Transovarial Transmission Index of Dengue Virus on *Aedes aegypti* and *Aedes albopictus* Mosquitoes in Malalayang District in Manado, North Sulawesi, Indonesia

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ABSTRACT

Introduction: Dengue Hemorrhagic Fever (DHF) is an infectious vector-borne disease caused by *Aedes sp* mosquitoes still cause serious health problem in Indonesia. Based on Manado Health Office Report, Malalayang was identified as dengue-endemic areas. In 2010, number of DHF cases in Malalayang is 211 cases with Incidence Rate (IR) 328 per 100,000 populations. Dengue viruses (DENV) survive in nature by two mechanisms; by horizontal transmission through infected vertebrates and mosquitoes, and by vertical (transovarial) transmission in the mosquitoes. Transovarial transmission is assumed as an important aspect in the maintanance of DENV during inter epidemic, but this problem has not been studied in Malalayang District, Manado. An effort to prevent and control DHF requires knowledge of an *Aedes sp* Dengue virus transovarial infection.

Objectives: To prove the existence of Dengue virus transmission in *Ae. aegypti* and *Ae. albopictus* mosquitoes and its relationship with the incidence of DHF in Malalayang District in Manado, North Sulawesi, Indonesia.

Methods: The method of this research was an observational analytic study with cross-sectional design. Study samples were unbloodfed *Aedes aegypti* and *Aedes albopictus* mosquitoes on the F1 generation from ovitrap placed in five selected villages based on the number of cases in the District Malalayang. The secondary data of DHF patients from Malalayang district was obtained from Health Office Manado and the Community Health Center in 2010. The presence of dengue antigen in head squashes preparation were detected using monoclonal antibody against dengue (DSSE10) based on immunohistochemical streptavidin biotin peroxidase complex (ISBPC) technique to confirm the presence of transovarial transmission of dengue virus both in *Ae. Aegypti* and *Ae. Albopictus*, and to obtain the data of transovarial transmission index. Fisher's Exact test and Pearson correlation are used to analyze those data.

Results: Transovarial transmission of Dengue virus in *Aedes sp* was found from 5 villages in Malalayang district with Transovarial Transmission Index (TTI) ranges 6.1%-17.1%. Statistic test showed significant differences in positive rate (p-value=0.00<0.05) on *Ae. aegypti* higher than *Ae. albopictus*. It is also known that there is no statistically significant correlation (p-value=0.528>0.05) between the *Aedes sp.* Dengue virus TTI and DHF IR in Malalayang district.

Conclusion: This study demonstrates the existence of Dengue virus transovarial transmission in *Aedes sp* in Malalayang district. *Ae. aegypti*'s TTI is higher than that of *Ae. Albopictus*, and no significant correlation between TTI and DHF IR in Malalayang district.

Keywords: DHF, transovarial transmission, *Ae. aegypti, Ae. albopictus*

INTISARI

Pendahuluan: Demam Berdarah Dengue (DBD) adalah penyakit infeksi yang ditularkan vektor yang disebabkan oleh nyamuk *Aedes sp* dan merupakan masalah kesehatan yang serius di Indonesia. Berdasarkan Laporan Dinas Kesehatan Manado, Malalayang diidentifikasi sebagai daerah endemis demam berdarah. Pada tahun 2010, terdapat 211 jumlah kasus DBD di Malalyang, dengan IR virus Dengue 328 per

100.000 population. Virus dengue (DENV) bertahan hidup di alam oleh dua mekanisme, oleh transmisi horisontal melalui vertebrata yang terinfeksi dan nyamuk, dan dengan vertikal (transovarial) transmisi dalam nyamuk. Transmisi transovarial diasumsikan sebagai aspek penting dalam memelihara DENV saat epidemi berlangsung, namun hal ini belum diteliti di Malalayang, Manado. Pengetahuan tentang infeksi virustransovarial *Aedes sp* Dengue diperlukan dalam upaya untuk mencegah dan mengendalikan penyakit DBD

Tujuan: Untuk membuktikan adanya penularan virus Dengue melalui *Ae. aegypti* dan *Ae. albopictus* dan hubungannya dengan kejadian DBD di Malalayang kabupaten di Manado, Sulawesi Utara, Indonesia.

Metode: Metode penelitian ini adalah penelitian observasional analitik dengan desain cross-sectional. Sampel penelitian adalah nyamuk *Ae. aegypti* dan *Ae. albopictus* generasi F1 yang belum pernah menghisap darah dari hasil pemasangan ovitrap di 5 desa terpilih berdasarkan jumlah kasus dengue di Kabupaten Malalayang. Data sekunder pasien DBD dari Kabupaten Malalayang diperoleh dari Dinas Kesehatan Manado dan Puskesmas pada tahun 2010. Keberadaan antigen dengue pada sediaan head squash sampel nyamuk dideteksi menggunakan antibodi monoklonal antidengue (DSSE10) berdasarkan teknik imunositokimia streptavidin biotin proxidase complex untuk membuktikan adanya transmisi transovarial virus dengue dan mendapatkan data indeks transmisi transovarial. Uji Exact Fisher dan korelasi Pearson digunakan untuk menganalisis data tersebut.

Hasil: Penularan transovarial virus Dengue pada nyamuk *Aedes sp* ditemukan dari 5 desa di kabupaten Malalayang dengan index transmisi transovarial (ITT) berkisar 6,1% -17,1%. Uji statistik menunjukkan *positive rates Ae. aegypti* terhadap virus dengue secara signifikan lebih tinggi daripada nyamuk Ae. albopictus (P<0.005) serta tidak ada hubungan yang signifikan secara statistik (*p-value* 0,528) antara ITT dengue virus dan angka insidensi (IR) DBD di Kabupaten Malalayang.

Simpulan: Penelitian ini membuktikan adanya transmisi transovarial virus dengue pada nyamuk *Aedes sp* di kabupaten Malalayang, dan ITT *Ae. aegypti* lebih tinggi dibandingkan dengan *Ae. albopictus*, serta tidak ada hubungan yang signifikan antara ITT dan angka insidensi DBD.

Kata Kunci: DBD, transmisi transovarial, *Ae. aegypti, Ae. albopictus*

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a disease transmitted through female *Aedes sp.* The disease become one of the most serious health problem in Indonesia and often cause an outbreak which leading to death. It was first discovered in Manado, North Sulawesi in 1973 then spread to various areas. Incidence Rate (IR) per 100,000 population per year in North Sulawesi over the last 5 years (2005-2009) was increased more than 40/100,000 population per year. Dengue cases in province reported highest in February 2010 with 615 cases[1].

In January 2010, dengue cases were reported in Manado, followed in Minsel, Minut, Bitung, Minahasa and Sangihe regencies. Based on the surveillance report activities in Manado Health Office, the highest DHF cases was reported in Malalayang district with 211 cases in 2010 with incidence rate of 328 per 100,00 population[2].

Effort to maintain hygiene in order to eradicate DHF has actually been declared by the Mayor of Manado since 1998 through "Clean Friday Movement", followed by "Clean and Green City" in 2002 and "JUMPA BERLIAN (clean environment in Friday morning) in 2006. The aim of those actions is to create a conductive environment and indirectly to suppress the occurrence DHF cases in Manado. Mayor of Manado has also issued a form letter No:440/D.02/75/I/2006 on eradication and DHF prevention. However, the local government programs were not optimally realized by both health personnel and the community[3].

Ae.aegypti mosquitoes, are major vector

borne disease for DHF. The mosquitoes are very anthropophilic and live closed to humans. Another species of Aedes, *Ae. albopictus*, is suggested for being a potential vector of DHF. Morbidity and mortality of dengue virus infection is influenced by the host immune status, the mosquito vector density, dengue virus transmission, malignancy (virulence) of dengue virus and local geographical conditions[4,5]. Dengue virus transmission generally occurs horizontally from human carrier dengue virus of its vector, *Aedes sp*, after propagation in the mosquito and transmitted to human recipients[6]. In addition, vertical transmission (transovarial) dengue virus by *Aedes sp* vector as a parent to the ovum (egg) in the uterus and then propagates in the eggs, larvae, pupa, and imago (adult). Transovarial transmission of dengue virus by its vector in endemic areas could be a causative key which responsible for the phenomenon of increasing cases of DHF. The results of observations made in India, an *Ae. albopictus* mosquito showed a high percentage for the vertical transmission of dengue virus by Indirect Fluorescence Antibody Test (IFAT) method[7].

Studies on natural transovarial transmission of dengue virus in Indonesia was first reported byUmniyati (2004)[8] in *Kelurahan* Klitren, Gondokusuman Sub-district, Yogyakarta based on Immunohistochemistry Streptavidin Biotin Peroxides Complex (ISBPC) method. The method used head squash preparation of one week old unbloodfed *Ae. aegypti* female mosquitoes with transovarial transmission index (TTI) 27.97%, which is then standardized by Umniyati (2004)[9]. Despite its qualitative nature, it is known to be sensitive, specific, reliable, and valid for diagnostic purposes of dengue virus infection in the *Ae. aegypti* mosquito[6]. This is more convenient and can be performed in places with less laboratories facilities.

The purpose of this study is to prove the existence of transovarial transmission of dengue virus in *Ae. aegypti* and *Ae. albopictus* mosquito and its relationship with the incidence of dengue in Malalayang district in Manado, North Sulawesi.

MATERIALS AND METHODS

The method of this research was an observational analytic study with cross-sectional design to find out the DHF incidence and to demonstrate the existence of Dengue virus transovarial transmission in the *Ae. aegypti* and *Ae. albopictus* mosquitoes in DHF endemic area in the same period.

Research site was in Malalayang district Manado municipality, a district with highest endemic cases for DHF in 2010. Five out of 9 villages were taken representing villages with high case of dengue in 2010. Secondary data of dengue patient was obtained from City Health Office and Community Local Health Centers, Malalayang District in 2010. This study used ovitraps for eggs collection, trays and cages for mosquito colonization[6] and immunohistochemical streptavidin biotin peroxidase complec (ISBPC) assay using monoclonal antibody against dengue virus (DSSE10) to detect Dengue viralantigen on head squash of mosquito specimen[8,9].

Ovitrap survey was then performed, and then followed with OI calculation. Eggs were reared to get F1 for Dengue virus examination with ISBPC technique. Positive rate of Dengue virus differences in *Ae. aegypti* and *Ae. albopictus* was analysed using Fisher's Exact Test, whereas to relationship TII and IR DHF was analysed using Pearson correlation test.

RESULTS AND DISCUSSIONS

Ovitraps were distributed in study area based on the number of DHF cases that occurred in the last 3 years per village in Malalayang district. The 5 endemic DHF villages that were selected represent the number of high, medium, and low endemic areas. Those 5 endemic DHF village were characterized by some garden and forest, except for densely Bahu village.

Ovitrap distribution was done in each house on Malalayang village II, Bahu village, Malalayang East I village, Batukota village and Malalayang district I. Sampling refers to the DHF Guideline Survey of Entomology according to WHO 2001 criteria[10]. Ovitraps were distibuted in Malalayang village I (58 houses), Bahu village (57 houses), Malalayang village II (57 houses), Malalayang East I village (57 houses), Batukota village (57 houses).

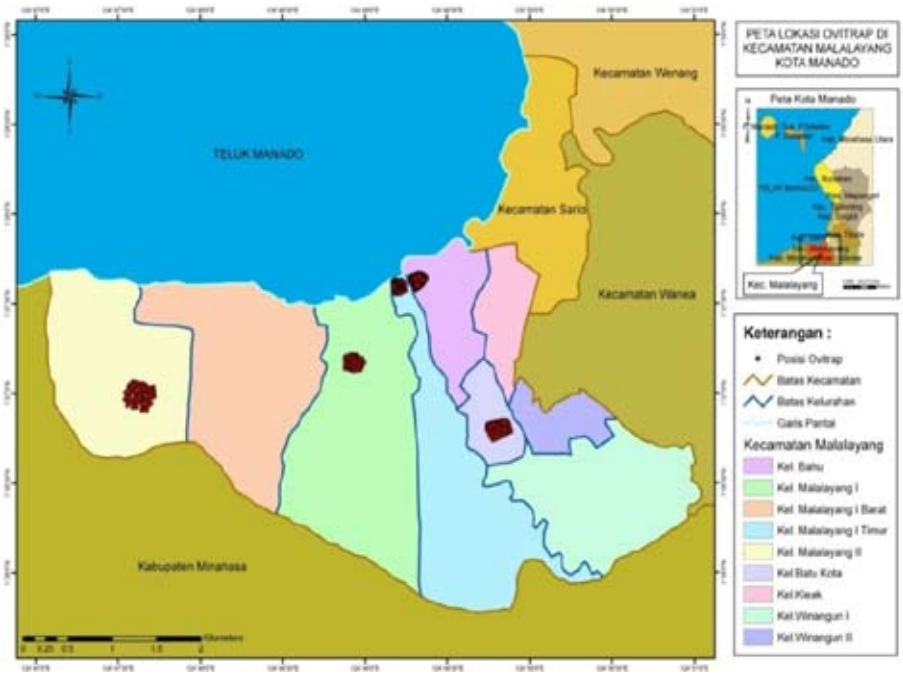

Figure 1. Ovitrap mounting location in five villages in Malalayang district

Table 1. Frequency distributions of positive *Aedes sp* ovitrap in Malalayang district Manado

Villages	Ovitrap distribution							
	I				O			
	BR	LR	Amount	Total	F	S	Amount	Total
Malalayang I	12	17	29	116	14	5	19	116
Bahu	9	13	22	114	19	17	36	114
Malalayang II	15	15	30	114	20	21	41	114
East Malalayang I	13	12	25	114	15	16	31	114
Batukota	12	13	25	114	22	21	43	114

Note : BR : Bath Room F : Front I : Indoor
LR : Living Room S : Side O : Outdoor

Table 2. Frequency distributions of positive and negative *Aedes sp* ovitraps and ovitrap index (OI) in DHF endemic villages in Malalayang district Manado

Villages	Ovitrap distribution							
	I				O			
	(+) egg	(-) egg	Amount	OI (%)	(+) egg	(-) egg	Amount	OI (%)
Malalayang I	29	87	116	25	19	97	116	16.3
Bahu	22	92	114	19.3	36	78	114	31.6
Malalayang II	30	84	114	26.3	41	73	114	35.9
East Malalayang I	25	89	114	21.9	31	83	114	27.2
Batukota	25	89	114	21.9	43	71	114	37.7

Note : I : Indoor OI : Ovitrap Index
O : Outdoor

Table 3. Positive *Aedes sp* ovitraps based on the position of ovitrapsindoors and outdoors in five DHF endemic villages

Ovitrap placement location	**Positive**	**Negative**	**Ovitrap (+) %**	**Attached**
Bathroom	61	225	21.3	286
Living room	70	216	24.5	286
Front	90	196	31.5	286
Besides	80	206	28	286
Total	**301**	**843**	**26.3**	**1144**

Four ovitraps were mounted per house, two inside the house (bathroom and living room) and the other two were outside the house (in front and besides of the house). Thus, totally 1144 ovitraps were mounted attached.

Table 2 showed positive ovitrap were found more at outdoors than indoors. Ovitrap distribution outcome in 5 villages in DHF endemic areas based on the position of the ovitraps (bathroom, living room, front and besides the houses) can be seen in the Table 3.

The above table shows the highest percentage of positive *Aedes sp* ovitrap was in front of the houses.

The ovitrap index (OI) result in DHF endemic areas in Malalayang district is higher outdoors than indoors. These results similar with the results of Hasyimi[9] who conduct research in several villages in Jakarta, OI is higher outdoors (36.4%) than indoors (33.5%) because Ae. aegypti prefer lay eggs outdoors than indoors. This mosquitoes plays role in Dengue virus transmission, because its life is inside and around the house, while *Ae. albopictus* live in the gardens, so have less contact with human[4].

After collection, the ovistraps containing eggs brought to the laboratory, then the eggs were hatched, and reared for obtaining F1 generation adults. *Aedes sp* eggs hatch into larvae around 1 to 4 days. The larvae stadium need approximately 7-8 days to become pupae, and the pupae need around 2-3 days to turn into adult mosquitoes. Usually, male mosquitoes appeared faster than female ones. Once all the mosquitoes turn into adult ones, the next process is to separate the adult mosquito by its species, *Ae. aegypti* or *Ae.*

albopictus. It is found that five endemic villages were dominated by *Ae. aegypti* mosquitoes, whereas *Ae. albopictus* was found but few. Moreover, most *Ae. albopictus* mosquitoes died after the age of two days, whereas *Ae. aegypti* mosquitoes were able to survive more than a week. Based on this condition, seven-day-old female *Ae. aegypti*, and two-day-old female *Ae. albopictus* were selected in this study. Thirty female *Ae. aegypti* mosquitoes were detected in each village and all *Ae. albopictus* in each village. Positive and negative control mosquitos were taken from Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada. The presence of dengue antigen on head squashes of intra thoracally - infected male *Ae. aegypti* mosquitoes were detected based on ISBPC technique using comercially monoclonal antibody against dengue as positive controls. Negative control tissue specimens without primary antibody were used as negative controls.

Dengue antigen was detected as brownish color in the cytoplasm of infected cells or as discrete brownish granular deposits scattered throughout brain tissue of positive controls, and positive samples. The negative result was shown as blue or purple colour throughout brain tissue of negative controls and negative samples (Figure 2).

Dengue virus detection results of unbloodfed *Ae. aegypti* samples on the F1 generation in 5

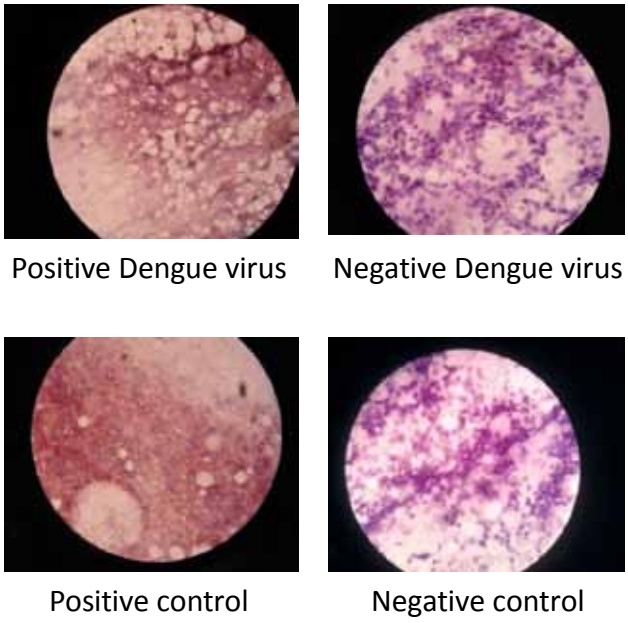

Figure 2. Head squashes of immunohisto chemical preparation of a positive sample and a positive control (left) showing positive reaction as brownish color in the cytoplasm of infected cells and descrete brownish colour deposits throughout brain tissues, and negative results (right) were shown as blue or purple colour throughout brain tissues of a negative sample and a negative control.

Table 4. The result of microscopic examination of dengue antigen on head squashes of unbloodfed *Ae. aegypti* samples on the F1 generation at 400x and 1000x magnification based on immunohistochemical assays using monoclonal antibody against dengue (DSSE10) as primary antibody in 5 DHF endemic villages in Malalayang District year 2011

No	Villages	Total		
		Sample	Positive	TTI (%)
1	Malalayang I	30	6	20
2	Bahu	30	3	10
3	Malalayang II	30	6	20
4	East Malalayang I	30	2	6.7
5	Batukota	30	3	10

TTI= Transovarial transmission index

Table 5. The result of microscopic examination of dengue antigen on head squashes of unbloodfed *Ae. albopictus* samples on the F1 generation at 400x and 1000x magnification based on the immunohistochemical assays using monoclonal antibody against dengue (DSSE10) as primary antibody in 5 DHF endemic villages in Malalayang District year 2011

No	Villages	Total		
		Sample	Positive	TTI (%)
1	Malalayang I	15	0	0
2	Bahu	0	0	0
3	Malalayang II	5	0	0
4	East Malalayang I	3	0	0
5	Batukota	22	1	4.5

Table 6. Dengue virus TII difference in *Ae. aegypti* and *Ae. albopictus* mosquitoes

No	Villages	TTI (%) *Ae. Aegypti*	TTI (%) *Ae. Albopictus*
1	Malalayang I	20	0
2	Bahu	10	0
3	Malalayang II	20	0
4	East Malalayang I	6.7	0
5	Batukota	10	4.5

Table 7. Dengue virus positive rate in *Ae. aegypti* and *Ae. albopictus* mosquitoes

Mosquito Spesies	Negatif Dengue virus	Positif Dengue virus	*p* value
Ae. Aegypti	130	20	0,00
Ae. Albopictus	44	1	(p<0,05)

Table 8. The result of microscopic examination of dengue antigen on head squashes of unbloodfed *Aedes sp* samples on the F1 generation at 400x and 1000x magnification based on the immunohistochemical assays using monoclonal antibody against dengue (DSSE10) as primary antibody in 5 DHF endemic villages in Malalayang District year 2011

No	Villages	*Ae. Aegypti*	*Ae. Albopictus*	Total *Aedes spp*	Positive	TTI (%)
1	Malalayang I	30	15	45	6	13.3
2	Bahu	30	0	30	3	10
3	Malalayang II	30	5	35	6	17.1
4	East Malalayang I	30	3	33	2	6.1
5	Batukota	30	22	52	4	7.7

Table 9. Dengue virus positive rate in *Ae. aegypti* and *Ae. albopictus* mosquitoes

Villages	IR year 2010	TII (%) *Aedes sp*.	p value
Malalayang I	72.5	13.3	0.528
Bahu	46.2	10	(p>0.05)
Malalayang II	31.2	17.1	
East Malalayang I	25.3	6.1	
Batukota	25.8	7.7	

DHF endemic villages can be seen in Table 4. The result showed that the highest TII in DHF endemic areas was in Malalayang I and Malalayang II with TII 20%, whereas, the the lowest TII was in East Malalayang I.

Dengue virus detection results of unbloodfed *Ae. albopictus* samples on the F1 generation in 5 DHF endemic villages can be seen in Table 5, whereas the differences between Ae. aegypti and *Ae. albopictus* Dengue virus TTI can be seen in the Table 6, and the differences between *Ae. aegypti* and *Ae. albopictus* Dengue virus positive rate can be seen in the Table 7.

Table 5 showed that transovarial transmission of dengue virus in *Ae. albopictus* only occured in Batukota with TTI of 4.5%. The result showed that TTI of *Ae. aegypti* was significantly higher than *Ae. albopictus*. The results were similiar to those performed by Wanti (2010) in Kupang, that found that *Ae. aegypti* TTI (20.14%) was higher than *Ae. albopictus* (8.33%)[11].

The result of Fisher`s Exact Test confirmed that the positive rates of *Ae. aegypti* was significantly higher than *Ae. albopictus* (Table 7). Based on the results of microscopic examination of the *Aedes sp* mosquito, the highest TTI in DHF endemic areas was in Malalayang II with TII 17.1%, followed by Malalayang I 13.3%, Bahu 10%, Matukota 7.7% and East Malalayang I village 6.1% (Table 8).

To assess the relationship between Aedes spp with DHF incidence rate (IR), Pearson correlation statistic test was done and *p-value* = 0.528 was obtained. It indicates that there was no significant correlation between the Dengue virus TTI on *Aedes sp* mosquitoes with the IR of DHF in 5 villages in Malalayang district, while the value of closeness of the relationship with r = 0.38 indicates the correlation was not strong. This is possible because of the ease transport between region led to increase population mobility, allowing the spread of dengue viruses from other regions. In this study, patients with DHF case data were not classified by patient age, so there was the posibility of cases derived from other areas because of the high population mobility. In contrast study result done by Sucipto (2009), patient case limited on children aged under five, assuming the case comes from the local site[12].

CONCLUSION

This study found the existence of Dengue virus transovarial with different TTI in each DHF endemic villages ranged from 6.1% to 17.1%. The Dengue virus transovarial transmission was different between *Ae. aegypti* and *Ae. albopictus*, with TTI in *Ae. albopictus* was lower than *Ae. aegypti*'s. Statistic test result showed no significant correlation between the Aedessp mosquito Dengue virus TTI with DHF IR in 5 DHF endemic villages in Malalayang district. Further research needs to be done with more number of villages, in order to get complete data from each endemic and sporadic DHF villages.

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