The Differences of the Prevalences and Serotypes of Dengue Virus on Aedes Aegypti Mosquitoes from Pagutan and Pagutan Timur in the Sub District of Mataram

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ABSTRACT

Introduction: Sub district of Mataram is one of endemic area of Dengue Haemoraghagic Fever in the West of Nusa Tenggara province, the number of dengue cases are reported increasing every years. Pagutan is a village that has been reported as higth case incidance area and Pagutan Timur as low case insidance area.
Objectives: The aims of this study is to determine the density of the Aedes aegypti mosquitoes and to know the prevalence and dominant serotypes of dengue virus in the Aedes aegypti mosquitoes from the village of Pagutan and Pagutan Timur Mataram sub district.
Methods: The Collection of Aedes aegypti mosquitoes were conducted by ovitrap provided indoor and outdoor of randomly selected houses. The mosquitoes density was determined by calculating the average number of mosquitoes emerged from eggs collected each houses. The prevalence of dengue virus were calculated by the percentage of mosquitoes that containing of dengue virus as examined by immunocytochemistry method, using the monoclonal antibody anti-dengue DSSE10, while dengue virus serotypes determined by Reverse Transcriptase-Polymerase Chain Reaction using Lanciotti specific primers.
Results: The density of Aedes aegypti mosquitoes from Pagutan is significantly higher than Pagutan Timur Village ($p<0.05$). The confirmed statistical analysis with Paired t test were obtained. The prevalence of dengue virus in Pagutan and Pagutan Timur are 18.4% and respectively 14.3%. The serotypes of dengue virus in Pagutan areas is dengue 1, dengue 2 and dengue 3, whereas in Pagutan Timur areas is dengue 1.
Conclusion: There is diffences of mosquitoes density, prevalence and the serotypes of dengue virus found in Aedes aegypti mosquitoes from Pagutan and Pagutan Timur in the sub district of Mataram.

Keywords: Mosquito density, Aedes aegypti, dengue virus, prevalence and serotype

INTISARI

Pendahuluan: Kabupaten Mataram merupakan salah satu area endemik Demam Berdarah Dengue di provinsi Nusa Tenggara Barat dengan kasus Dengue dilaporkan meningkat tiap tahun. Pagutan merupakan desa yang dilaporkan memiliki insidensi kasus yang tinggi dan Pagutan Timur merupakan area dengan insidensi kasus yang rendah.
Tujuan: Penelitian ini bertujuan untuk menentukan kepadatan nyamuk Aedes aegypti dan untuk mengetahui prevalensi dan serotipe virus dominan pada nyamuk Aedes aegypti dari desa Pagutan dan Pagutan Timur Kabupaten Mataram.

Metode: Pengumpulan nyamuk Aedes aegypti dilakukan dengan ovitrap yang diletakkan di dalam dan luar ruangan di rumah-rumah yang dipilih secara acak. Kepadatan nyamuk ditentukan dengan menghitung rerata jumlah nyamuk yang muncul dari telur yang dikumpulkan dari tiap rumah. Prevalensi virus dengue dihitung dengan persentase nyamuk yang mengandung virus dengue seperti yang diuji dengan metode imunositokimia, menggunakan antibodi monoklonal anti-dengue DSSE10, sedangkan serotipe virus dengue ditentukan dengan Reverse Transcriptase-Polymerase Chain Reaction menggunakan primer spesifik Lanciotti.
Hasil: Kepadatan nyamuk Aedes aegypti dari Pagutan lebih tinggi secara bermakna daripada dari desa Pagutan Timur ($p<0,05$). Kemaknaan ini dikonfirmasi oleh analisis statistik dengan uji T berpasangan. Prevalensi virus dengue di Pagutan dan Pagutan Timur berturut-turut ialah 18,4% dan 14,3%. Serotipe virus dengue di area Pagutan ialah dengue 1, dengue 2 dan dengue 3, sementara di area Pagutan Timur ialah dengue 1.
Simpulan: Terdapat perbedaan kepadatan nyamuk, prevalensi dan serotipe virus dengue pada nyamuk Aedes aegypti dari Pagutan dan Pagutan Timur di Kabupaten Mataram.

Kata kunci: Kepadatan nyamuk, Aedes aegypti, virus dengue, prevalensi dan serotipe

INTRODUCTION

Dengue virus is the cause of dengue fever (DD) and dengue hemorrhagic fever (DHF) diseases, which is transmitted through the *Aedes aegypti* mosquitoes' bites. To date, DHF is still a major public health problem in tropical and sub tropical regions. Globally, 2.5 billion people who live in more than 100 countries are at risk of dengue virus infection and the number of cases is 20 million every year[1].

In Indonesia, the first reported dengue case was in Surabaya in 1968 with 58 patients and 24 of them were reported dead. However, diagnostic tools for detection of dengue virus were only available in 1972 and the dengue virus had spread widely to several areas in 1980. The increase of dengue cases are caused by several influencing factors, those are: mosquitoes as the vector, human, dengue virus and environment[2,3].

Dengue viruses have been classified into serotype: Dengue 1, Dengue 2, Dengue 3 and Dengue 4. All of the serotypes were found in several regions in Indonesia and the most dominant is dengue serotype 3. In Indonesia, Dengue 3 is associated with severe cases, whereas the most common cause of hemorrhagic shock is Dengue 2[4].

The pathogenicity of DHF and dengue shock syndromes have not been clearly understood. The reference of theory that is still used is the secondary heterologous infection hyphotesis Halstead (1969), which suggests that patients who undergo a second infection with different serotypes of dengue virus have greater risk of dengue fever and dengue shock syndrome[5].

Dengue virus is transmitted by *Aedes aegypti* as the main vector of dengue fever. *Aedes aegypti* mosquito is antropophylic, endophylic, and endophagic, thus it is always close to human. It spreads widely throughout Indonesia, especially in urban and densely populated residential areas. The population density of mosquitoes in an area will increase the risk of transmission of DHF[6,7].

Dengue virus is maintained in nature through two mechanisms, the first is by the horizontal transmission trans viremia patient to another transmitted by *Aedes aegypti* mosquitoes and the second is by vertical or transovarial transmission from infected *Aedes aegypti* mosquitoes to the next generation. The study conducted by Joshi *et al.* (2002) proved that the transovarial transmission could occur up to 7 generations[8,9].

Dengue virus is present in the mosquito's body lifetime, thus it is a transmitter of dengue virus all through its life. However, not all mosquitoes are susceptible to dengue virus infection. Therefore, the data about the proportion of mosquitoes that are infectious of dengue virus is very crucial to identify the patterns and transmission dynamics of DHF in endemic areas. The specific data needs to

be available at all times for planning the eradication of DHF, especially in relation with vector control. So the discontinuation of dengue virus transmission by vectors is one of the success keys of dengue prevention because a safe and effective vaccine for dengue virus to date is still in development stage[3,8].

The study conducted by Umniyati (2009) was about to the prevalence of dengue virus infection on *Aedes aegypti* mosquitoes in endemic areas at Yogyakarta during the end of 2006. The samples used were *Aedes aegypti* mosquitoes which were caught on the field with a back pack aspirator instrument and *Aedes aegypti* mosquitoes which were collected from the egg stage, larva, pupa, which were maintained until adulthood. The study result showed that there was no difference between the transovarial infections index with the *Aedes aegypti* infections index to dengue virus[10].

Incidence of DHF in the Mataram city was first reported in 1986 and is increasing every year. District of Mataram is one of the districts that are located in the city of Mataram and it has the highest number of DHF cases compared to the other districts. The data from the Department of Health in Mataram show an increase of dengue cases in the last three years (2008 - 2010), i.e. 239 cases found in 2008, 332 cases found in 2009 with four people died and there are 403 cases in 2010 with two people died[11].

Cases of DHF have been mostly reported by people who live in the Pagutan Village, while other village that is Pagutan Timur Village has a relatively low number of cases. However, the exact data about the population density of *Aedes aegypti* mosquitoes, prevalence of dengue virus in *Aedes aegypti* mosquitoes and the predominant dengue virus serotype among the *Aedes aegypti* mosquitoes in the Pagutan and Pagutan Timur Villages are not available up to date.

Finding out the presence of dengue virus in the blood of the population in a region is rarely done because DHF is an acute disease. The approach of detecting the presence of dengue virus in the mosquito population of *Aedes aegypti* found in an area is very useful for predicting the presence of dengue virus in the area.

At present, the developed methods to detect dengue virus are Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) technique and streptavidin-biotin peroxidase immuno-cytochemistry Complex (SBPC) using specific monoclonal antibodies. The immunocyto-chemistry method is very sensitive and specific although qualitative, while the RT-PCR can be used to determine the serotype of dengue virus in mosquitoes, samples from patients suspected of dengue and autopsy tissues in a rapid, precise and specific way[12,13.14].

The aims of this study are to know the difference of the population density of *Aedes aegypti* mosquitoes, the difference of dengue virus prevalence in *Aedes aegypti* mosquitoes and the dominant serotypes of dengue virus in *Aedes aegypti* mosquitoes in the Pagutan and Pagutan Timur Villages in Mataram District.

MATERIALS AND METHODS

This study is a descriptive study with a cross-sectional design. Samples of adult *Aedes aegypti* female mosquitoes were collected by ovitraps that were provided indoor and outdoor of randomly selected houses in Pagutan and Pagutan Timur Village. The number of houses that were provided with ovitraps was in accordance with the entomologist survey of DHF guidelines which followed the criteria from WHO, i.e. 59 houses in each of Pagutan and Pagutan Timur Villages[15].

The number of ovitrap provided in each house in the Pagutan and Pagutan Timur was determined based on the number of buildings located in the area, as determined by FUNASA (Fundacao Nacional de Saúde). Based on the number of buildings, the ovitraps provided in each house were two, one indoor and the other outdoor[16].

The setting up of ovitrap was carried out during January - February 2012, and then incubated in the laboratory based on the houses where the ovitrap was set up until the adult *Aedes aegypti* mosquitoes

emerged. The amount was then calculated to produce the population density of *Ae. aegypti* mosquitoes in the Pagutan and Pagutan Timur villages. The existence of adult *Aedes aegypti* mosquitoes was maintained by feeding them sugar solution 10 %.

The numbers of *Aedes aegypti* mosquitoes in this study were 490 mosquitoes that were taken from representative houses in the Pagutan and Pagutan Timur villages. Each village had 245 mosquitoes. The criteria of *Aedes aegypti* mosquitoes used in this study were: female mosquito, mean of age was 7 days and did not suck blood.

Aedes aegypti mosquitoes were separated between the head and thorax. The head of a mosquito was used for head squash preparation. To determine the prevalence of dengue virus in *Aedes aegypti* mosquitoes, the mosquitoes were examined by immuno-cytochemistry with Streptavidin-Biotin Peroxidase Complex method, using the monoclonal antibody anti-dengue DSSE10 as primary antibodies. Mosquito thoraxes were grouped by neighborhood in the Pagutan and Pagutan Timur villages, and then extracted using the QIAamp ® Viral RNA reagent Mini Kit (QIAGEN, Cat. No.52904). To find out the types of dengue virus serotypes in the *Aedes aegypti* mosquitoes, the extracted RNA was examined by two-step RT-PCR method using Lanciotti specific primers (Table 1). The PCR products were electrophoresed on 2% agarose gel with 0.5 x TBE buffer and analyzed by marker 100 bp DNA ladder. The expected band sizes for each dengue virus serotype are as shown in Table 1.

Table 1. Primer for detection the RNA dengue virus[13].

Primer	Sequence	Position of Genom	Size of DNA amplification result (in bp)
D1	5'-TCAATATGCTGAAACGCGCGAGAAACCG -3'	134–161	511
D2	5'-TTGCACCAACAGTCAATGTCTTCAGGTTC -3'	616–644	511
TS1	5'-CGTCTCAGTGATCCGGGG -3'	568–586	482
TS2	5'-CGCCACAAGGGCCATGAACAG -3'	232–252	119
TS3	5'-TAACATCATCATGAGACAGAGC -3'	400–421	290
TS4	5'-CTCTGTTGTCTTAAACAAGAGA -3'	506–527	392

RESULTS

Population density of *Aedes aegypti* mosquitoes

In this study the population density of mosquitoes was known from the number of mosquitoes that emerged from eggs in the ovitraps which were provided in the houses in Pagutan and Pagutan Timur Villages. Table 2 shows the numbers of *Aedes aegypti* mosquitoes in the Pagutan Village are 945 mosquitoes, with the average numbers of mosquitoes in each house are 16 mosquitoes, whereas in the Pagutan Timur there are 695 mosquitoes, with the average numbers of mosquitoes in each house are 11.8 mosquitoes.

Table 2. The differences in the number of adult *Aedes aegypti* mosquitoes that emerged from eggs in ovitraps which were provided in the Pagutan and Pagutan Timur Villages in Mataram District.

Location of research	Number of houses with ovitraps	*Aedes aegypti* mosquitoes		Number of mosquitoes	Mosquitoes average in each house
		Female	Male		
Pagutan village	59	402	543	945	16
Pagutan Timur village	59	308	387	695	11,8

Table 3 shows the differences in the number of adult *Aedes aegypti* mosquitoes that emerged from eggs from the results of the ovitraps provided in houses in each neighborhood in the Pagutan and Pagutan Timur Villages in Mataram District, and the average number of mosquitoes in each house.

Table 3. The differences in number of adult *Aedes aegypti* mosquito that emerged of egg from results of *ovitraps* provided at each neighborhood in the Pagutan and Pagutan Timur Village at Mataram District.

Pagutan Village				**Pagutan Timur Village**			
Neigh-Borhood	Number of houses with ovitraps	Number of mosquitoes	Mosquitoes average in each house	Neigh-borhood	Number of houses with ovitraps	Number of mosquitoes	Mosquitoes average in each house
1	8	151	18,9	1	8	99	12,4
2	10	221	22,1	2	7	111	15,9
3	10	184	18,4	3	10	122	12,2
4	8	78	9,7	4	8	89	11,1
5	8	148	18,5	5	8	124	15,5
6	8	110	13,7	6	10	59	5,9
7	7	53	7,6	7	8	91	11,4
Total	59	945	16	Total	59	695	11,8

The prevalence of dengue virus in *Aedes aegypti* mosquitoes

Figure 1 shows the results of microscopic examination *Aedes aegypti* head squash preparations by using immunocytochemistry with Streptavidin-Biotin Peroxidase Complex method, using the monoclonal antibody anti-dengue DSSE10 as primary antibodies. At the time of *Aedes aegypti* head squash preparations' staining, positive and negative controls were included. Positive controls were the *Aedes aegypti* mosquito from the Parasitology Laboratory, Faculty of Medicine, Universitas Gadjah Mada, did not suck blood which were injected with dengue 3 virus and were incubated for 7 days. Negative controls were made of *Aedes aegypti* mosquitoes that were not injected with dengue virus and did not suck blood.

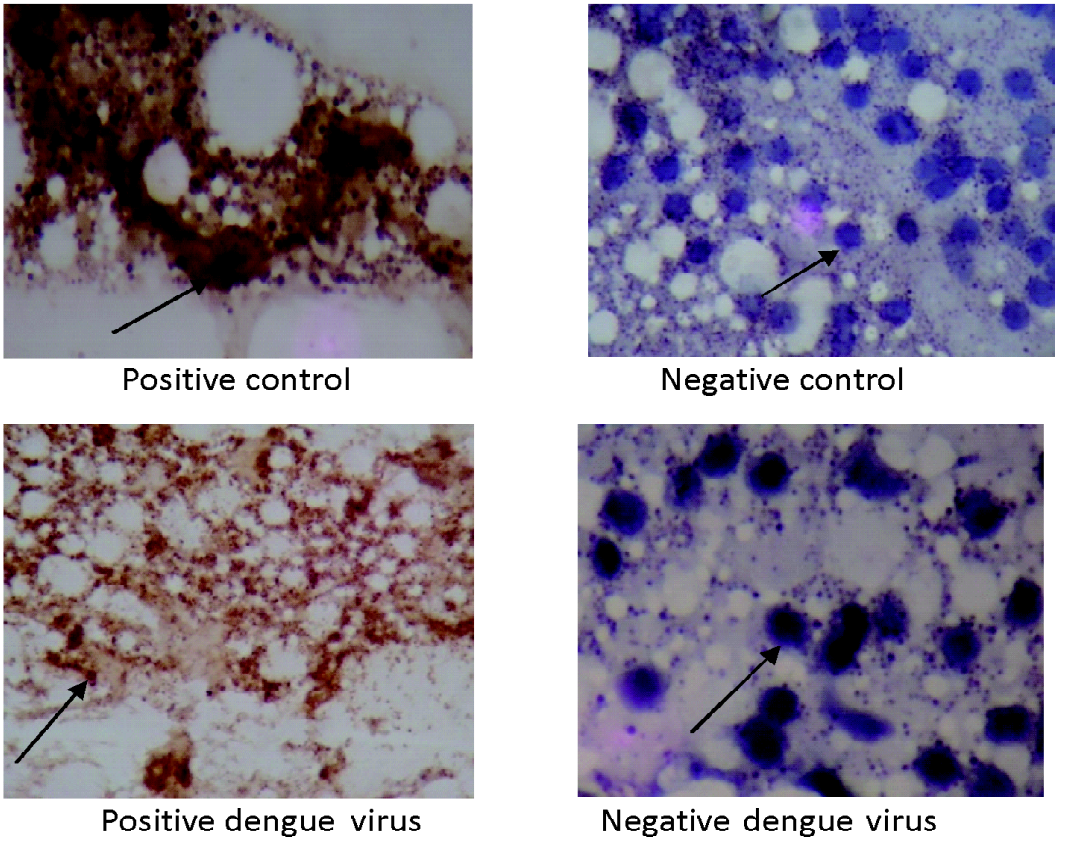

Figure 1. Head squashes immunocytochemical preparations of the *Aedes aegypti* mosquito infected with positive virus, positive control and negative control in microscopic picture.

Table 4 shows the differences in the prevalence of dengue virus in *Aedes aegypti* mosquitoes in the Pagutan and Pagutan Timur villages.

Table 4. The prevalence of dengue virus in *Aedes aegypti* mosquitoes from the Pagutan and Pagutan Timur villages in Mataram District.

Location of research	Number of *Aedes aegypti* mosquitoes	Dengue virus		Prevalence (%)
		Positive	Negative	
Pagutan village	245	45	200	18,4
Pagutan Timur village	245	35	210	14,3

Table 5 shows the differences in the prevalence of dengue virus in *Aedes aegypti* mosquitoes in the Pagutan and Pagutan Timur villages and are grouped according to neighborhood.

Table 5. Differences in the prevalence of dengue virus in *Aedes aegypti* mosquitoes from each of the Neighborhood in the Pagutan and Pagutan Timur Village in Mataram District

Pagutan Village				Pagutan Timur Village			
Neigh-borhood	Number of *Ae. aegypti* mosquitoes		Prevalence (%)	Neigh-borhood	Number of *Ae. aegypti* mosquitoes		Prevalence (%)
	Examined	Positive dengue virus			Examined	Positive dengue virus	
1	47	11	4,5	1	30	7	2,9
2	61	11	4,5	2	45	10	4,1
3	54	9	3,7	3	52	13	5,3
4	15	6	2,4	4	29	0	0
5	41	4	1,6	5	44	5	2
6	20	4	1,6	6	18	0	0
7	7	0	0	7	29	0	0
Total	245	45	18,4	Total	245	35	14,3

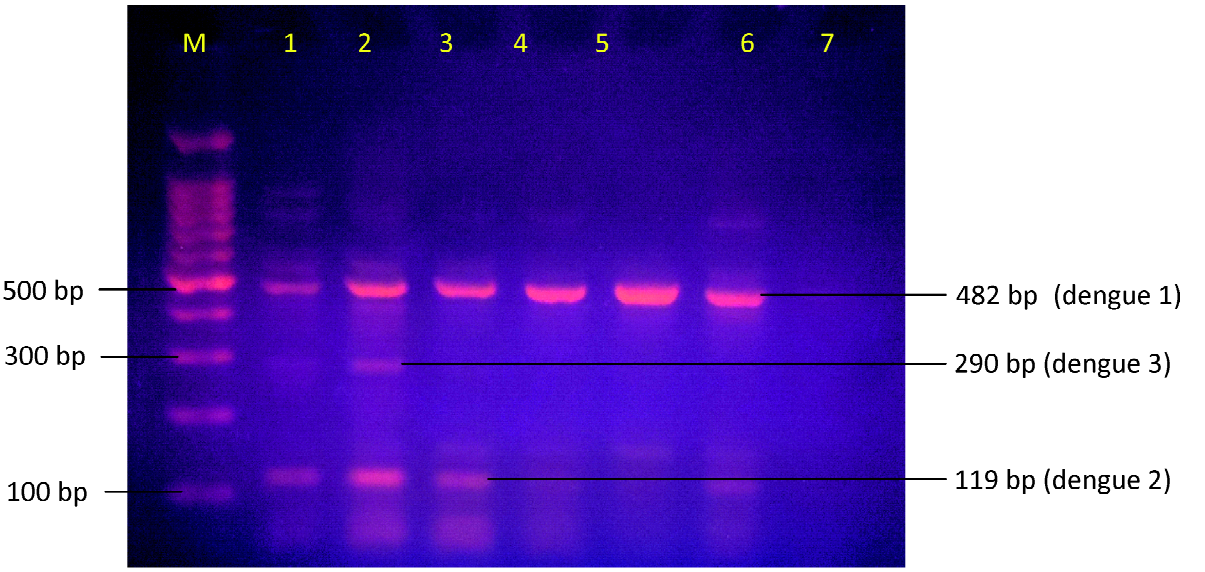

Figure 2. The results of electrophoresis products of two-step RT-PCR using Lanciotti primers to detect virus dengue serotype in *Aedes aegypti* mosquitoes from Pagutan Village in Mataram District on agarose gel 2%. M: marker 100 bp DNA ladder; line 1 and line 3: dengue 1 with size of band 482 bp and dengue 2 with size of band 119 bp; line 2: dengue 1 with a size of band 482 bp, dengue 2 with size of band 290 bp, and dengue 3 with size of band 290 bp; line 4, line 5 and line 6: dengue 1 with size of band 482 bp; line 7: negative.

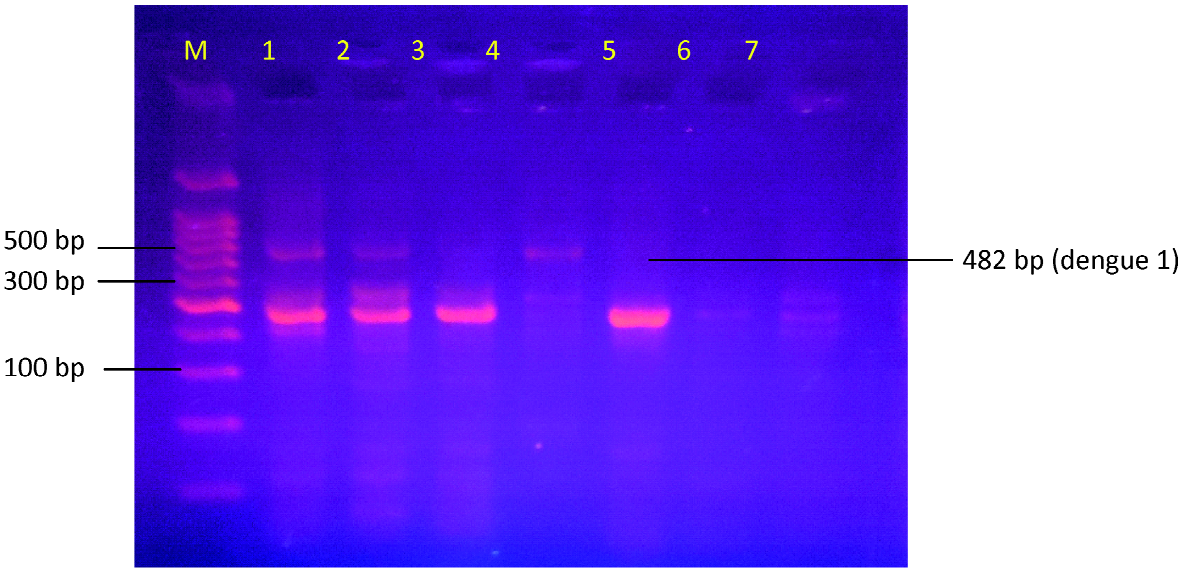

Figure 3. The results of electrophoresis products of two-step RT-PCR using primers Lanciotti primers to detect dengue virus serotype in *Aedes aegypti* mosquitoes from Pagutan Timur Village in Mataram District on agarose gel 2%. M: marker 100 bp DNA ladder; line 1, line2, line 3 and line 5: dengue 1 with a size of band 482; line 4, line 6 and line 7: negative.

Serotypes of dengue virus in *Aedes aegypti* mosquitoes

Figure 2 shows the results of electrophoresis of two-step RT-PCR products on *Aedes aegypti* mosquitoes from Pagutan Village and grouped by Neighborhood. On the line 1 are *Aedes aegypti* mosquitoes from Neighborhood 1, line 2 from Neighborhood 2, line 3 from Neighborhood 3, line 4 from Neighborhood 4, line 5 from Neighborhood 5, line 6 from Neighborhood 6, and line 7 from Neighborhood 7.

Figure 3 shows the results of electrophoresis of two-step RT-PCR products on *Aedes aegypti* mosquitoes from the Pagutan Timur Village in Mataram District and grouped by Neighborhood. On the line 1 are *Aedes aegypti* mosquitoes from Neighborhood 1, line 2 from Neighborhood 2, line 3 from Neighborhood 3, line 4 from Neighborhood 4, line 5 from Neighborhood 5, line 6 from Neighborhood 6, and line 7 from Neighborhood 7.

DISCUSSION

The population density of *Aedes aegypti* mosquito in a high-risk area of dengue virus occurs through horizontal transmission from infectious human to *Aedes aegypti* mosquitoes or otherwise and through vertical or transovarial transmission via *Aedes aegypti* mosquitoes gravid females that are infective to the eggs in their uterus and propagates consecutively in the embryonic egg, larva, pupa to adult (imago) as a medium to reproduce its life. Thus, human can be infected by dengue virus when the adult mosquitoes first emerge out of the pupa and then bite and suck blood[3].

The result of the statistical test confirmed by t test shows that p value is 0.019 ($p < 0.05$). This suggests that there is a significant difference between the population density of *Aedes aegypti* mosquitoes in the Pagutan village compared with Pagutan Timur village, so the risk of dengue virus transmission occuring horizontally

and vertically or transovarial is higher in the Pagutan village than in Pagutan Timur village.

The prevalence of dengue virus in *Aedes aegypti* mosquitoes from Pagutan and Pagutan Timur Villages was 18.4%, and 14.3% respectively. Statistically the difference was not significant ($p > 0.05$). Another similar study conducted previously by Riandini (2010) in Pekan Baru City, Province of Riau stated that there was no significant difference between the index of transovarial transmission of dengue virus in endemic areas compared to the sporadic DHF, with different mosquito population density and egg. The index of transovarial transmission in endemic and sporadic areas was 75% and 74.6% respectively[17].

The presence of infectious *Aedes aegypti* mosquitoes in nature causes the unbreakable chain of transmission of dengue virus, as evidenced by Angel and Joshi (2008) in their research in India. In their study, vertical and transovarial transmission of dengue virus in the *Aedes aegypti* mosquitoes are found every year in the summer, spring, autumn and winter. Another study conducted by Joshi *et al.* (2002) shows that transovarial dengue virus infection in the *Aedes aegypti* mosquito has been found in India until 7 generations with the index of infection is high (12.6%)[8,12,18].

The difference of dengue virus prevalence in each village is caused by mosquito susceptibility to dengue virus. Mosquitoes are considered vulnerable, if after being infected orally by dengue virus and incubated for 2 weeks, they show a positive outcome of dengue virus in their brain tissues from the results of head squash preparations by immuno-fluorescence method. However the susceptibility test of mosquitoes for virus dengue was not conducted in this the study.[19]

Types of dengue virus serotypes found in the Pagutan village is dengue 1, dengue 2 and dengue 3. The presence of several different serotypes of dengue virus in an area can be caused by the large number of DHF cases in the area. This is in accordance with the Halstead hypothesis (1969), which suggests that patients who experience a second infection with different serotypes of dengue virus have a greater risk for DHF and SSD suffering[5].

The finding of three types of different serotypes of dengue virus can be caused by double infection or 2 types of dengue virus serotypes are present in a dengue patient in the area. Double infection can occur because the patient is bitten by two different type of *Aedes aegypti* mosquitoes and the types of viruses carried are different too or in the mosquito's body is found two types of different serotypes of the dengue virus[20].

The existence of transovarial transmission allows an *Aedes aegypti* mosquito to carry 2 different serotypes of dengue virus, which means the mosquito has been carrying the dengue virus since the egg stage. So when that mosquito bites DHF patients, it can suck other dengue virus serotypes, and if that mosquito bites another person it will cause that person to be infected by two different serotypes of dengue viruses[21].

In Indonesia, dengue virus serotype that is associated with severe cases is dengue 3 while the cause of most shock syndrome is dengue 2. In this study, the serotype found in the Pagutan Timur village is dengue 1, but statisticaly the prevalence of dengue virus is not different with the Pagutan village and the number of DHF cases in 2012 in the each of the village is same. This condition can be caused by the presence of asymptomatic patients who are dangerous source of dengue virus transmission because dengue virus-infected people have no symptoms or only show mild symptoms of fever, but can freely move to another area[1,4].

CONCLUSION

The population density of *Aedes aegypti* mosquitoes in Pagutan village was significantly higher than in Pagutan Timur village with an average number of 16 *Aedes aegypti* mosquitoes in each house in the Pagutan village and 11, 8 mosquitoes in Pagutan Timur Village. The

prevalence of dengue virus in *Aedes aegypti* mosquito population in Pagutan and Pagutan Timur Village was 18.4%, and 14.3% respectively; statistically this difference was not significant. The serotypes of dengue virus in Pagutan Village were dengue 1, dengue 2 and dengue 3, whereas in Pagutan Timur Village the dengue virus serotype was dengue 1. It is recommended for future studies to be done about the prevalence and serotypes of dengue virus in adult *Aedes aegypti* male mosquitoes and patients of DHF in Pagutan and Pagutan Timur Villages. It is suggested to the community in Pagutan Villages to be more watchful against *Aedes aegypti* mosquitoes because three different serotypes of dengue virus have been found among them, therefore secondary infection is likely to occur.

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