to 8.6 and water with salinity concentration of 0-0.7%.

The most recent research conducted in Brazil was showed that *Ae. aegypti* is able to adapt to certain salinity conditions in littoral, coastal, and highland areas.[9] Meanwhile, data generated from experimental studies in Semarang City was showed that *Ae. aegypti* can develop both in various water pH conditions from pH 4 to pH 10 and in water salinity ranging from 0% to 6%.[8]

North Semarang sub-district, one of the areas in Semarang City lying on the coast of the Java Sea, has high salt content in both soil and water compare to other regions. This condition should have made Semarang Utara sub-district free from DHF endemic because high Na Cl concentrations resulted in an imbalance between larval body fluid and medium brood fluid. The difference in osmosis pressure causes the mortality of larvae on Instar II.[8] However, data from the Semarang City Health Office showed that some villages in the Semarang Utara sub-district have been categorized as DHF endemic areas.

North Semarang sub-district borders with the Java Sea in the north, with Semarang Tengah sub-district in the south, with Semarang Timur sub-district in the east, and with Semarang Barat sub-district in the west. Semarang Utara sub-district consists of 9 villages, 89 sub villages, and 708 neighborhoods. Within 10.9 km^2, in 2014, the population density was 11,272 per million, and the number of household was 32,000 each of which had 4 family members, even one house might dwell more than one households; one of the most populated are in Semarang.

Given this situation, research needs to be done to describe epidemiological conditions of the incidence of DHF from the perspective of the characteristics of the people, of the place, and of the time. The characteristic of the people was described by age, occupation, and history of the patient's activity before being diagnosed to be exposed to DHF. Meanwhile, as environmental factors are important to detect the presence of DHF vectors, vector density was observed. In addition, the characteristic of the time studied was related to climate at the time the DHF occurred in patients. Therefore, the aim of this study was to describe the epidemiology of the incidence of DHF in the coastal area of Semarang, based on characteristic and behavior of cases, also bionomic of the vector.

METHOD AND MATERIAL

This study took place in the coastal area in Semarang Utara sub-district of Central Java Province and was conducted in 2017. The population was those who exposed to DHF and Dengue Shock Syndrome (DSS) as many as 62 patients. Total sampling method was used to describe the Semarang Utara sub-district.

As this study described the epidemiological conditions of the incidence of dengue in the Semarang Utara sub-district, larvae survey was used to determine the density of vectors in a particular region. The number of houses surveyed was calculated using purposive sampling method.

The theory used to determine the number of houses was within the mosquito fly distance is ± 100 meters; therefore, it was estimated that mosquitoes could transmit the dengue virus at a radius of 100 meters around the sufferer. As a result, surveyed larvae was carried out on 4 houses around the patient's home, either from the North, East, West, or south and the number of houses to be surveyed was 248 houses. The cases of DHF was diagnosed by medical team in hospital and supported by clinical laboratory test.

Research variables measured in this study were age, occupation, illness history, behavior to eradicating mosquitoes nest (EMN), type of mosquito breeding place, water source, container location, water salinity, distance from the shoreline, Water salinity was measured by refracto meter equipment. House Index (HI), as well as Container Index (CI). HI is a percentage of houses identified positive larvae per total of examined houses. CI is a percentage of containers identified positive larvae per total of examined containers.

Bivariate analysis was carried out in testing HI; while, Pearson/Rank Spearman correlation test was used to test the incidence of DHF, all of which were analyzed using ArcGIS version 10.3 software. The data analyzed was exhibited the

coordinates of DHF cases and HI as well as the urban CI where the cases were located. Furthermore, the distance of the house of dengue cases to the North Semarang coastline were also described.

RESULT AND DISCUSSION

The incidence of DHF in the Coastal Area of North Semarang Sub-District

Semarang City Health Office reported 62 cases to be diagnosed both DHF and DSS in Semarang Utara sub-district in 2017. In the field, 55 cases of DHF were found; while, 7 other cases were not found as the sufferers had been moved to another place.

Table 1. Characteristics of DHF cases in north semarang sub-district in 2017

Characteristics	Frequency (n= 55)	Percentage (%)
1. Age (year)		
a. 1-4	7	12.7
b. 5-9	10	18.2
c. 10-14	14	25.5
d. 15-44	17	30.9
e. > 45	7	12.7
2. Occupation		
a. Jobless	40	72.7
b. Labor	1	1.8
c. Trader	9	9
d. Retired/Housewife	5	9.1
3. Education		
a. Not yet school	11	20
b. Not finish Elementary School	1	1.8
c. Elementary School	23	41.8
d. Secondary School	8	14.5
e. High School/ Vocational High School	8	14.5
f. University	4	7.3
4. Home distance from coastline		
a. ≤ 100 meter	5	9.1
a. > 100 meter	50	90.9

Based on Table 1. DHF cases in Semarang Utara sub-district were exposed to people aged from 2 years to 88 years, with the most age being 11 years (9.1%). The largest age group exposed (30.9%) is the age group of 15-44 years. As many as 72.7% of the people suffering from dengue cases in Semarang Utara sub-district have not worked, 41.8% complete elementary schools or are taking elementary education.

Case history of DHF was obtained through an in-depth interview to 55 respondents of the DHF patients or their family using open questions about activities carried out by DHF patients before being diagnosed with DHF. The result of the interview was showed that there were patients carrying out activities outside Semarang Utara sub-district before being diagnosed with DHF, whether they were traveling outside the district or out of town more than two days. In addition, there were patients who mostly spend their daily activities outside the Semarang Utara sub-district due to work or school. The fact also was showed that there were school-age who previously did not exposed to; yet, after being contacted with their friends who suffered from DHF, they started to be infected. From the distance of the house to coastline, 5 sufferers' houses were standing right to the coastline. In general, the distance of the house to the coastline ranging from 0 meter to 2,844 meters with 1,311.42 meters in average.

Behavior to Eradicating Mosquito Nests (PSN) in North Semarang Sub-District

To find out the behavior to eradicating mosquito nests (PSN), interviews were conducted with 55 respondents whose families were DHF sufferers.

Table 2. Behavior of Eradicating Mosquito Nests (PSN)

Behavior	Yes	Percentage (%)	No	Percentage (%)
Covering water container inside the house	9	16.4	46	83.6
Covering water container outside the house	49	89.1	6	10.9
Routinely draining water container	41	74.5	14	25.5
Brushing water container	25	45.5	30	54.5
Disposing of used goods	47	85.5	8	14.5
Recycling of used goods	5	9.1	50	90.9
Using insect repellent	47	85.5	8	14.5
Using bed nets	29	52.7	26	47.3
Using abate powder	8	14.5	47	85.5
Maintaining fish larvae eaters	8	14.5	47	85.5
Window/ventilation	45	81.8	10	18.2
Enough lighting	31	56.4	24	43.6
Hanging clothes	23	41.8	32	58.2
Another family hung clothes	23	41.8	32	58.2

Based on Table 2, the results of the interview showed that 90.9% of respondents did not recycle used goods, and 85.5% did not use abate powder. These habits might increase the risk of dengue vector mosquitoes to explode.

Most of the water container was made from plastic (41.8% respondents), and 61.8% respondents used non-tap water; instead, they are used water from deep well water, dug well water, and supplied water from Tanah Mas housing complex. As much as 50.9% of the water samples taken contained 2 % salt which was categorized as brackish water (the category of water according to the salt content in a row, namely water < 0.5 -; 0.5-30 %; and > 30 % is fresh water, brackish water, and salt water).[10]

Table 3. Characteristics and conditions of water container

Characteristic	Frequency	Percentage (%)
1. Container Material (n=55)		
· Plastic	23	41.8
· Ceramic	20	36.4
· Cement	12	21.8
2. Water Source (n=55)		
· Non Tap Water	34	61.8
· Tap Water	21	38.2
3. Salinity (n=5)		
· 0 ‰	11	20.0
· 1 ‰	28	50.9
· 2 ‰	13	23.6
· 3 ‰	3	5.5
4. *House Index* (n=184)		
· Low (HI < 10%)	6 villages	
· High (HI ≥ 10%)	3 villages	
5. *Container Index*		
· Low (CI < 5%)	6 villages	
· High (CI ≥ 5%)	3 villages	

Based on Table 3, to determine the density of dengue mosquito vector in each village in North Semarang sub-district, 184 houses scattered in all villages were surveyed. The results of HI and CI were grouped into high and low categories according to WHO provisions.[11,12] There were 3 villages with low HI and CI namely Bulu Lor, Plombokan, and Purwosari Villages; while, the other 6 villages had high HI and CI, namely Tanjungmas, Dadapsari, Kuningan, Bandarharjo, Panggung Kidul, and Panggung Lor.

Figure 1 shows villages with the value of the container index (CI) in each village in the sub-district of North Semarang. There are 3 villages with CI in the low category (CI < 5%), namely Bulu Lor, Plombokan, and Purwosari; while, the other 6 villages have high category of CI values (≥ 5% CI), namely Tanjungmas, Dadapsari, Kuningan, Bandarharjo, Panggung Kidul, and Panggung Lor.

Figure 1 shows villages with the value of the container index (CI) and HI in each village in the sub-district of Semarang Utara. There are 3 villages with CI in the low category (CI < 5%), namely Bulu Lor, Plombokan, and Purwosari; while, the other 6 villages have high category of CI values (≥ 5% CI), namely Tanjungmas, Dadapsari, Kuningan, Bandarharjo, Panggung Kidul, and Panggung Lor.

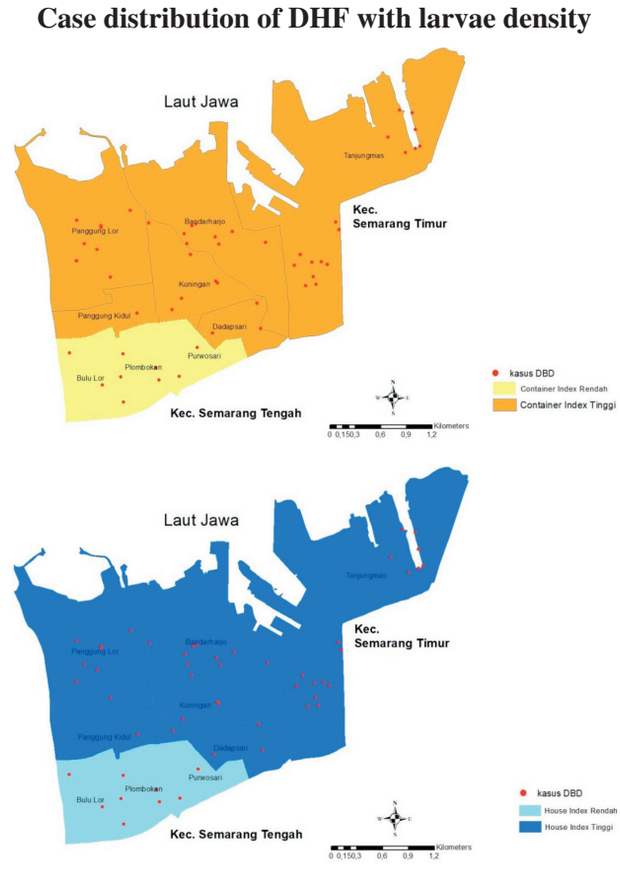

Figure 1. HI, CI, and dengue cases in North Semarang Sub-district

DISCUSSION

There were five homes of DHF sufferers in North Semarang Sub-district standing right on the coastline. Of the five houses, two houses were identified to be positive for having mosquito larvae. The closer distance between the house and the beach allows the mixing of ground water with seawater to make the region's water source brackish.[14] If the salt content of a water source is high, mosquito larvae will not be able to develop.[11,15] However, the fact showed that mosquito larvae were found in that region. After analyzing the salt content using refractometer, it was found that the salinity ranged from 2-3 ‰. Water is categorized as brackish water if it exceeds 0.5‰, so it can be concluded that the *Ae. aegypti* mosquito could live in brackish water.[11,15]

Other research finding reported that there was a significant change in ion transportation by anal papillae of the mosquito larvae living in the salt water.[16,17] The morphological and physiological changing showed that *Aedes aegypti* mosquito had been able to adapt to the

changing environment of the breeding place, especially the one having high content of salt.

Through in-depth interviews, it was identified that the water source used by residents in Tanjungmas area came from 1-2 main sources channeled by pipes to homes. The deep well water source was used by most of the residents. Water from the deep well should not have had salt or fresh water. However, in reality there had been a change in the quality of fresh groundwater to brackish in deep wells in the North Semarang region. This phenomenon was related to seawater intrusion or ancient salt dissolution trapped in sediment when rock sedimentation took place.[14]

Increased levels of seawater could increase the number of mosquitoes tolerant to salt content and allow mosquito vectors adaptation that were not tolerant to salt content to be tolerant to brackish water. This explained the increase in dengue cases in the coastal areas. The increased population living in the coastal area is predicted to be 134 people/ km^2 by 2050.[11,15,18] Therefore, if the control of vectors in coastal areas was not implemented properly, there would be an increase in dengue cases in that particular areas. An area is considered to be at high risk for dengue transmission if the container index is š 5% and the house index is š 10%,[19] based on which villages of Tanjungmas, Dadapsari, Kuningan, Bandarharjo, Panggung Kidul dan Panggung Lor were at high risk to DHF spreading. As shown in Figure 1, there was a tendency for DHF cases to be in the villages with high HI and CI. However, the results of the correlation test showed that the HI value did not correlate to the incidence of DHF in North Semarang sub-district. This proved that the HI value was not the main risk factor for the spread of DHF in the North Semarang sub-district. This finding was in line with the research result conducted in Sendangmulyo village of Semarang city.[20]

The interviews with patients concluded that the patients were infected with DHF after traveling outside the sub-district. Therefore, the spread of DHF in North Semarang sub-district did not originally come from inside of the sub-district but also from outside of the sub-district area; therefore, the high and low HI values did not affect the incidence of DHF.

CONCLUSION

DHF cases in the coastal areas of North Semarang occur in all villages areas both in and off the coast. DHF cases mostly occur in the 6-16 years age group, namely school age. The incidence of DHF is not related to monthly rainfall. The distribution of dengue cases in the North Semarang Sub-district is not related to high population density, House Index, and Container Index.

Aedes aegypti can live and breed in the coastal areas; even though, the water sources used show higher salt content or tend to be brackish. No *Aedes albopictus* is found at the coastal location. Survival *Aedes aegypti* can affect the transmission of DHF in the coastal areas of North Semarang. No exception in controlling DHF and its vector, coastal communities also need to carry out actively in PSN activities in their area.

CONFLICT OF INTEREST

There is no conflict of interest occurred in this study, both among researchers, and communities. This research has obtained permission from the Kesbangpolinmas office, the Health Office, as well as the sub-district to RT units to carry out the research. Respondents involved in the interview were provided informed consent to get their approval.

ACKNOWLEDGEMENT

This research could run well because of the help of various parties; therefore, we would like to extend our appreciation to Undip graduate Director for the approval of the research topic, Semarang City Health Office which provided data on DHF sufferers, as well as respondents who participated in this study.

REFERENCES

1. Gede, Wempi. *Kontainer Larva Aedes sp di Desa Saung Naga Kabupaten Oban Komesing ulu Sumatra Selatan tahun 2012*", Jurnal Aspirator, 2013; 5(1):16-22
2. Kementrian Kesehatan RI. *Demam Berdarah Dengue, Situasi 2011 dibanding 2010*. Jakarta: Kementrian Kesehatan RI, 2011.
3. Dinas Kesehatan Kota Semarang. *Profil Kesehatan Kota Semarang Tahun 2012*. Semarang: Dinkes Kota Semarang, 2013
4. Dinas Kesehatan Kota Semarang. *Profil Kesehatan Kota Semarang Tahun 2013*. Semarang: Dinkes Kota Semarang, 2014
5. Dinas Kesehatan Kota Semarang. *Profil Kesehatan Kota Semarang Tahun 2014*. Semarang: Dinkes Kota Semarang, 2015
6. Dinas Kesehatan Kota Semarang. *Profil Kesehatan Kota Semarang Tahun 2015*. Semarang: Dinkes Kota Semarang, 2016
7. Dinas Kesehatan Kota Semarang. *Data HEWS DBD Tahun 2017*. Semarang: Dinkes Kota Semarang, 2017
8. Anggraini TS, Cahyati WH. Perkembangan Aedes Aegypti pada Berbagai PH Air dan Salinitas Air. Higeia Journal of Public Health Research and Development. 2017; 1(3): 1-10.
9. Arduino MB, Mucci LF, Serpa LLN. Effect of Salinity on the Behaviour of Aedes Aegypti populations from Coast and Plateau of Southheasternn Brazil. Journal Vector Borne Disease.2015; 52: 79-87.
10. Ramasamy R, Surendran SN, Jude PJ, et. Al. Larval Development of Aedes Aegypti and Aedes albopictus in Peri-Urban Brackish Water and Its Implications for Transmission of Arboviral Disease. PLoS Neglected Tropical Disease.2011; 5(11).
11. WHO. Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever. India: Regional Office if Southe-East Asia. 2011
12. WHO. Dengue and severe dengue. 2014. Available from : *http://www.who.int/mediacentre/factsheets/fs117/en/*
13. Bakti H, Naily W, Lubis RF, et.al. Penjejak Keluaran Air Tanah Lepas Pantai (KALP) di Pantai Utara Semarang dan Sekitarnya dengan Radon. Riset Geologi dan Pertambangan. 2014; 24(1) : 43-51
14. Jude PJ, Tharmasegaram T, Sivasubramaniyam G, et.al. Salinity-toerant larvae of mosquito vectors in the tropical coast of Jaffna, Sri

Lanka and the effect of salinity on the toxicity of Bacillus thuringiensis to Aedes aegypti larvae. *Parasites & Vectors* 5, 2012: 269
15. Clark T.M, Bradley T.J. Malpihian tubules of larval *Aedes aegypti* are hormonally stimulated by 5-hydroxytryptamine in response to increased salinity. Insect Biochemistry And Physiology. 1997; 34(2):123-141
16. Donini A, Gaidhu M.P, Strasberg DR, et.al. Changing salinity induces alterations in hemolymph ion concentration and Na^+ and Cl^- transport kinetics of the anal papillae in the larval mosquito, *Aedes aegypti*. The Journal of Experimental Biology. 2007; 210: 983-992
17. United Nation Environment Programme (2007) Global Programme of Action for Protection of the Marine Environment from Land-based Activities 2007: Physical alteration and destruction of habitats. UNEP. Nairobi, Kenya. Available: http://gpa.unep.org/content.html?id=199&ln = 6
18. Joharina AS, Widiarti. Kepadatan Larva Nyamuk sebagai Indikator Penularan Demam Berdarah Dengue di Daerah Endemis di Jawa Timur. Jurnal Vektor Penyakit. 2014; 8(2): 33-4.
19. Saraswati LD, Martini M. Hubungan Kepadatan Jentik dengan Penyakit DBD di Kelurahan Sendangmulyo Kota Semarang melalui Pendekatan Analisis Spasial. Jurnal Kesmas Indonesia. 2012; 5(1): 52-64.
20. Saraswati LD, Martini M. Hubungan Kepadatan Jentik dengan Penyakit DBD di Kelurahan Sendangmulyo Kota Semarang melalui Pendekatan Analisis Spasial. *Jurnal Kesmas Indonesia* 5(1), 2012: 52-64.

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Research Report

DETECTION OF *HELICOBACTER PYLORI* INFECTION IN CHRONIC GASTRITIS BIOPSY SPECIMEN USING WARTHIN-STARRY AND MODIFIED GIEMSA STAIN IN DR SOETOMO HOSPITAL SURABAYA

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ABSTRACT

Helicobacter pylori *is a bacteria that commonly cause chronic gastritis. Identification of its infection is essential for eradication treatment. Detection of H.pylori bacteria in gastric biopsy specimen by histology method is a diagnostic tool that widely accepted because it is superior to serology examination. Although the bacteria can be seen in routinely Hematoxylin-Eosin staining, modified-Giemsa and Whartin-Starry stain was commonly used to identify the bacteria more clearly. Whartin-Starry stain gives more contrast to the bacteria but modified-Giemsa stain is preferable at many centres because it is a cheaper and simple method. This study aim is to explore the differences of two stain method to identify H.pylori. Paraffin blocks from gastric biopsy patients with chronic gastritis in the year 2017 were retrieved from Anatomic Pathology Laboratory Dr. Soetomo Hospital Surabaya. Thirty paraffin blocks were taken randomly and were made into microscopic slides for staining with Warthin-Starry and modified-Giemsa stain concomitantly. Specimen with Whartin-Starry stain found 19 out of 30 were positive for H.pylori while in modified-Giemsa stain only 16 out of 30 specimen were positive for H.pylori. Detection of H.pylori Warthin-Starry stain give more chance to obtain positive result because it use silver technique that coat the bacteria making it is more clearly visible in microscopic examination.*

Keywords: *H.pylori detection, H.pylori infection, Warthin-starry, modified Giemsa, chronic gastritis.*

ABSTRAK

Helicobacter pylori adalah bakteri penyebab gastritis khronis yang umum dijumpai. Identifikasi infeksi H.pylori diperlukan untuk acuan terapi eradikasi. Deteksi H.pylori pada spesimen biopsi gaster dengan metode histopatologi merupakan teknik diagnostik yang diterima secara umum mengingat teknik ini lebih akurat dibandingkan dengan pemeriksaan serologi. Walaupun bakteri H.pylori dapat terlihat pada pengecatan rutin Hematoxylin-Eosin, akan tetapi umumnya digunakan pengecatan tambahan modified-Giemsa atau Whartin-Starry untuk melihat bakteri dengan jelas. Pengecatan modified-Giemsa lebih disukai di banyak sentra laboratorium oleh karena lebih murah dan lebih mudah dikerjakan akan tetapi pengecatan Whartin-Starry dapat melihat bakteri lebih jelas dengan kontras yang lebih baik. Penelitian ini bertujuan untuk membandingkan adanya perbedaan hasil identifikasi H.pylori pada kedua jenis pengecatan tersebut. Blok parafin biopsi lambung diambil dari pasien dengan gastritis kronis yang diperiksa di Laboratorium Patologi Anatomik Rumah Sakit Dr. Soetomo Surabaya pada tahun 2017. Tiga puluh blok parafin diambil secara acak untuk dibuat slide mikroskopis dan dilakukan 2 jenis pewarnaan yakni Warthin-Starry dan modified-Giemsa. Pada pengecatan Whartin-Starry bakteri H.pylori terdeteksi pada 19 dari 30 spesimen sedangkan pada pengecatan modified-Giemsa hanya 16 dari 30 spesimen yang menunjukkan adanya H.pylori. Deteksi infeksi H.pylori dengan pengecatan Whartin-Starry memberikan hasil positif yang lebih banyak dibandingkan dengan pengecatan modified-Giemsa. Pereaksi perak pada reagen Whartin-Starry dapat membuat bakteri menjadi terlihat lebih jelas pada pengamatan mikroskopis.

Kata kunci: *deteksi H.pylori, infeski H.pylori, pewarnaan Warthin-starry, pewarnaan modified giemsa, gastritis kronis.*

INTRODUCTION

H.pylori is a bacteria that closely related with chronic gastritis and dyspepsia. Chronic gastritis case and dyspepsia are commonly found in daily practice. Beside causing chronic gastritis, *H.pylori* infection plays some important role in the presence of gastric malignancy either in the form of gastric carcinoma or gastric lymphoma.[1,2] According to the American College of Gastroenterologists guidelines on management of *H.pylori* infection[3], the diagnostic method *H.pylori* infection detection comprises of urea breath test and gastric endoscopic biopsy. Serology method should be avoided. Nevertheless if the serologic test gave positive results, it should be confirmed with a test that identify an active infection such as the urea breath test or stool antigen test. Urea breath test, stool antigen test, histology examination with special staining for *H.pylori* organisms, and bacteria culture are considered to be the gold standard tests for diagnosis of *H.pylori* infection.[4,5]

Culture is not routinely used for initial diagnosis of *H.pylori* infection but is required for antibiotic susceptibility testing if physicians suspect antibiotic resistance in patients who have previously failed therapy.[5] For stool antigen test, Mayo Medical Laboratories utilizes the POCone Infrared Spectrophotometer. Performance characteristics for this instrument have not been established for persons under age 3. For patients 3 to 17 years, age, weight and height must be included in test request for appropriate result interpretation.[6]

The gold standard diagnostic test for *H.pylori* infection is to find the bacteria through direct smear or *H.pylori* culture.[4] Cross reaction with other antigens often encountered in serology test with possibly to obtain false positive result. Several staining methods of biopsy specimen are proposed to detect *H.pylori* infection, usually start with routinely stain hematoxylin-eosin to more specific stain using monoclonal antibody for immunohistochemistry testing.[7] Whartin-Starry stain is used mainly to detect Spirochetes bacteria with silver impregnation methods. Whartin starry has some advantage compare to other methods because it gives more contrast color (bacteria stained black in yellow background).[8] This method can improve sensitivity of its detection. On the other hand, Giemsa stain is a common method for examining blood smear. In tissue, Giemsa stain can detect microorganism that appears dark blue in pink-pale blue background. A modification of Giemsa stain was proposed to reduced the staining time and it was known as modified-Giemsa technique.[9] Modified-Giemsa stain is a non-silver stain that commonly use to detect *H pylori* infection in many health centre including Dr. Soetomo general hospital. The staining methods are quite simple and the reagent is easy to obtain and cheap. Sometimes it fails to detect microorganism due to lack of contrast in dirty background. It must be used by experienced experts. There are still a debate which stain should be used to detect *H.pylori* as a routine procedure.[10] This study aim is to compare the result of these two staining methods for detection of *H.pylori* infection in gastric biopsy specimens and to find out if there is difference result of *H.pylori* identification in gastric biopsy tissue using Whartin–Starry stain compared to modified-Giemsa stain.

MATERIAL AND METHOD

Thirty paraffin blocks from patients who diagnosed clinically as chronic gastritis were retrieved from Anatomic Pathology laboratory archives at Dr. Soetomo hospital Surabaya in the year 2017. Two microscopic slides were made from each paraffin blocks by slicing the tissue 6 µm thick and placed on object glass. The first slide was stained with modified-Giemsa technique according to Bancroft[9] which have done routinely in the laboratory while the other slides stained with Warthin-Starry for spirochetes (Bio-optica cat no.04-040903). In modified-Giemsa stain, the *H.pylori* bacteria were identified as a reddish purple microorganism in the background of other cells that was stained blue and pale-blue. Warthin-Starry stain give more contrast colour. The bacteria were stained black while the background cells are stained yellow. The two microscopic slides were assessed by one pathologist and *H.pylori* infection scoring was made according to updated Sidney classification system.[11] *H.pylori* infection in gastric tissue biopsy were score as follows: score +1 when there were sparse bacteria found in specimen (mild density of bacteria), score +2 when there were some bacteria (moderate density) and score +3 when there were many bacteria in tissue (marked bacteria were found).[12] This study has been approved by Health-Research Ethical Commission in Dr. Soetomo General Hospital.

RESULT AND DISCUSSION

On examination of 30 gastric biopsy specimens with Warthin-Starry stain, 19 tissue biopsies were found positive for *H.pylori* bacteria while specimens with modified-Giemsa stain only 16 biopsies tissue were found positive for *H.pylori* (Table 1).

Table 1. Comparison of *H.pylori* bacteria examination with Warthin-Starry and modified-Giemsa stain

	Warthin-Starry with positive bacteria	**Warthin-Starry with negative bacteria**
Modified-Giemsa with positive bacteria	16	0
Modified-Giemsa with negative bacteria	3	11

Detection of *H.pylori* in gastric biopsy specimen has been accepted worldwide to diagnose *H.pylori* infection. Culture method is gold standard for infection detection but *H.pylori* culture did not routinely used due to its complexity and need a longer period to obtain the result.[4] Until this date, majority of microbiologic laboratory in the world are not equipped to perform *H.pylori* culture. Instead of culture, PCR technique for detection of *H.pylori* may be considered as a gold standard, but PCR technique also has a limitation due to genetic sharing of other microbes which can gives false positive result and the presence of PCR inhibitors can give false negative result in specimen with low bacterial count.[13]

Histological examination from gastric biopsy specimen has been a method of choice for identifies *H.pylori* infection. There are several staining methods that can be used to identify the presence of the bacteria which can be classified into two groups: silver-based stain and common histochemistry stain. Silver-based stain such as Whartin-Starry stain has an advantage over other stain since it can detect *H.pylori* bacteria even if its morphology has been altered by proton pump inhibitor and antibiotic administration, which can alter the bacteria morphology to cocoid and short bacillary forms. Proton pump inhibitor administration can cause *H.pylori* migrate into deeper portion of oxyntic glands, making its detection is impossible without a silver-based or immunohistochemical stain.[14]

Modified-Giemsa stain is a histochemistry stain that has been performed in lots of laboratory because it is cheap and simple method. The lack of contrast is a disadvantage of this technique. Silver-based stain such as *H.pylori* silver stain and Whartin-Starry stain give more clearly visible bacteria. So it can be detected easily on histological examination. The disadvantage of this technique it is more expensive than modified-Giemsa stain and it takes longer period to do the staining protocol.[15]

Immunohistochemistry with specific antibody has been proposed to be used as a gold standard to detect *H.pylori* infection.[8] But according to the study of Patel, *et al.* there is no difference result for *H.pylori* detection by immunohistochemistry technique compared with modified-Giemsa stain. The limitation of immunohistochemistry technique is more expensive than any other stain including Whartin-Starry and it took longer period than any other stain. It also needs a control specimen to be done with every slide making it is more complicated.[13]

Table 1 is showed that there were 3 cases of *H.pylori* infection detected by Warthin-Starry stain which did not detected by modified Giemsa stain. The discordance occurs due to lack of contrast between the color of micro-organism and background color. Using Whartin-Starry stain one can easily direct to get the presence of the bacteria because it provides black color of bacteria in yellow background. Furthermore, the silver reagent in Whartin-Starry stain gives a good result for detecting *H.pylori* because the organism are coated with the silver stain and therefore look larger, making their identification easier.[15]

Assessment of *H.pylori* infection in gastric biopsy specimen has performed according to Updated Sidney System scoring. The method is to detect *H.pylori* bacteria throughout entirely biopsy specimen and making the score by counting the number of bacteria in one high power field which give most number of bacteria.[11] The result of *H.pylori* scoring according to Updated Sidney System was presented in Figure 1.

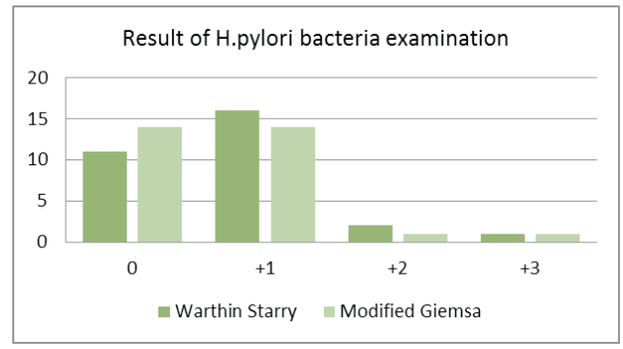

Figure 1. Result of *H.pylori* bacteria examination with Warthin-starry dan modified Giemsa stain.
Score 0 = no bacteria detected, score +1 = only sparse bacteria detected, score +2 = moderate bacteria count detected, score +3 = many bacteria detected.

Figure 1 is showed that Warthin-starry stain gives more results of identified bacteria compared to modified-Giemsa stain. Three cases show *H.pylori* score 0 on modified Giemsa staining (no bacteria detected) while these cases show sparse *H.pylori* bacteria (score +1) on Warthin-starry staining. There were also 1 case which gives *H.pylori* score +1 with modified Giemsa staining while it gives score +2 with Warthin-starry staining.

H.pylori infection can be easily detected with Whartin-starry stain compare to that of modified Giemsa stain either in low density or high density bacteria as shown at Figure 2 and Figure 3.

Figures 2 and Figure 3 are showed various *H.pylori* score in gastric biopsy specimen. This study was showed that there were some cases when the presence of *H.pylori* infection is either undetectable or detectable in smaller amounts in modified-Giemsa stain compare to Whartin-Starry stain. In Warthin-starry stain the spirochete bacteria wall were react with silver nitrate impregnation so that the bacteria will appear black within a yellow background. According to Glickman, *H.pylori* bacteria can not be detected if it present in a small number.[16]

The gold standard diagnosis of *H.pylori* infection in gastritis is made by finding *H.pylori* bacteria in gastric tissue. Gastric tissue specimens are generally obtained by endoscopic biopsy techniques. This technique is a minimally invasive but it can see directly at the morphology of the gastric tissue followed by tissue endoscopic biopsy for pathology examination.[17]

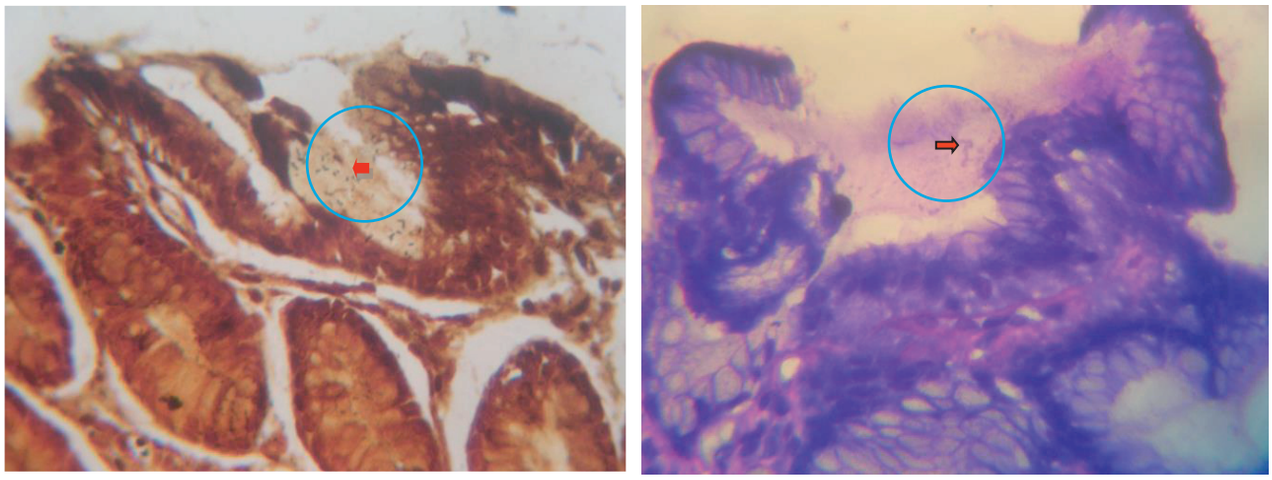

Figure 2. Gastric biopsy specimen with high density of H.pylori bacteria (+3). Specimen stianed with Whartin-starry (left) and modified Giemsa (right). Thebacteria were found on gastric mucous layer (➡) (400 X magnification).

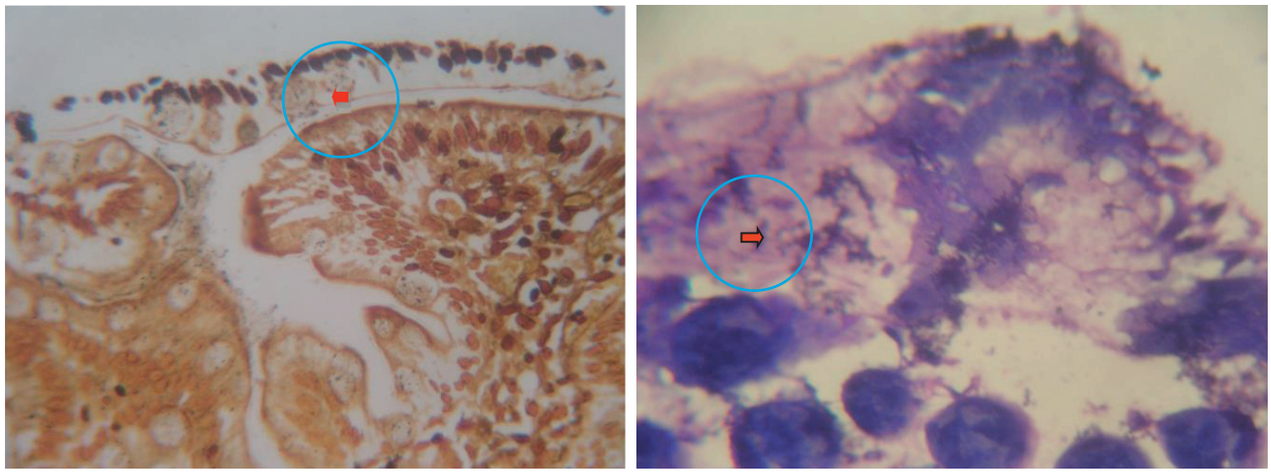

Figure 3. Gastric biopsy specimen with low density of H.pylori bacteria (+1). Specimen stianed with Whartin-starry (left) and modified Giemsa (right). Thebacteria were found on gastric mucous layer (➡) (400 X magnification).

The presence of *H.pylori* bacteria in gastric biopsy can be detected by culture, PCR technique and *H.pylori* visualization techniques with histochemical stain to indentify directly *H.pylori* by light microscope.[7] The histochemical technique of gastric biopsies has an advantage over culture methods because it can directly see *H.pylori* in a relatively simple and faster way. Therefore, culture methods are not commonly used for *H.pylori* detection. Histochemical staining techniques are also easier to perform and have less cost than PCR molecular techniques. The histochemical technique has become the standard diagnostic of *H.pylori* infection in many health centers.[4]

Routine hematoxyllin-eosin staining has been used in *H.pylori* examination but in several cases the bacteria can not be visualized without special stain.[18] Histochemical special stain for *H.pylori* detection include Gimenez, Toluidine blue, Romanowski, Genta and Giemsa stain which can be used for visualization of with various modifications (modified-Giemsa) for a more rapid work-up time.[4] Silver-based stain such as Warthin-Starry stain has advantage compare to common histochemistry stain since it uses silver impregnation technique to give black color to *H.pylori* bacteria. In Warthin-starry, *H.pylori* bacteria will appear dark brown, making them easier to see and can increase the sensitivity of *H.pylori* detection in gastric biopsy tissue.[14] This study was performed on gastric endoscopic biopsy specimens in patients with chronic gastritis. In this study, 19 samples (63.33%) were detected as *H.pylori* positive on Warthin-Starry stain from 30 biopsy specimens of chronic gastritis patients. Adlekha, et al reported *H.pylori* infection in 329 of 530 (62%) chronic gastritis patients in Kerala India.[19] The positivity of *H.pylori* from India is similar to this study. Adlekha took a gastric biopsy specimen from patients with dyspepsia complaints as many as 530 patients in 2010–2012. On microscopic slides biopsy material performed Hematoxylin-Eosin and modified-Giemsa staining. *H.pylori* examination was

performed by a Pathology specialist and the results were presented in grading (mild, moderate and severe) infections in accordance with the updated Sydney grading system.[11]

Immunohistochemistry technique using monoclonal antibody anti-*H.pylori* can gives more specific result. However, this technique rarely be done in many Pathology laboratory due to higher cost of antibody-based detection.[20] According to Rodger Haggit recommendation: immunohistochemistry stain should not be use as a routine procedure. It was only performed in gastric biopsy specimen with chronic inflammation with negative finding on *H.pylori*.[21] Detection of *H.pylori* bacteria by special stain has been served as a routinely *H.pylori* detection in tissue specimen.[13]

The results of this research showed difference of *H.pylori* detection due to increased sensitivity detection by Warthin-Starry stain compared to modified-Giemsa stain. Warthin-starry was found more specimens with positive result than modified-Giemsa stain in 3 out of 30 cases. The presence of *H.pylori* that is undetected by the observer is largely due to lack of color contrast in modified-Giemsa stain. Bacteria are missed from observation in *H.pylori* infection with mild intensity due to the unclear color. In infections with moderate to severe intensity, this result found 100% concordance result of *H.pylori* stained with modified-Giemsa compared to Warthin-Starry.

CONCLUSION

A study has been conducted to find the difference of *Helicobacter Pylori* detection in gastric biopsy tissue with Warthin-starry and modified Giemsa staining. Detection of *H.pylori* Warthin-starry stain give more possibility to obtain positive result because it use silver technique that coat the bacteria making it is more clearly visible in microscopic examination.

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Conflict of interest. There is no conflict of interest for this research. The manuscript has not been published previously.

REFERENCES

1. Watari J, Chen N, Amenta PS, Fukui H, Oshima T, Tomita T, *et al.* Helicobacter pylori associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. World J Gastroenterol. 2014; 20(18): 5461–5473.
2. Barbosa AJA. Detection of *H.pylori* in endoscopic gastric biopsies: a routine research that goes far beyond the laboratory limits. J Brasileiro de Patologia e Medicina Lab. 2015;51(2):70-71.
3. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG Clinical Guideline:Treatment of Helicobacter pylori Infection. Am J Gastroenterol 2017 Feb; 112: 212–238.
4. Talebi Bezmin Abadi A. Diagnosis of Helicobacter pylori Using Invasive and Noninvasive Approaches. J Pathog. 2018 May 22; 2018:9064952. doi: 10.1155/2018/9064952..
5. Garza-González E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla, FJ. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol. 2014 Feb; 20(6): 1438–1449.
6. Shimomaya T. Stool antigen tests for the management of Helicobacter pylori infection. World J Gastroenterol. 2013 Dec; 19(45): 8188-8191
7. Ramis IB, de Moraes EP, Fernandes MS, Mendoza-Sassi R, Rodrigues O, Juliano CRV, *et al.* Evaluation of diagnostic methods for the detection of Helicobacter pylori in gastric biopsy specimens of dyspeptic patients. Braz J Microbiology. 2012; 43(3):903–908.
8. Lee JY and Kim N. Diagnosis of Helicobacter pylori by invasive test: histology. Ann Transl Med. 2015; 3(1):10-17.
9. Morris GB, Ridgway EJ, Suvarna SK. Traditional stains and modern techniques for demonstrating microorganisms in histology. In: Suvarna SK, Layton C, Bancroft JD, editors. Bancroft's theory and practice of histological techniques 8th ed. Philadelphia: Churchill Livingstone/Elsevier; 2013, p.263.
10. Smith SB, Snow AN, Perry RL, Qasem SA. Helicobacter pylori: To Stain or Not to Stain? Am J Clin Pathol. 2012; 137:733-738.
11. Sipponen P, Price AB. The Sydney System for classification of gastritis 20 years ago. J Gastroenterol Hepatol. 2011 Jan;26 Suppl 1:31-4.
12. Rugge M, Pennelli G, Pilozzi E, Fassan M, Ingravallo G, Russo VM, Di Mario F. Gastritis: the histology report. Dig Liver Dis. 2011 Mar;43 Suppl 4:S373-84.
13. Patel SK, Pratap CB, Jain AK, Gulati AK, Nath, G. Diagnosis of Helicobacter pylori: What should be the gold standard? World J Gastroenterol. 2014 Sep; 20(36): 12847–12859.
14. Genta RM and Lash RH. Helicobacter pylori-negative Gastritis: Seek, Yet Ye Shall Not Always Find. Am J Surg Pathol. 2010; 34(8):e25-e34.
15. Farouk WI, Hassan NH, Ismail TR, Daud IS, Mohammed F. Warthin-Starry staining for the detection of Helicobacter pylori in gastric biopsies. Malays J Med Sci. 2018;25(4):92–99.
16. Glickman JN, Noffsinger A, Nevin DT, Ray M, Lash RH, Genta RM. Helicobacter infections with rare bacteria or minimal gastritis: Expecting the unexpected. Dig Liver Dis, 2015; 47(7):549-55.
17. Wang YK, Kuo FC, Liu CJ, Wu MC, Shih HY, Wang SSW, *et al.* Diagnosis of *Helicobacter pylori* infection: Current options and developments. World J Gastroenterol. 2015 Oct; 21(40): 11221–11235.
18. Versalovic J. Helicobacter pylori: Pathology and diagnostic strategies. Am J Clin Pathol. 2003 Mar;119(3):403-12.
19. Adlekha S. Chadha T, Krishnan P, Sumangala B. Prevalence of Helicobacter pylori infection amongst patients undergoing upper gastrointestinal endoscopy in a medical college hospital in Kerala, India. Ann Med Heatlh Sci Res. 2013 Oct-Des; 3(4): 559-563.
20. Wang XI, Zhang S, Abreo F, Thomas J. The role of routine immunohistochemistry for Helicobacter pylori in gastric biopsy. Ann Diagn Pathol. 2010 Aug;14(4):256-9.
21. Batts KP, Ketover S, Kakar S, Krasinskas AM, Mitchell KA, Wilcox R, *et al.* Appropriate use of special stains for identifying Helicobacter pylori: Recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society. Am J Surg Pathol. 2013 Nov;37(11):e12-22.

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Research Report

IDENTIFICATION AND PREVALENCE OF GASTROINTESTINAL PARASITES IN BEEF CATTLE IN SIAK SRI INDRAPURA, RIAU, INDONESIA

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ABSTRACT

Gastrointestinal (GI) parasites infection are one of the major constraints cattle farm in tropical countries including Indonesia and some of GI parasites in cattle have the potential to transmit to humans. This study was aimed to identify and determine the level of prevalence of gastrointestinal (GI) parasites in beef cattle in Siak Sri Indrapura District, Riau Province, Indonesia. This research was conducted on 100 beef cattle consisted of, respectively, 32, 34 and 34 cattles from Bungaraya, Sabak Auh, and Dayun sub-district. The characteristic of sample such as age and sex cattle, cage management, feed and drinking water were recorded. Native, sedimentation and sucrose flotation methods were used to find protozoa and eggs worm. We assessed GI parasites based on finding eggs worm, protozoan cyst and coccidia oocysts in stool samples and identification of GI parasite was based on the morphology and size of the eggs worm and cysts or oocysts of protozoan. The result showed that all of 100 feces samples that examined 100% positive infection for parasites. There were eleven types of gastrointestinal parasites that have been identified, 6 genera of protozoan and 5 genera of worms. The prevalence of gastrointestinal parasites in beef cattles in Siak Sri Indrapura District were Blastocystis sp. *(100%),* Entamoeba sp. *(90%),* Eimeria sp. *(53%),* Giardia sp. *(7%),* Balantidium coli *(4%),* Cryptosporidium sp. *(2%),* Oesophagustomum sp. *(45%),* Toxocara vitulorum *(20%),* Moniezia expansa *(9%),* Trichuris sp. *(5%), and* Fasciola sp. *(4%). In conclusion, Siak Sri Indrapura Riau is an endemic GI parasite and this can threaten the health of livestock and potentially as a zoonotic transmission.*

Keywords: *Identification, Prevalence, Beef cattle, Gastrointestinal (GI) parasites, Siak Sri Indrapura Riau Indonesia*

ABSTRAK

Infeksi parasit gastrointestinal (GI) merupakan salah satu kendala utama pada peternakan sapi di negara-negara tropis termasuk Indonesia dan beberapa parasit GI pada sapi berpotensi menular ke manusia. Penelitian ini bertujuan untuk mengidentifikasi dan mengetahui tingkat prevalensi parasit gastrointestinal (GI) pada sapi potong di Kabupaten Siak Sri Indrapura, Provinsi Riau, Indonesia. Penelitian ini dilakukan pada 100 sapi potong yang terdiri atas 32 sapi dari Kecamatan Bungaraya, 34 sapi dari Kecamatan Sabak Auh, dan 34 sapi dari Kecamatan Dayun. Dilakukan pencatatan karakteristik sampel seperti umur dan jenis kelamin, manajemen kandang, pakan dan air minum. Deteksi parasit menggunakan metode natif, sedimentasi dan apung dengan sukrosa. Sapi dinyatakan terinfeksi parasit gastrointestinal berdasarkan pada penemuan telur cacing, kista protozoa dan ookista koksidia pada feses dan identifikasi parasit didasarkan pada morfologi dan ukuran telur, kista atau ookista protozoa. Hasil penelitian menunjukkan bahwa 100 sampel feses yang diperiksa 100% positif terhadap infeksi parasit. Terdapat 11 genus parasit gastrointestinal yang terdiri atas 6 genus protozoa dan 5 genus cacing. Prevalensi parasit gastrointestinal yang ditemukan pada sapi potong di Kabupaten Siak Sri Indrapura adalah Blastocystis sp. *(100%),* Entamoeba sp. *(90%),* Eimeria sp. *(53%),* Giardia sp. *(7%),* Balantidium coli *(4%),* Cryptosporidium

sp. *(2%),* Oesophagustomum sp. *(45%),* Toxocara vitulorum *(20%),* Moniezia expansa *(9%),* Trichuris sp. *(5%), dan* Fasciola sp. *(4%). Kesimpulannya, Siak Sri Indrapura Riau merupakan daerah endemik parasit gastrointestinal dan ini dapat mengancam kesehatan ternak dan berpotensi sebagai penularan zoonosis.*

Kata kunci: *Identifikasi, Prevalensi, Sapi potong, Parasit Gastrointestinal (GI), Siak Sri Indrapura Riau Indonesia*

INTRODUCTION

Gastrointestinal parasites infections are the major cause of gastroenteritis in livestock throughout the in world.[1] It is have impacts on public and animal health around the world, mainly in developing countries.[2] Some gastrointestinal parasites in cattle have the potential to transmit zoonoses to humans. The gastrointestinal parasites in cattle including *Trichuris* spp., *Strongyloides* sp., *Criptosporidium parvum*, *Balstocystis* sp., *Giardia* sp. and hookworms are zoonotic.[3,4,5,6] According to Marskole *et al.* "gastrointestinal parasites cause considerable global economic losses as a consequence of reduced weight gain, digestive disturbance, lowered production, impaired reproductive performance, abnormalities in infected organs, and mortality in infected animals".[2]

Riau Province has the potential for livestock development and Siak Sri Indrapura District is a center for developing cattle in Riau. However, health surveillance of livestock is still lacking, through this study a survey of the prevalence of parasitic gastrointestinal diseases was carried out and also to determine the presence of zoonotic parasitic infections in beef cattle in Siak Sri Indrapura District.

MATERIAL AND METHODS

Study Area

Beef cattles from three Sub-district: Bungaraya, Sabak Auh and Dayun, Siak Sri Indrapura District, Riau Province, Indonesia were targeted for sampling. The reason for sampling at the location is because in Bungaraya, Sabak Auh and Dayun sub-districts are a breeding center. The geographical position of Siak Sri Indrapura District is located at N10º 16' 30" E100º 54' 21" (**Figure 1**).

Faecal Samples Collection

Fresh stools (feces) were collected directly from the ground and after defecation without disturbing the animals and the study was also explained orally to all participants (the farmers). One hundred fresh stools were collected, using gloves from 32 cattles from Bungaraya sub-district, 34 cattles from Sabak Auh sub-district and 34 cattles from Dayun sub-district. Collection sampel was conducted during 7-26 January 2018. All stool samples were collected in labeled urine container sterile and were preserved in 2.5% potassium dichromate. The characteristic of sample such as age and sex cattle, cage management, feed and drinking water were recorded.

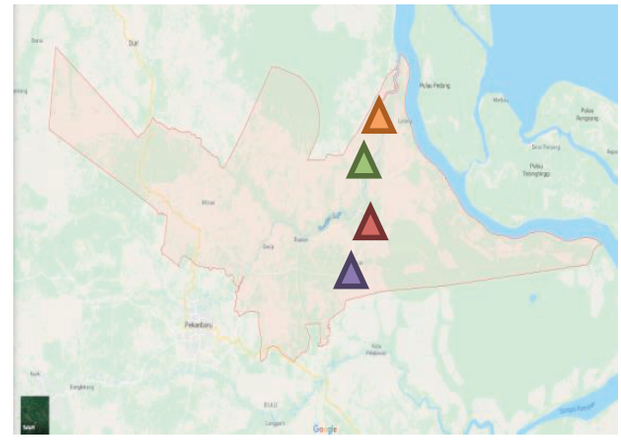

Figure 1. Map of location of sampling point. Pink color is Siak Sri Indrapura District. The shape of the triangle in the picture shows the sampling location: red color (district center of Siak Sri Indrapura); purple color (Dayun sub-district); green color (Bungaraya sub-district); and orange color (Sabak Auh sub-district).

Examination of Faecal Samples

Stool samples were analyzed at the Laboratory in Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Airlangga University, Surabaya Indonesia. Samples were examined for eggs worm, cysts and oocysts for protozoa by native, sedimentation and sucrose flotation methods. For native examination, the stool sample was taken and placed on the glass object and covered and than observed under microscope. For sedimention, filtrate was centrifugated 1.500 rpm for 5 minutes (by centrifuge HC 1180T 8 HOLE WITH TIMER, China), supernatant was removed. This step was repeated until three time. Sediment was taken slightly and placed in a slide to observed under microscope. Then, remaining sediment was diluted in sucrose solution and centrifuged at 1.500 rpm for 10 minutes. Floated was added sucrose solution until end of tube and was covered by a cover glass. The cover glass was transferred to object glass, and observed under light microscope at 100x and 400x magnification. Identification of GI parasites were based on the morphology and size of the eggs, cysts or oocyts.[7]

RESULT AND DISCUSSION

The characteristics of stool samples were provided in **Table 1**. The age of cattles ranged from 3 months to 3 years, mostly more than one year, the majority of beef

cattle population are females, extensive management and the feed was grass and drinking was well water. Out of the three locations, the characteristics of stool samples in Siak Sri Indrapura District were almost the same, only the management in Sabak Auh sub district was better, extensive management is less than the other two places.

Table 2 and **Table 3** were showed the kind of the GI parasites that found. Eleven parasites were found, 6 genera of protozoa and 5 genera of worm. The six genera of protozoan were *Blastocystis* sp. (100%), *Entamoeba* sp. (90%), *Eimeria* sp. (53%), *Giardia* sp. (7%), *Balantidium coli* (4%), *Cryptosporidium* sp. (2%), and the five genera of worms were *Oesophagustomum* sp. (45%), *Toxocara vitolorum* (20%), *Moniezia expansa* (9%), *Trichuris* sp. (5%), and *Fasciola* sp. (4%). Almost of cattles infected by more than one genus GI parasites, only one cattle (from Dayun) infected with one genus parasite, *Blastocystis* sp. and there are mixed infections in the sample examined for up to seven genera parasites. **Figure 2** was showed the morphologically of the gastrointestinal parasites found in this study using a light microscope.

Table 1. Characteristics of Stool Samples in Siak Sri Indrapura, Riau

Characteristic Samples		Number of Beef Cattle in each Sub District (head/%)			Total Number (head/%) (n=100)
		Bungaraya (n=32)	Sabak Auh (n=34)	Dayun (n=34)	
Age	3 - 6 months	3(9.38%)	0(0%)	1 (2,94%)	4 (4%)
	>6 months – 1 year	9(28.12%)	14(41.18%)	5(14.70%)	28(28%)
	>1-2 years	11(34.37%)	15(44.12%)	15(44.12%)	41(41%)
	>2-3 years	9(28.12%)	5(14.71%)	13(38.23%)	27(27%)
Sex	Male	8(25%)	11(32.35%)	8(23.53%)	27(27%)
	Female	24(75%)	23(67.65%)	26(76.47%)	73(73%)
Cage Management	Intensive	8(25%)	16(47.16%)	2(5.88%)	26(26%)
	Semi Intensive	2(6.25%)	7(20.59%)	10(29.41%)	19(19%)
	Extensive	22(68.75%)	11 (32.35%)	22(64.71%)	55(55%)
Feed and drinking water	Grass, well water	0(0 %)	16(47.06%)	0(0 %)	16(16%)
	Grass, well water + salt	21(65.62%)	0(0 %)	0(0 %)	21(21%)
	Grass + waste of tofu, well water	0(0 %)	18(52.94%)	0(0 %)	18(18%)
	Grass + stump of oil palm+ rice bran, well water	8(25%)	0(0 %)	28(82.35%)	36(36%)
	Grass + stump of oil palm+ rice bran, pool water	0(0 %)	0(0 %)	6(17.65%)	6(6%)
	Grass + stump of oil palm+ rice bran, well water	3(9.38%)	0(0 %)	0(0 %)	3(3%)

Table 2. Prevalence of Gastrointestinal Parasites in Beef Cattles in Siak Sri Indrapura, Riau

Types Parasite		Number of positive (Prevalence %)			Total Number of positive (Prevalence %) (n=100)
		Bungaraya Sub District (n=32)	SabakAuh Sub District (n=34)	Dayun Sub District (n=34)	
Protozoa	*Blastocystis* sp.	32 (100%)	34 (100%)	34 (100%)	100 (100%)
	Amoeba sp.	25(78.13%)	34 (100%)	31(91.18%)	90 (90%)
	Eimeria sp.	30 (93.75%)	19 (55.88%)	4 (11.76%)	53 (53%)
	Giardia sp.	5 (15.63%)	0 (0%)	2 (5.88%)	7 (7%)
	Balantidium sp.	3 (9.38%)	0 (0%)	1(2.94%)	4 (4%)
	Cryptosporidium sp.	2 (6.25%)	0 (0%)	0 (0%)	2 (2%)
Eggs Worm	*Oesophagustomum* sp.	24(75%)	14 (41.18%)	7(20.59%)	45 (45%)
	Moniezia expansa	4(1.25%)	1(2.94%)	4 (11.76%)	9 (9%)
	Toxocara vitulorum	4(1.25%)	7 (20.59%)	9 (26.47%)	20 (20%)
	Trichuris sp.	2(6.25%)	0 (0%)	3(8.82%)	5 (5%)
	Fasciola sp.	1(3.13%)	0 (0%)	3(8.82%)	4 (4%)

Table 3. Prevalence of Gatrointestinal Parasites Infection in Beef Cattle Based on Single or Mix Infection

Infection (Single/Mix)	Number of positive (%)			Prevalence (%) (n=100)
	Bungaraya (n=32)	Sabak Auh (n=34)	Dayun (n=34)	
Single infection parasite	0 (0%)	0 (0%)	1 (2.94%)	1 (1%)
Mix infection with two parasites	3 (9.37%)	11 (32.35%)	16 (47.05%)	30 (30%)
Mix infection with three parasites	6 (%)	8 (23.52%)	8(23.52%)	22 (22%)
Mix infection with four parasites	11 (18.75%)	12 (35.29%)	5 (14.70%)	28 (28%)
Mix infection with five parasites	9 (28.13%)	3 (8.82%)	3 (8.82%)	15 (15%)
Mix infection with six parasites	2 (6.25%)	0 (0%)	1 (2.94%)	3 (3%)
Mix infection with seven parasites	1 (3.13%)	0 (0%)	0 (0%)	1 (1%)

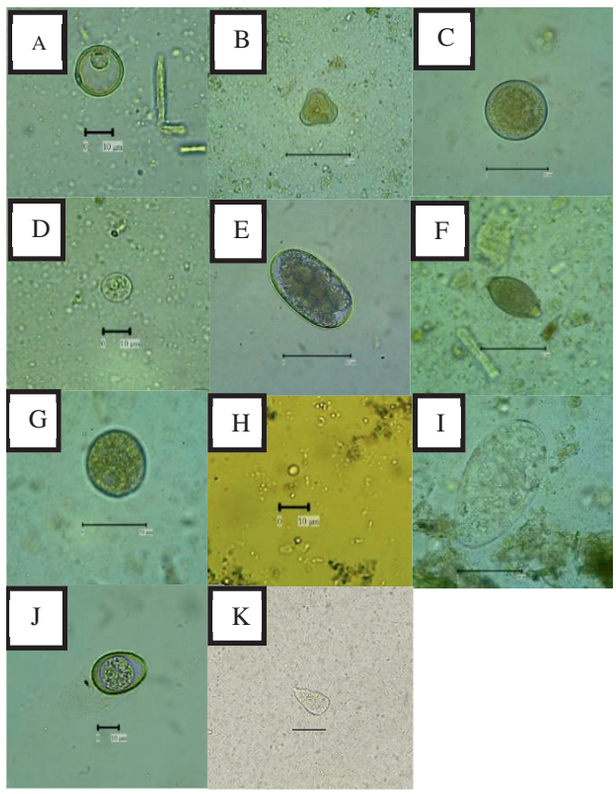

Figure 2. Morphological features of gastrointestinal parasites in beef cattle in Siak Sri Indrapura district, Riau Province. A). *Blastocystis* sp.; B). *Moniezia expansa*; C). *Toxocara vituorum*; D). *Entamoeba* sp.; E). *Oesophagustomum* sp.; F). *Trichuris* sp.; G). *Balantidium* sp.; H). *Cryptosporidium* sp.; I). *Fasciola* sp.; J) *Eimeria* sp.; K). *Giardia* sp.

This study is the first assessment of the prevalence of GI parasites in beef cattles in Siak Sri Indrapura District, Riau Province, Indonesia. The prevalence of GI parasites in beef cattles has been investigated in Riau Province by Rozi *et al.* it done in Tenayan Raya Pekanbaru Municipality and "they only emphasized on trematode worms, *Parampistomum* sp. and *Fasciola* sp".[8] The prevalence of *Fasciola* sp. infection in this study (4%) was much lower than their findings (50.43%; 49.02% in female and 60.71% in male). Although the prevalence is low, *Fasciola* infection must be aware because it has potential to infect humans. To date, *Fasciola* infection has been identified in human in many countries, with higher prevalence in farming communities in low income countries.[9]

In addition to *Fasciola* eggs worm, this study also found another parasites that are potentially zoonotic and its can be a source of transmission to human. They were *Blastocystis* sp., *Giardia* sp., *Balantidium* sp., *Cryptosporidium* sp., *Toxocara vitolorum* and *Trichuris* sp.

Blastocystis sp. is a parasite of the digestive tract of humans, livestock, birds, rodents, reptiles, dogs, pigs, cats and other animals.[10] The prevalence of *Blastocystis* sp. was high in this study, 100%. It raises the question, whether *Blastocystis sp*. actually a commensal protozoan or a pathogen. According to Parija and Padukone,[11] at date, understanding of the taxonomy, biology and pathogenicity of *Blastocystis* sp. were not fully clear yet, although *Blatocystis* sp. has been identified 100 years ago, but, in the recent decades, many researchers have focused their research on the pathogenicity of *Blastocystis* sp. Several research have reported that *Blastocystis* infection has potential to be a zoonotic parasite, base on finding the same subtype in both animals and human,[12] among 17 subtypes of *Blastocystis* in mammals and birds, 9 subtypes (ST1-9) can infect humans.[13] Badparva *et al.*[6] reported that the most common subtype *Blastocystis* in cattle were ST5, followed by ST3 and ST6. It means that cattle with Blastocyst positive can be a source of transmission to human.

The prevalence of *Giardia* sp. and *Cryptosporidium* sp. in beef cattles in this study were 7% and 2%, respectively. It was lower than research conducted in Bangladesh by Ehsan *et al.*[14] they reported that the prevalence of *Giardia* sp. and *Cryptosporidium* sp. infection in calf in Bangladesh were 22% and 5%, respectively. Evidence that *Giardia* sp. and *Cryptosporidium* sp. are zoonotic parasites have been reported by several researchers. Wegayehu *et al.*[3] reported that while *Cryptosporidium* sp. and *Giardia duodenalis* infected children and cattle in Ethiopia. The prevalence of *Giardia* sp. infection in children was significantly associated with contact with cattle and manure. Both, direct transmission of *Giardia* sp. and *Cryptosporidium* sp. between cattle and their handlers (farmers) and indirect transmission through water ponds were also investigated by Ehsan *et al.*[14]

Balantidium infection (Balantidiosis) is a zoonotic disease and it can infect humans and animal through the fecal-oral route. According to Wisesa *et al.*[15] and Hussin and Al-Samarai,[16] cattles are highly susceptible to balantidiosis. The prevalence of *Balantidium* sp. infection in beef cattle in Siak Sri Indrapura Riau was lower (4%) compared in Bali cattle in Bali Province, it was 17.19%.[15] While, Hussin and Al-Samarai[16] showed the prevalence of *B. coli* in cattle and their breeder in Baghdad Iraq were 29.50% and 9.09%, respectively. Although considered an opportunistic pathogen, *Balantidium coli* can cause severe illness. Randhawa *et al.*[17] reported case chronic cattle diarrhoea due to *Balantidium coli* infection.

In this study, the prevalence of *Amoeba* sp. was found in 90 (90%) beef cattles. It was higher than parasite infection rate than that reported in Korea.[18] Until now, the role of *Amoeba* sp. in cattle is still not understood.

More than 50% the prevalence of *Eimeria* sp. in beef cattle in Siak Sri Indrapura Riau. It was higher than the prevalance of *Eimeria* sp. in Bali female cattle in Nusa Penida Bali, which was 12%,[19] and in Bali and West and East Nusa Tenggara, was 9.6%.[20] While in Bandung West Java, the prevalence of *Eimeria* sp. infection in dairy cattle was 44.75%.[21] *Eimeria* sp. infection (coccidiosis) is responsible for major economic losses in animal husbandry worldwide.[22]

The prevalence of *Moniezia expansa* in the city of Siak is 9%, where the prevalence is higher compared to the prevalence of *Moniezia expansa* in Bali and Rambon cattle in the Morowali district, Central Sulawesi, which was 2.5% and lower to the prevalence of *Moniezia* sp. in slaughterhouses in Pontianak City, West Kalimantan, at 11.25%.[23,24] In addition to *Taenia saginata*, a group of *cestoda* worm that can infect humans.[25] The results of the study in 2018, *Moniezia expansa* which infects livestock (cattle, sheep and goats), have also been reported in humans.[26]

The prevalence of *Toxocara vitulorum* infection in beef cattle in Siak Sri Indrapura Riau was higher (20%) compared with Saraswati *et al.*[27] finding in Bali cattle in Bali Province (2.2%). The role of *T. vitulorum* in toxocariasis in humans is still not understood,[28] although *T.vitulorum* larvae were carried out in somatic migration mice.

Toxocariasis (*Toxocara vitulorum*) which attacks cattle can be transmitted through oral fecal contamination, placenta (transplacenta) and milk (transmamary). *Toxocara* infection in animals can cause digestive tract disorders such as diarrhea, vomiting, constipation, intestinal damage to death. Besides infecting cows, *Toxocara vitulorum* can also infect spotted deer (*Axis axis*). *Toxocara* sp. infection causes diarrhea, loss of appetite, thinness and anemia, and even *Toxocara vitulorum* can cause pneumonia due to larval migration in the organs of the lung, liver organ damage and toxemia. Humans can be infected with Toxocariasis if they are eaten by infective eggs contaminated with dog, cat, livestock and soil feces, then the larvae hatch and then migrate through tissues and organs. It can cause visceral migrating larvae or larvae to migrate the eyes if they are trapped in a vein behind the eye which can cause permanent eye damage. The larvae was found in tissues (lung, liver and kidney) and milk are considered a source of transmission in humans.[29]

Mohd-Zain *et al.*[30] collected samples from playgrounds in Malaysia and found 95.7% *Toxocara* eggs and 88.3% another nematode. This prevalence shows very high contamination of local soil by parasitic eggs and can be a source of transmission to humans.[30]

Prevalence of *Oesophagustomum* sp. in beef cattle in Siak Sri Indrapura was 45% and this prevalence was higher than the prevalence of *Oesophagustomum* sp. in Badung Regency, Bali in 2011 and in the province of Bali in 2014, respectively, 1.85% and 0.27%.[31,32] *Oesophagostomum* spp. in ruminants was zoonotic worms that can cause a risk of environmental pollution and can lead to infection in humans.[33]

Prevalence of *Trichuris* sp. in Siak City, Riau is 5% of a total of 100 samples. This prevalence is higher when compared to the prevalence in Bali Province (1.5%) and lower than the prevalence of trichurosis in Bojonegoro District at 7.22% in the dry season and 5.19% in the rainy season. [32,34] The prevalence of trichurosis found in cattle in Bhutan is 4.31%,[35] and in Costa Rica (7.8-14.5%).[36] Meanwhile, the prevalence of trichinosis in dairy cows using intensive maintenance management is found to be much lower at 0.63% in Thailand,[37] and 1.2% in Ethiopia.[38] Prevalence of *Trichuris* sp. in Dayun sub-district is higher than in the regencies of Bungaraya and Sabak Auh by 8.82%. This is because livestock that are grazed have the potential to be infected with parasites higher than those who are fed in cages.[39] This statement also supports the results of the prevalence of *Trichuris* sp. in Sabak Auh District was 0%. More than 47% livestock management in the Sabak Auh Sub-district, was intensive management (Table 1). *Trichuris* was resistant to changes in temperature and humidity. On dry highlands, wet highlands (also Dayun sub-district), semi dry lands (Bungaraya and SabakAuh), *Trichuris* eggs can survive and develop for several years and cause infection in cattle.[32]

The advantages of intensive management were also shown in negative results (0%) in Sabak Auh for some parasites such as *Giardia* sp., *Balantidium coli*, *Cryptospodidium* sp., and *Fasciola* sp. (**Table 2**). Whereas that parasites have been discussed previously are zoonotic.

CONCLUSION

In conclusion, Siak Sri Indrapura District, Riau Province Indonesia is an endemic gastrointestinal parasite area and it can threaten the health of livestock and potentially as a zoonotic transmission.

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REFFERENCE

1. Ananta SM, Suharno, Hidayat Adi, Matsubayashi M. Survey on gastrointestinal parasites and detection of *Cryptosporidium* spp. on cattle in West Java, Indonesia. Asian Pac J. Trop Med. 2014; 7 (3): 197-201. doi: 10.1016/S1995-7645(14)60020-1
2. Marskole P, Verma Y, Dixit AK, Swamy M. Prevalence and Burden of Gastrointestinal Parasites in Cattle and Buffaloes in Jabalpur, India. Vet World. 2016; 9 (11): 1214–1217.
3. Wegayehu T, Adamu H, Petros B. Prevalence of *Giardia duodenalis* and *Cryptosporidium* species infections among children and cattle in North Shewa Zone, Ethiopia. BMC Infect Dis. 2013;13: 419.
4. Youn H. Review of zoonotic parasites in medical and veterinary fields in the Republic of Korea. Korean J Parasitol. 2009; 47 (Suppl.): S133-141.
5. Bawm S, Htun LL. Parasitic zoonoses in livestock and domestic animals of Myanmar and neighbouring countries. Asian J Anim Vet Adv [Internet]. Science Alert; 2015; 10 (11): 740–751. Available from: http://dx.doi.org/10.3923/ajava.2015.740.751.
6. Badparva E, Sadraee J, Kheirandish F. Genetic diversity of *Blastocystis* isolated from cattle in Khorramabad, Iran. Jundishapur J Microbiol. 2015; 8 (3): 4–7.
7. Meutchieye F, Kouam MK, Miegoue E, Nguafack TT, Tchoumboue J, Teguia A, Theodoropoulos G. A survey for potentially zoonotic gastrointestinal parasites in domestic cavies in Cameroon (Central Africa). BMC Vet Res [Internet]. BioMed Central; 2017 Dec 26 [cited 2018 Sep 12]; 13 (1): 196. Available from: http://bmcvetres.biomedcentral.com/articles/10.1186/s12917-017-1096-2
8. Rozi F, Handoko J, Febriyanti R. Infestation of Luke Worm (*Fasciola* sp.) and Stomach Worm (*Paramphistomum* sp) on Adult Bali Cattle in Tenayan Raya Pekanbaru Municipality. J Sain Veterine. 2015; 33 (1): 8–15.
9. Nyindo M A-HL. Fascioliasis: An ongo ing zoonotic trematode infection. Biomed Res Int. 2015: 1–27.
10. Yoshikawa H, Tokoro M, Nagamoto T, Arayama S, Asih PBS, Rozi IE, *et al.* Molecular survey of Blastocystis sp. from humans and associated animals in an Indonesian community with poor hygiene. Parasitol Int. 2016; 65 (6): 780–784.
11. Parija SC, Padukone S. *Blastocystis*: Pathogen or passenger? An evaluation of 101 years of research. Trop Parasitol. 2016; 6 (2): 163–164.
12. Osman M, Bories J, El Safadi D, Poirel MT, Gantois N, Benamrouz-Vanneste S, *et al.* Prevalence and genetic diversity of the intestinal parasites *Blastocystis* sp. and *Cryptosporidium* spp. in household dogs in France and evaluation of zoonotic transmission risk. Vet Parasitol [Internet]. Elsevier B.V.; 2015; 214 (1–2):167–170. Available from: http://dx.doi.org/10.1016/j.vetpar.2015.09.015
13. Cian A, El Safadi D, Osman M, Moriniere R, Gantois N, Benamrouz-Vanneste S, *et al.* Molecular epidemiology of *Blastocystis* sp. in various animal groups from two French zoos and evaluation of potential zoonotic risk. PLoS One. 2017; 12 (1): 1–29.
14. Ehsan AM, Geurden T, Casaert S, Parvin SM, Islam TM, Ahmed UM, *et al.* Assessment of zoonotic transmission of *Giardia* and *Cryptosporidium* between cattle and humans in rural villages in Bangladesh. PLoS One. 2015;10 (2):1–11.
15. Wisesa IBGR, Siswanto FM, Putra TA, Oka IBM, Suratma NA. Prevalence of *Balantidium*sp in Bali Cattle at different areas of Bali. Int J Agric For Plant. 2015; (1): 49–53.
16. Hussin AG, Al-samarai FR. Prevalence of *Balantidium coli* in cattle and cattle breeders in some regions of Baghdad in Iraq. Bang J Anim. Sci. 2016; 30–34.
17. Randhawa SNS, Singla LD, Randhawa CS. Chronic cattle diarrhoea due to *Balantidium* coli infection-a clinical report. J Vet Parasitol. 2010; 24 (2): 197–198.
18. Ismail HAHA, Jeon HK, Yu YM, Do C, Lee YH. Intestinal parasite infections in pigs and beef cattle in rural areas of Chungcheongnam-do, Korea. Korean J Parasitol. 2010; 48 (4): 347–349.
19. Indraswari AAS, Suwiti NK, Apsari IAP. *Eimeria auburnensis* and *Eimeria bovis* of Protozoa Gastrointestinal Infected on Female Bali Cattle in Nusa Penida. Bul Vet Udayana. 2017; 9 (1): 112–116.
20. Mastra IK, Saraswati, NKH, Sutawijaya IMG, Yunanto. Surveilance and Monitoring of Gastrointestinal Parasite Infection in Bali Cattle in Bali, West Nusa Tenggara and East Nusa Tenggara. Bul BBVet Denpasar. 2014; 26 (85): 1–12.
21. Sufi IM, Cahyaningsih U, Sudarnika E. Prevalence and Risk Factor of Coccidiosis in Dairy Cattle in Bandung District. JKH. 2016; 195–199.
22. Heidari H, Sadeghi-Dehkordi Z, Moayedi R, Gharekhani J. Occurrence and diversity of *Eimeria species* in cattle in Hamedan province, Iran. Vet Med (Praha). 2014; 59 (6): 271–275.
23. Widnyana IGN. Prevalence of Gastrointestinal worm parasite infection in Bali cattle and Rambon Cattle in Wosu Village, Bungku subdistrict, Morowali district. J Ilmiah Agro Pet. 2013; 10 (2): 1-8.
24. Tantri N, Setyawati TR, Khotimah S. Prevalence and Intensity of Parasitic worm eggs in cow (Bos sp.) feces in cow (*Bos* sp.) feces in Pontianak city sloughterhouse (West Kalimantan). J Protobiont. 2013; 2 (2): 102-106.
25. CDC. DPDx - Laboratory identification of parasites of public health concern. Center for Disease Control and Prevention. 2017.
26. Squire SA. Gastrointestinal helminths in farmers and their ruminant livestock from the Coastal Savannah zone of Ghana. Parasitol Res [Internet]. Parasitology Research; 2018; Available from: https://doi.org/10.1007/s00436-018-6017-1
27. Saraswati, NKH, Yunanto, Sutawijaya IGM. *Toxocara vitolorum* Prevalence in Bali Cattle in Bali Province. Bul BBVet Denpasar. 2015; 17 (86): 1–10.
28. Oguz B. Genetic Characterization of *Toxocara vitulorum* in Turkey by Mitochondrial Gene Markers (cox1). Acta Sci Vet. 2018; 46 (Januari): 1-6.
29. Indriyati L. Inventarization of Parasite Nematodes in Plant, Animal, and Human. Enviro Sci. 2017; 13 (3): 195–207.
30. Mohd-Zain SNM, Rahman R, Lewis JW. Stray animal and human defecation as sources of soil-transmitted helminth eggs in playgrounds of Peninsular Malaysia. J Helminthol. 2015; 89 (6): 740–747.
31. Arsani NM, Mastra IK, Saraswati NKH, Yunanto, Sutawijaya IGM. Epidemiology of Helminthiasis in Cattle in Bali Province. Bul Veteriner, BBVet Denpasar. 2015; 27 (87): 1–11.
32. Pramasudha AAR, Nyoman AS, Ida BMO. The Prevalence of *Trichuris* spp. In Bali Cattle According to the Layout Geographic of Bali Province. Udayana Veterinary Bulletin. Udayana Vet Bull. 2015; 7 (2): 202–208.
33. Karshima SN, Maikai BV, Kwaga JKP. Helminths of veterinary and zoonotic importance in Nigerian ruminants: A 46-year meta-analysis (1970-2016) of their prevalence and distribution. Infect Dis Poverty. Infectious Diseases of Poverty; 2018; 7 (1): 1–15.
34. Winarso A, Fadjar S, Yusuf R. Prevalence of Trichurosis in Beef Cattle in Kasiman Subdistrict, District of Bojonegoro, East Java. J Kaji Vet. 2015; 3 (2): 225–230.
35. Tshering G, Dorji N. Prevalence of Gastrointestinal Parasites in Free Range Cattle; a Case Study in Haa District Bhutan. J Anim Heal Prod. 2013; 1 (4): 36–37.
36. Jimenez AE, Fernandez A, Alfaro R, Dolz G, Vargas B, Epe C, *et al.* A cross-sectional survey of gastrointestinal parasites with dispersal stages in feces from Costa Rican dairy calves. Vet Parasitol. 2010; 173 (3–4): 236–246.
37. Jittapalapong S, Sangwaranond A, Nimsuphan B, Inpankaew T, Phasuk C, Pinyopanuwat N, *et al.* Prevalence of Gastro-Intestinal Parasites of Dairy Cows in Thailand. Kasetsart J Nat Sci. 2011; 45: 40–45.
38. Hiko A, Wondimu A. Occurrence of nematodiasis in Holstein Friesian dairy breed. J Vet Med Anim Heal. 2011; 3 (January): 6–10.
39. Bilal MQ, Hameed A, Ahmad T. Prevalence of gastrointestinal parasites in buffalo and cow calves in rural areas of Toba Tek Singh, Pakistan. J Anim Plant Sci. 2009; 19 (2): 67–70.

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Research Report

MTT FORMAZAN REPLACED WST-8 AS A BETTER SIMPLE SCREENING METHOD FOR DETECTION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

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ABSTRACT

We have previously developed a new method using a new formazan substrate WST-8, as a simple and rapid screening test for detection of glucose-6-phosphate dehydrogenase (G6PD) deficiency accomplished by the naked eye. However, it was little difficult to distinguish between faint orange colors developed by heterozygous females and pink colors of normal hemolyzed blood, since both have similar tones, but this was the only simple and rapid screening test can be applied in the field. To solve this problem, we established a newer and simple screening method has been established by replacing a different formazan substrate, MTT (3-(4,5-dimethyl-2- thiazolyl)-2,5-diphenyl-2H tetrazolium bromide) in combination with a hydrogen carrier, 1-methoxy phenazine methosulfate to replace WST-8. MTT formazan exhibits a purple color, thus allowing for the ability to easily distinguish the pink colors of hemolyzed blood. However, MTT has been reported to react with hemoglobin non-specifically and to interfere with the interpretation of the color reaction. In our examinations by mixing MTT with hemolyzed blood, we found that the non-specific reaction was very slow, and that the addition of a small amount of blood (5 ~ 10 µl) into a reaction mixture (800 µl) did not interfere the reaction of G6PD activity. In this new MTT method, a strong purple color was generated in normal blood samples at 20~30 min after incubation, which could be distinguished by the naked eye from G6PD-deficient blood samples with less than 50% residual activity and has the same sensitivity and negative predictive value as WST-8 (ca. 85%). In addition, quantitative measurement using a spectrophotometer was also possible despite the fact that MTT formazan is water-insoluble.

Keywords: *G6PD-deficiency, new screening method, formazan substrate, MTT, purple color development*

ABSTRAK

Kami sebelumnya telah mengembangkan metode tes skrining sederhana dan cepat untuk mendeteksi defisiensi glukosa-6-fosfat dehidrogenase (G6PD) menggunakan substrat formazan WST-8 yang dapat diamati langsung dengan mata telanjang. Namun mengalami sedikit kesulitan dalam membedakan antara warna oranye pudar yang dihasilkan oleh perempuan heterozigot dan warna merah muda yang disebabkan oleh hemolisis pada darah normal karena memiliki warna dasar yang sama, tetapi ini merupakan satu satunya tes skrining cepat yang dapat digunakan di lapangan. Untuk mengatasi hal ini, kami mengembangkan metode skrining G6PD baru dan sederhana dengan menggunakan substrat formazan lain, yaitu MTT (3-(4,5-dimethyl-2- thiazolyl)-2,5-diphenyl-2H tetrazolium bromide) yang dikombinasikan dengan1-methoxy phenazine methosulfate sebagai pengganti WST-8 . Formazan MTT akan menghasilkan warna ungu, sehingga dengan mudah dapat dibedakan dengan warna merah muda yang disebabkan oleh hemolisis pada darah normal. Walaupun disebutkan bahwa MTT dapat bereaksi non-spesifik dengan hemoglobin dan mengganggu interpretasi reaksi warna. Namun dari hasil penelitian kami dengan mencampurkan MTT dengan darah hemolisis, menunjukkan bahwa reaksi non-spesifik yang terjadi sangat lambat, dengan demikian bila penambahan hanya dengan sejumlah kecil sampel darah (5 ~ 10 µl) ke dalam campuran reaksi

(800 µl) tidak akan mengganggu reaksi aktivitas G6PD. Dengan metode MTT yang baru ini, akan menampilkan warna ungu yang kuat pada sampel darah normal dalam waktu 20 ~ 30 menit setelah inkubasi sehingga dengan mata telanjang dapat langsung dibedakan dengan sampel darah defisiensi G6PD dengan aktivitas enzim kurang dari 50% dan memiliki sensitivitas dan nilai prediksi negatif yang sama dengan WST-8 (sekitar 85%). Selain itu pengukuran kadar G6PD secara kuantitatif dapat dilakukan dengan menggunakan spektrofotometer walaupun disebutkan bahwa formazan MTT tidak larut dalam air.

Kata kunci: *defisiensi G6PD, metode skrining baru, substrat formazan, MTT, perubahan warna ungu.*

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most frequent hereditary disorders, with an estimated 400 million people affected worldwide, particularly in tropical areas including malaria endemic regions.[1] The G6PD gene spans 18 kb on the X chromosome (Xq28), containing an open reading frame of 1,545 base pairs encoded in 13 exons and 12 introns. To date, more than 400 G6PD biochemical variants have been described, and 186 mutations among them have been discovered at the molecular level.[2]

The most frequent clinical manifestation of G6PD deficiency is acute hemolytic anemia, which is usually triggered by taking specific oxidative drugs such as primaquine.[1] Primaquine has been used for the radical treatment of vivax malaria and for gametocytocidal action against falciparum malaria. Primaquine-induced hemolytic crisis is thus a serious problem in chemotherapeutic malaria control efforts. Therefore, primaquine should be administered to malaria patients only after normal G6PD activity is confirmed.

A number of surveys on malaria and G6PD deficiency of individuals living in malaria endemic areas of Southeast Asian countries [3-10] have been done using the Acridine Orange staining method for rapid diagnosis of malaria[11-12] and the WST-8 method[13] for rapid detection of G6PD deficiency. By using these methods, the results of a blood examination could be informed within 30 mins to the malaria patients and prescribed antimalarial drugs, including primaquine, on-site if their G6PD activity was normal.

Presently, several screening methods for detection of G6PD-deficiency in the field have been reported. The fluorescent spot test [14-15] is the most widely used screening method. However, this method requires an ultraviolet lamp in a dark room, and since it provides only a qualitative result, it is very difficult to identify heterozygous females. Other methods, such as the Formazan ring method [16] and the Sephadex gel method,[17] that do not require any equipment or electricity have been used in epidemiological studies.[18-22] Both of these methods have used a formazan substrate, MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide) and a hydrogen carrier, phenazine methosulfate (PMS). Unfortunately, both methods also provide only qualitative results and, thus, it was extremely difficult to diagnose heterozygous females. In addition, PMS is strongly photo-sensitive, and special attention is needed to protect against exposure to ordinary light during screening.[19]

Another formazan method, using WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitro phenyl)-5-(2,4-disulfophenyl)-2H tetrazolium monosodium salt) and 1-methoxy PMS [13] have been reported to overcome the disadvantages in the MTT/PMS methods. The 1-Methoxy PMS is a photo-resistant hydrogen carrier, and WST-8 formazan is highly water-soluble; both are easy to assay qualitatively and quantitatively. However, this method also has a disadvantage: a faint orange color developed by 30~50% G6PD residual activity (*i.e*, heterozygous female samples) is quite similar in tone to the pink color of hemolyzed blood, which is not so easy to distinguish by the naked eye, making it difficult to confidently identify heterozygous females.[9]

As MTT formazan exhibits a purple color, the WST-8 in previous method[13] was replaced by MTT formazan to be easier distinguish faint orange colors from pink colors (Figure 1). A newer rapid screening and detection method of G6PD deficiency by using MTT/1-methoxy PMS and naked eye without the interference of non-specific reactions between MTT and hemoglobin is reported herein.

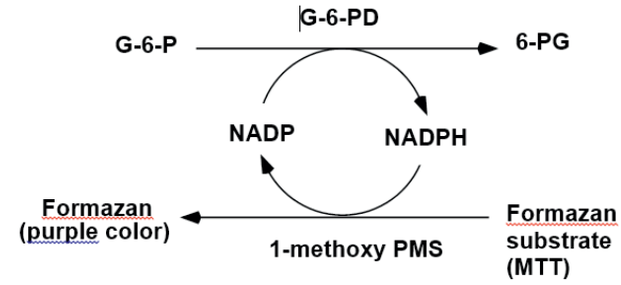

Figure 1. Principle of chemical reactions for detection of G6PD activity by using a formazan substrate, MTT

MATERIALS AND METHODS

Chemicals

Glucose-6-phosphate (G6P) and nicotinamide adenine dinuleotide phosphate (NADP) were obtained from Boehringer Co. (Mannheim, Germany). MTT, 1-methoxy PMS and the WST-8 diagnostic kit were purchased from Dojindo Laboratories (Kumamoto, Japan).

Preparation of Reaction Mixtures for the MTT Method

The transparent type of microcentrifuge tube should be used in this method. The reaction mixture in a 1.5-ml microcentrifuge tube consisted of: (1) 20 µl of the substrate mixtures containing 50 mM G6P, 4 mM NADP in 400 mM Tris-HCl buffer with 100 mM $MgCl_2$ (adjusted pH to 7.2~7.5), (2) 20 µl of 5 mM MTT in H_2O, (3) 20 µl of 1 mM 1-methoxy PMS in H_2O, and (4) 740 µl of H_2O. These substrates and dye solutions can be stored at least for 6 months at 4 °C in the dark or for several years at –20 °C.

Procedures

Normal blood (G6PD activity, 9.0 IU/g Hb), hemizygous male blood (1.0 IU/g Hb) and heterozygous female blood (4.1 IU/g Hb) were obtained from Indonesian donors (the senior author and two volunteers, respectively). Written informed consents were obtained from the two volunteers after the explanation of this study to them based on the guidelines ofthe Declaration of Helsinki. All IUs were measured by an ultraviolet spectrophotometric method using a biochemical assay kit (345-B, Trinity Biotech, Ireland).

The reaction was commenced after adding 5 µl of whole blood to the reaction tube and mixing by shaking several times. The reaction tube was then left to stand and color photographs were taken at various intervals. Development of purple color was observed by measuring the absorbance at 550 nm[23] of the reaction tubes at various intervals using an ultraviolet spectrophotometer, Hitachi U-2800 (Tokyo, Japan).

Examination of the non-specific reaction between MTT and hemoglobin was performed by mixing 5~25 µl normal blood into 800 µl of 0.125 mM MTT in H_2O (the same concentration of MTTs in the reaction mixture for the screening method) and the color change was observed at different intervals.

RESULTS

Non-specific Reaction with Hemoglobin

Fairbanks and Beutler [23]and Hirono *et al.*[17] have reported that MTT reacts with hemoglobin non-specifically, and its dark red or brown color strongly interferes with the interpretation of the color reaction. In our examinations of a 1.5-ml tube containing 800 µl of 0.125 mM MTT in H_2O, addition of 20~25 µl blood reacted with MTT albeit very slowly and the color of hemoglobin was changed to dark red at 6 hrs after incubation (Figure 2). Subsequently, small brownish precipitates were formed in the tubes at 8~10 hrs after incubation. However, these non-specific reactions were not observed when a small amount of blood (5~10 µl) was loaded (see tube 1 in Figure 3D). These results indicated that the interference by the non-specific reaction was negligible when 5~10 µl of blood were mixed with the 800-µl reaction mixture. Indeed, absorbance at 550 nm in the negative control did not change even in the presence of the same concentration of MTT (figure 4).

Qualitative Findings

Figure 3 shows the development of a purple color that was generated by different G6PD activities in 1.5-ml tubes. Appearance of the purple color in normal blood was observed at about 10 min after incubation at room temperature. At 30 min after incubation, a dark purple color in the normal blood sample (tube 4 in Figure 3A) was clearly distinguished from only a faint purple color produced by heterozygous female sample (tube 3 in Figure 3A). At 1 hr (Figure 3B) and 2 hrs (Figure 3C) after incubation, purple colors in the normal blood and heterozygous female blood became stronger, respectively, but at 3 hrs after incubation, small aggregates of MTT formazan formed, and the intensity of the purple color gradually decreased as the aggregates precipitated (tube4 in Figure 3D). Hemizygous male blood showed no change in color (tube 2 in Figure 3A-D), nor did the negative control (tube 1 in Figure 3A-D). However, the samples of heterozygous female blood (tube 3 in Figure 3A-D) showed a slow color development toward purple, and it was possible to visually differentiate them from positive and negative controls.

Time-course of Purple Color Development

Figure 4 shows the time-course of the purple color development in normal blood and in the negative controls without substrate. The color reaction of the normal blood reached a maximum after a 1.5~2-hr incubation, while the color development in the negative control did not occur during the 3-hr incubation (Figure 4). At 3 hrs after incubation, however, absorbance in the normal blood sample decreased as the formazan aggregates precipitated to the bottom of the tube. These results indicated that a quantitative measurement was possible until 2 hrs after incubation although MTT formazan was water-insoluble. All results taken together suggested that judgment of G6PD activity by the new MTT method should be performed between 30 to 60 min of incubation, particularly for accurate identification of heterozygous female samples.

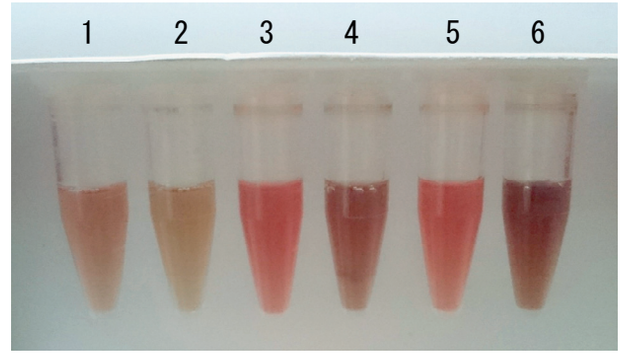

Figure 2. Development of dark red color by non-specific reactions between MTT and hemoglobin at 6 hrs after incubation.

Amount of blood loaded; 15 µl in tubes 1-2; 20 µl in tubes 3-4; 25 µl in tubes 5-6. Tubes 1, 3 and 5, controls without MTT; tubes 2, 4 and 6, 0.125 mM MTT in 800 µlH_2O. Note that hemoglobin colors are changed to dark red in tubes 4 and 6.

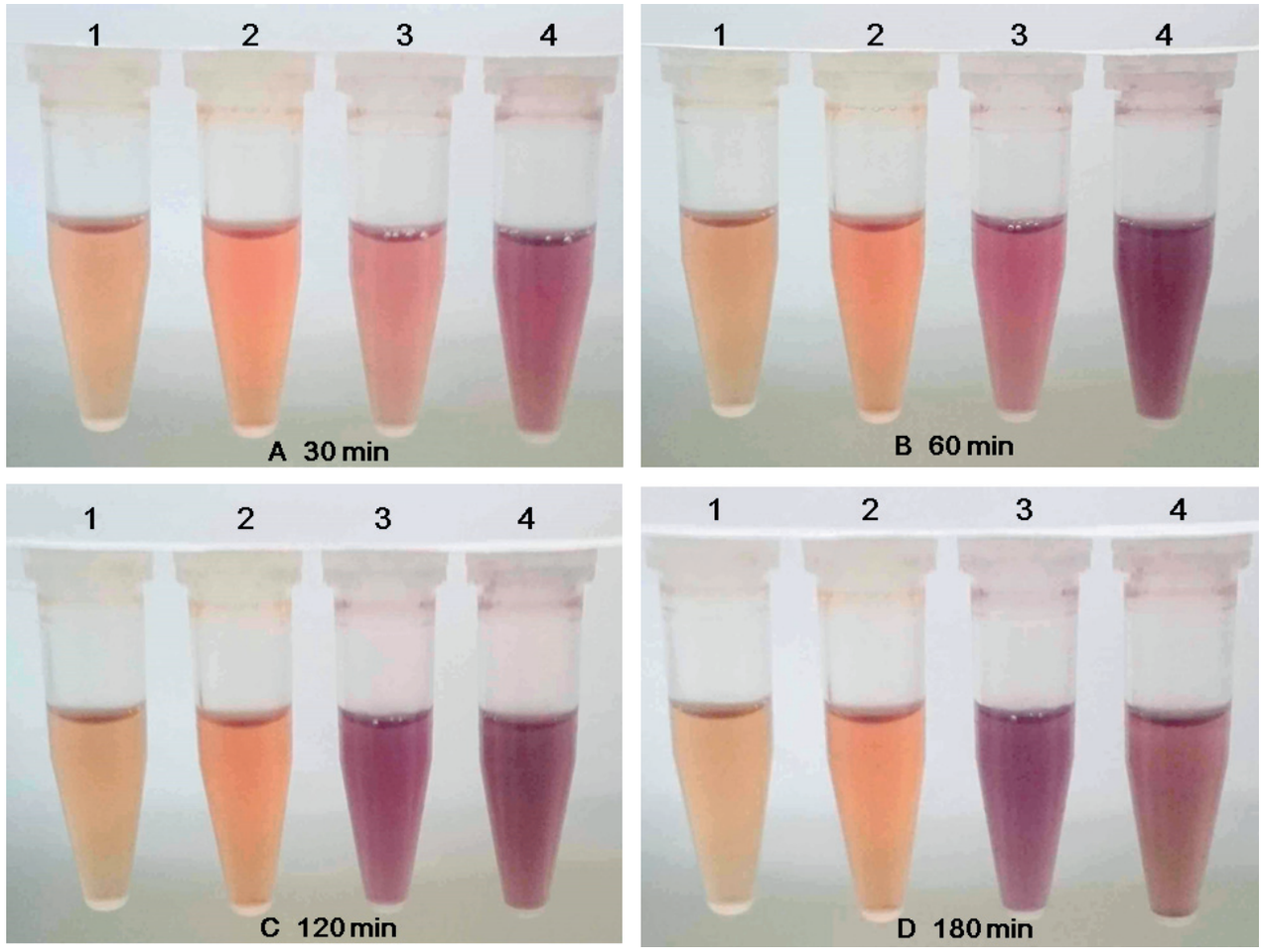

Figure 3. Purple color development in reaction tubes with blood samples of different G6PD activities. Five µl blood is loaded in each tube.
Tube 1, normal blood without the substrates (negative control); tube 2, hemizygous male; tube 3, heterozygous female; tube 4, normal blood (positive control).
Note that at 30 min after incubation, development of a strong purple color is seen in the positive control (tube 4), while a weak color development in tube 3 can be distinguished from the negative control (tube 1). At 3 hrs after incubation, the purple color of the positive control (tube 4) decreased in compared to those at 1~2 hrs since the water-insoluble MTT formazan gradually aggregates and then precipitates at the bottom of the tube. No change in color was observed in the negative control (A-D), even in the presence of MTT.

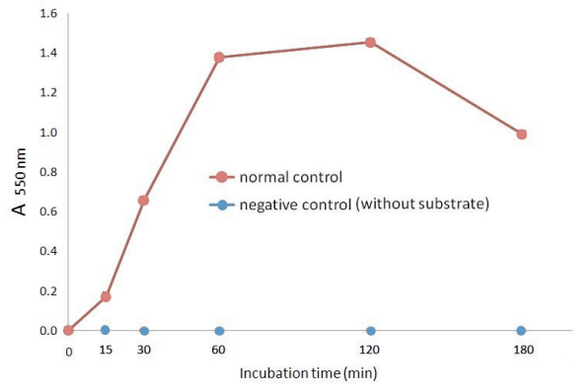

Figure 4. Time-course of purple color development at 550 nm absorbance as measured by ultraviolet spectrophotometer

G6PD activities corresponding to normal blood sample (tube 4 in Figure 3). Values represent the means of three determinations. At 3 hours after incubation, the absorbance at 550 nm decreased due to the precipitation of formazan aggregates. Note that no change in absorbance was seen in the negative control in the presence of MTT.

DISCUSSION

The International standard method for detection of G6PD-deficiency is UV spectrophotometric assay by measurement of absorbance at 340 nm using an UV spectrophotometer with a biochemical assay kit (345-B, Trinity Biotech, Ireland). But this method can be performed only at special hospitals or special institutions. A number of methods for rapid diagnosis of G6PD-deficiency have

been reported. Among them, the fluorescent spot test,[14-15] some MTT formazan methods [16-17, 22] and the WST-8 formazan method [13] have been adopted for application in the field. Recently, two rapid chromatographic diagnostic test kits are commercially available, *i.e*, BinaxNow G6PD (Alere Inc., USA)[24] and the CareStart G6PD (Access Bio, USA).[25] Both are qualitative assays utilizing formazan color development, but quantitative point-of-care tests are currently under development and validation.[26]

In the MTT/PMS methods, many researchers have attempted to resolve the interference problem caused by the non-specific reaction using many techniques for separation of MTT from hemoglobin in reaction mixtures, such as absorption of G6PD enzyme on anion-exchange cellulose paper,[23] of hemoglobin on cation-exchange cellulose paper,[16]and of G6PD enzyme absorbed on DEAE-Sephadex gel,[17] or dissolving all reagents in agar plates and separating from blood (the Formazan ring method[16]). However, our research on non-specific reactions revealed that many special efforts mentioned above are unnecessary. Interestingly, we found that the interference caused by the non-specific reaction can be neglected as a small amount of blood sample is loaded, and that a quantitative measurement is also possible, similar to that of the WST-8 method. Therefore, the new MTT method does not require any technique for separation of MTT from hemoglobin, and the only necessary action is to simply mix reagents in reaction tubes.

All MTT methods are basically qualitative assays due to the fact that the MTT formazan is water-insoluble. Extraction of the produced formazan by organic solvents, such as ether-acetone solution, dimethyl sulfoxide (DMSO) or sodium dodecyl sulphate, is possible.[23] As shown in Figure 4, however, a quantitative assay is possible by the MTT method without the extraction process. Nonetheless, the new MTT method may be more practical if it is used for screening for G6PD deficiency in malaria endemic regions by the naked eye without any equipment. MTT is a cheaper dye than WST-8, and it is more widely commercially available worldwide than WST-8.

Our field trial using the MTT method among the Dayak and the Melayu peoples in Batang Lupar District, Kalimantan Island was successful (unpublished). In this surveillance, 26 deficient individuals among 416 volunteers have been detected. Among those, 22 venous blood samples were confirmed mutations by sequencing. These results may indicate that this new screening method using MTT/1-methoxy PMS is a better method for field detection of G6PD deficiency than the WST-8 method since exhibits a strong purple color which shows production of MTT formazan and describes the high activity of G6PD enzyme which could be easily distinguished by the naked eye.

CONCLUSIONS

We found that the interference by non-specific reactions between MTT and hemoglobin can be neglected as a small amount of blood sample is loaded. Therefore, MTT could be used as a formazan substrate, instead of WST-8, for better rapid screening of G6PD deficiency. This method is easy, rapid and reliable screening method, especially for field application.

CONFLICT OF INTEREST

We have no conflict of interest to declare.

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REFERENCES

1. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet.2008; 371: 64-74.
2. Minucci A, Moradkhani K, Hwang MJ, Zuppi C, Giardina B, Capoluongo E. Glucose-6-phosphate dehydrogenase (G6PD) mutations database: review of the"old" and update of the new mutations. Blood Cells Mol Dis. 2012; 48: 154-65.
3. Matsuoka H, Arai M, Yoshida S, Tantular IS, Pusarawati S, Kerong H, et al. Five different glucose-6-phophate dehydrogenase (G6PD) variantsfound among 11 G6PD-deficient persons in Flores Island, Indonesia.JHum Gen. 2003; 48:541-4.
4. Matsuoka H, Wang J, Hirai M, Arai M, Yoshida S, Kobayasi T. et al. Glucose-6-phosphate dehydrogenase (G6PD) mutations in Myanmar: G6PD Mahidol (487G>A) is the most common variant in the Myanmar population. J Hum Gen. 2004; 49: 544-7.
5. Jalloh A, van Thien H, Ferreira MU, Ohashi J, Matsuoka H, Kanbe T, et al. Rapidepidemiologic assessment of glucose-6-phosphate dehydrogenase (G6PD) deficiency in malaria-endemic areasinSoutheast Asia using a novel diagnostic kit. Trop Med Int Health. 2004; 9:615-23.
6. Matsuoka H, Nguon C, Kanbe T, Jalloh A, Sato H, Yoshida S, et al. Glucose-6-phosphate dehydrogenase (G6PD) mutations in Cambodia: G6PD Viangchan (871G>A) is the most commonvariant in the Cambodian population. JHumGen. 2005; 50: 468-72.
7. Kawamoto F, Matsuoka H, Kanbe T, Tantular IS, Pusarawati S, Kerong H, et al. Further investigations of glucose-6-phosphate dehydrogenase variants in FloresIsland, eastern Indonesia. JHum Gen. 2006; 51: 952-7.
8. Matsuoka H, Thuan DTV, van Thien H, Kanbe T, Jalloh A, Hirai M, et al. Seven different glucose-6-phosphate dehydrogenase variants including a new variant distributed in Lam Dong Province in southern Vietnam. Acta Med Okayama. 2007; 61: 181-5.
9. Tantular IS, Matsuoka H, Kasahara Y, Pusarawati S, Kanbe T, Tuda JSB, et al. Incidence and mutation analysis of glucose-6-dehydrogenase deficiency in eastern Indonesian populations. Acta Med Okayama. 2010; 64: 367-73.
10. Kawamoto F, Matsuoka H, Pham NM, Hayashi T, Kasahara Y, Dung NT, et al. Further molecular analysis of G6PD deficiency variants in southern Vietnam and a novel variant designated as G6PD Ho Chi Minh (173 A>G; 58 Asp>Gly): Frequency distributions of variants compared with those in other Southeast Asian countries. Acta Med Okayama. 2017; 71: 325-32.

11. Kawamoto F. Rapid diagnosis of malaria by fluorescence microscopy using light microscope and interference filter. Lancet. 1991; 337: 200-2.
12. Kawamoto F, Billingsley PF. Rapid diagnosis of malaria by fluorescence microscopy. Parasitol Today. 1992;8: 69-71 pmid: 15463575
13. Tantular IS, Kawamoto F. An improved, simple screening method for detection ofglucose-6-phosphatedehydrogenase deficiency. Trop Med Int Health. 2003; 8: 569-74.
14. Beutler E. A series of new screening procedures for pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency, and glutathione reductase deficiency. Blood. 1966; 28: 553-62.
15. Beutler E, Mitchell M. Special modification of the fluorescent screening method for glucose-6-phosphate dehydrogenase deficiency. Blood. 1968; 32: 816-8.
16. Fujii H, Takahashi K, Miwa S. A new simple screening method for glucose-6-phosphate dehydrogenase deficiency. Acta Haematol Jap 1984:47: 185-8.
17. Hirono A, Fujii H, Miwa S. An improved single-step screening method for glucose-6-phosphate dehydrogenase deficiency. Jap J Trop Med Hyg. 1998; 26:1-4.
18. Hirono A, Ishii A, Kere N, Fujii H, Hirono K, Miwa S. Molecular analysis of glucose-6-phosphate dehydrogenase variants in the Solomon Islands. American J Hum Gen. 1995; 56:1243-5.
19. Tantular IS, Iwai K, Lin K, Basuki S, Horie T, Htay HH, et al. Field trials of a rapid test for G6PD deficiency in combination with a rapid diagnosis of malaria. Trop Med Int Health. 1999; 4, 245-50.
20. Ishii A, Nagai N, Arai M, Kawabata M, Matsuo T, Bobogare A, et al.Chemotherapeuticmalaria control as a selective primary health care activity in the Solomon Islands. Parasitologia. 1999; 41: 383-4.
21. Iwai K, Hirono A, Matsuoka H, Kawamoto F, Horie T, Lin K, et al. Distribution of Glucose-6-phosphate dehydrogenase mutations in Southeast Asia. Hum Gen. 2001; 108: 445-9.
22. Suryantoro P. Glucose-6-phosphate dehydrogenase (G6PD) deficiency in Yogyakarta and its surrounding areas. Southeast A J Trop Med Pub Health. 2003; 34 Suppl. 3: 138-9.Fairbanks VF, Beutler E. A simple method for detection of erythrocyte glucose-6-phosphate dehydrogenase deficiency (G-6-PD spot test). Blood. 1962; 20: 591-601.
23. Osorio L, Carter N, Arthur P, Bancone G, Gopalan S, Gupta SK, et al.Performance of BinaxNOW G6PD deficiency point-of-care diagnostic in P. vivax-infected subjects. Am J Trop Med Hyg. 2015; 92: 22-7.
24. Goo YK, Ji SY, Shin HI, Moon JH, Cho SH, Lee WJ, et al. First evaluation of glucose-6-hosphate dehydrogenase (G6PD) deficiency in vivax malaria endemic regions in the Republic of Korea. PLoS One. 2014; 9: e97390.
25. Bancone G, Gornsawun, Chu CS, Porn P, Pal S, Bansil P, et al. Validation of the quantitative point-of-care CareStart biosensor for assessment of G6PD activity in venous blood. PLoS One. 2018; 13: e0196716.

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