

MAYA INDEX (SANITATION INDICATOR) AND BIONOMIC OF VECTOR RELATED
TO DENGUE INCIDENCE IN TEMBALANG SUB DISTRICT SEMARANG

Martini, Sri Yuliawati, Retno Hestningsih, Praba Ginanjar

Epidemiology Department, Faculty of Public Health, Diponegoro University, Indonesia

Corresponding Author: tinihen65@yahoo.co.id

Abstract

Introduction: Dengue is one of the major public health problems in Semarang. In 2013, Dengue incidence was 134.09/100.000 population in Semarang. This study aimed to determine maya index and vector density related to Dengue incidence.

Methods: This was a cross sectional study. Sample consisted of 12 villages located in Tembalang Subdistrict. Data collection used observation sheet, ovitrap, GPS and PCR. Larvae density was measured according to house index (HI), container index (CI) and Breteau Index (BI). Maya Index described of sanitation level measured together with larva survey.

Results: The results showed HI = 21.17%, CI = 50%, and BI = 13.8. The indexes revealed Tembalang area had a high risk of dengue. There were two species of DHF vector found in this study, i.e. *Aedes albopictus* and *Ae. aegypti*. Proportion of *Ae. albopictus* was half of *Ae. aegypti*. Maya index, which indicates hygiene and sanitation, showed 23% houses in Tembalang Subdistrict had poor sanitation. To control the vector population, it is important to conduct breeding places elimination (*pemberantasan sarang nyamuk/PSN*), both indoors and outdoors, through active participation of the community.

Key words: DHF, maya index, vector, *Aedes* sp

Introduction

Incidence of Dengue Hemorrhagic Fever (DHF) increases every year especially in Semarang. World Health Organization reported that more than 40% of the world population have potential risk of dengue, and estimated about 50 million new cases infected per year (Regis *et al*, 2009). Ministry of Health (MoH) Indonesia reported DHF in Indonesia increased by 65.70/100.000 population in 2010 (Kemenkes 2011). According to MoH, DHF cases in 2015 should not exceed 51/100.000 population. On the other hand, Intergovernmental Panel on Climate Change (IPCC) estimated DHF incidence in Indonesia will increase in the future (Mc Michael, 2003).

Central Java Province is one of the DHF endemic area in Indonesia. Provincial Health Office of Central Java reported an increase of 19.29/100.000 DHF cases in 2012 compared to 15.3/100.000 in 2011. During 2011-2012, all 35 regencies/cities in Central reported DHF cases. Semarang City, one of cities in Central Java, always has high incidence of DHF. Throughout

Indonesia, Semarang was in the second rank of DHF cases for two consecutive years (2011-2012). DHF incidence in Semarang in 2012 was 83.78/100.000 population.

Recently, phenomenon of transovarial transmission is growing. With transovarian, *Ae. aegypti* may maintain the existence of dengue virus, even in the absence of susceptible host or poor climate condition (Soegijanto, 2006). Tribuwono *et al.* (2006) declare that transovarial transmission has already occurred in Semarang City. Method to detect the virus on mosquitos using immunohistochemical was developed by Umniyati (2009), and used for any study in Indonesia.

DHF is closely related to environment. The changing of one or more environment factors can modify mosquitos' life cycle, which eventually affects DHF incidence. Specific environment characteristics influence DHF endemicity in an area (Widayani, 2007). According to Suroso (2003), factors influencing morbidity and mortality of DHF are: host immunity, density of mosquitos' population, dengue virus transmission, virus virulence, and local geographic condition. In epidemiological point of view, DHF was influenced by environment, virus and host characteristics. Host factor consists of susceptibility and the immune response. Environment factor consists of geographic (altitude, rain fall, wind, humidity, season) and demographic (density, mobility, behavior, culture, socioeconomic) conditions.

Vectoral capacity and density of *Aedes* spp are also a determinant of DHF transmission. Vectoral capacity is largely controlled by intrinsic, vector factors; for example, vector competence and host preference are, to various degrees, controlled by genetics (Anderson and Rico-Hesse, 2006). Vector density indicates frequency of vector control, and expresses in House Index (HI), Breteau Index (BI), Container Index (CI), and Maya Index (MI). MI is the newest entomological indicator that describes mosquito habitat (breeding places) and potential risk to the vector growth (Lozano, 2002). Maya index grouped the mosquitos breeding place to the controllable sites (CS) and disposable sites (DS).

Tembalang is one of villages in Tembalang Subdistrict, where Diponegoro University located. As a consequence, Tembalang village is visited and occupied by people from different areas. High population mobility, both leaving and coming, can determine high DHF transmission pattern. In Tembalang, DHO of Semarang City reported 166.89 cases/100.000 population in 2014. Efforts on DHF control have been done but not yet brought the optimal result. The failure in DHF prevention mostly related to lack of human resource to deal with field operation and technology, vector observation method, poor technical and managerial operation on vector control (Mardihusodo, 2005; Uzcategui, *et al.*, 2001). Vector control plays an important role in DHF prevention since neither medicine nor vaccine are available for DHF. Vector control can be done mainly by understanding the vector's habitat, competency and susceptibility towards dengue virus (Abednego, 1996).

This study intended to describe level of vulnerability to DHF based on maya index and bionomic. The results are expected to be implemented by Primary Health Cares in Semarang to control and decrease DHF cases.

Method

The study was a cross sectional study. The study was conducted in Tembalang Subdistrict as one of endemic subdistricts with high endemicity of DHF. That sub district had 12 villages. Sample was total village. Every village was selected 15-20 houses to measure the variables. The

criteria for houses selection per village was the house of DHF patients reported in 2013 and their neighbour.

The variables of the study were larvae density, bionomic of the vector, and Maya Index. To identify of the vector, ovitraps were placed indoor and outdoor. It used a attractant made from hay solution 10%. Larvae density was measured by house index (HI), container index (CI) and Breteau Index (BI). Maya Index described of sanitation level measured together with larva survey. Maya Index was counted by formula, consist of index of Hygiene Risk (HRI) and Breeding Risk (BRI). Type of breeding places and present of larvae were recorded from the house selected, both indoor and outdoor the houses. HRI was determined by dispossable sites (DS), and BRI was determined by controllable sites (CS).

Data collection used observation sheet, ovitrap, GPS and PCR. The data was analysed descriptively by mapping and tabulating.

Results and Discussion

Tembalang Subdistrict is one of 16 subdistricts located in Semarang. Tembalang area is 3,871.765 ha and consists of 12 villages. There were 145,991 inhabitants in 2013, with 73,810 males and 72,181 females. Tembalang has two primary health center i.e. Puskesmas Rowosari and Puskesmas Kedungmundu. In 2013, Semarang Health Office reported Tembalang Subdistrict had 204.81/100.000 DHF incidents and CFR 0.6%. Tembalang is the one of subdistricts and endemic areas with high incidence of DHF in Semarang.

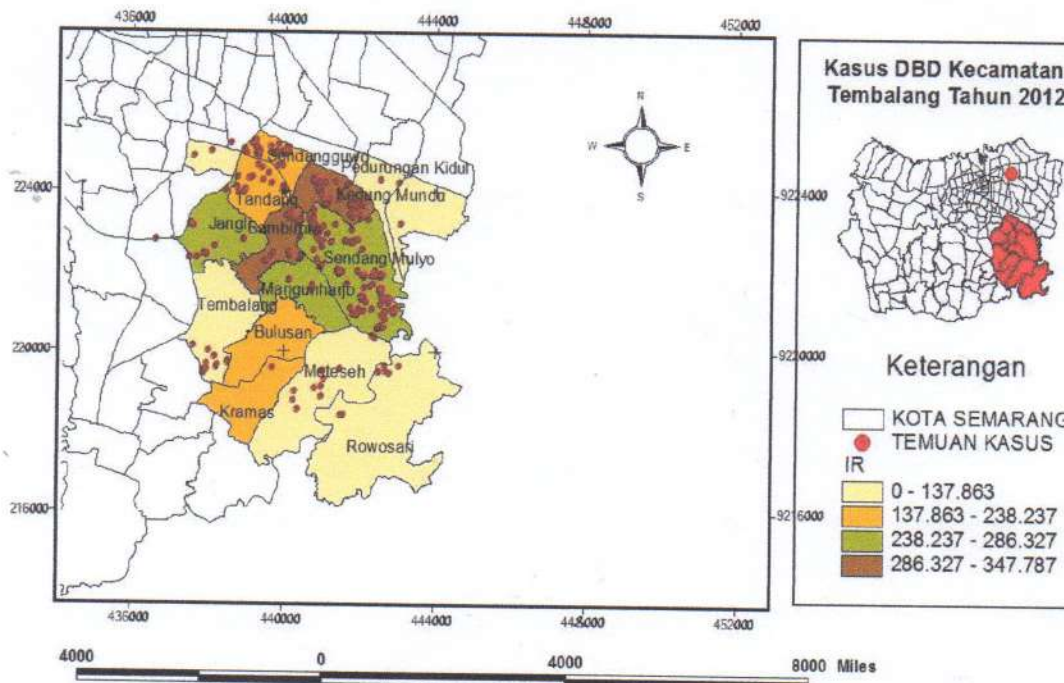


Figure 1. Distribution of DHF cases in 12 villages of Tembalang sub district

Most of DHF cases found in Sendangmulyo Village. Bulusan and Kramas villages were included in medium cases, however this should not be ignored since population density is increasing in these two areas. High distribution of DHF cases was reported in Sambiroto and Kedungmundu Villages. PCR results showed dengue virus was only found in *Aedes aegypti* mosquito from Sambiroto Village. After electrophoresis, the positive sample showed 342 bp band, which indicated Den-1 type virus. This should be a concern because Sambiroto is a residential area with high population density. We performed rearing of mosquitoes trapped from fields in laboratory to produce first filial generation (F1). Evidence from virology identification from F1 mosquitoes using PCR indicated there was transovarian transmission of dengue virus. Joshi (2002) even revealed that dengue virus was persistently transmitted through transovarian until F7.

Our survey showed vector density per 100 houses, expressed in HI, BI, and CI, in Tembalang Subdistrict was very high, 21.2%, 50.8%, and 13.8 respectively (Tabel 1). Mosquitoes identification resulted a relatively high proportion of *Ae. albopictus* population (32.16%) in Tembalang, which constituted half of *Ae. aegypti* (67.84%). Oviposition of *Ae. aegypti* indicated the mosquito prefers both indoor (67.84%) and outdoor (46.17%) the houses. Similarly, *Ae. albopictus* was also found indoor (32.16%) and outdoor (53.83%).

Table 1. Vector density and species type in Tembalang Subdistrict Semarang

Factors related to mosquito vector	
1. Entomological indexes:	
a. House index (%)	21.2
b. Container index (%)	50.8
c. Breteau index	13,8
2. Number of <i>Aedes</i>	
a. <i>Aedes aegypti</i>	
- Indoor (%)	66.3
- Outdoor (%)	45.2
b. <i>Aedes albopictus</i>	
- Indoor (%)	33.7
- Outdoor (%)	54.8

Based on the mosquitos eggs captured in the area, *Ae. aegypti* still be found mostly inside the house rather than outside the house. In the ovitrap, it was also found the mix breeding between both mosquitos in the same ovitrap, both inside and outside the house. The density of *Aedes sp* was dominated by *Ae.aegypti*. One third of *Ae.aegypt* was also identified outside the house. *Ae. Albopictus* was only 12% out of the total mosquitos identified in the ovitrap. More than three quarter was found outside the house. Based on the sex identified from the eggs in the rearing ovitrap, it showed almost equal sex between male and female both for *Ae. aegypti* (female : 69.65% dan male 72.54%) and *Ae. albopictus* (48.47% and 51.52%).

The analyses were conducted toward the mosquito ability to be vectors from the point of view of binomic characteristic, reproduction ability, and mosquitos susceptibility towards dengue virus. The result shows that the vector density in Semarang is relatively high. The mosquito density is measured using index like HI, CI, and BI. All index is over the health program standard (HI and CI < 5% and BI < 20%).

Environment factor like temperature and humidity are supporting the vector in Semarang. Temperature will influence the metabolism process. Low temperature is influencing the eggs growth, on the contrary, increasing temperature can decrease vector size and adult mosquitos size. Small body size will will increase metabolism speed, food consumption, and as a result will increase the eggs produced (Wongkoon, 2007). Temperature and humidity measured in studied region with the average temperature of 32°C and 64%. The measurement was taken during the survey.

Bionomic characteristic of the mosquitos is generally specific for the certain environment. It can be seen from the competition of *Ae. aegypti* and *Ae. albopictus* in the studied region. *Ae. aegypti* dominated all area in Tembalang sub-district Semarang, but *Ae. Albopictus* still can be found both inside and outside the house. *Ae. aegypti* domination shows that the vector was usually found doing activity outside the house. It indicated that there is changing behavior of *Ae. aegypti* to the *peri-domestic* that is behavior changing from inside the house into outside the house (Capinera, 2008). *Ae. albopictus* population can also be found inside the house. Larva survey result found the breeding place of *Ae. albopictus* in the water dispenser container inside the house, mainly in the village where the plants is still cultivated in the yard. This land use suits to the real habitat of *Ae. albopictus* which is the forest species that after all had adapted with the housing or human environment both in rural and urban (village and cities) (Santoso, 2008). The eggs and vector of *Ae. albopictus* was also found in the house. The movement of *Ae. albopictus* from outside into inside the house is predicted to increase the chance of female *Ae. albopictus* to get blood (Nur Aida, 2008; Rosilawati, 2007). Hamady (2010) added that the migration of *Ae. albopictus* into the housing complex can increase vector life span. As a result it can increase its capacity as a vector both from the vector-host contact and the vector density. The behavior changing pattern mainly in *Ae. albopictus* from ecotype semidomestic to domestic was also reported in India by a researcher Rao (2010). It was found more than one species or mixed breeding in some ovitrap both inside and outside the house. Chen *et al* (2006) also got this finding with mixed breeding between 10%-32%. Moreover, *Ae. aegypti* dan *Ae. albopictus* ratio is higher inside the house (25.79) compared with outside the house (2.85%).

From the data of gonotrophic cycle measured in the previous research (Martini *et al*, 2012), it was noted that gonotrophic cycle was about 2.6 – 2.8 days. It means that after mosquitos 3 day reproduction activities, they go back to normal looking for blood. Similary it is with the potency to bite and transmit dengue virus from the sufferer to the healthy one. The research found that biology cycle from egg to adult mosquito take 12 days. The implication of this characteristic is closely related to the mosquito age. The age of mosquito is closely depended on temperature and humidity of the environment. As it was explained by Cook *et al*, (2006) that mosquitos age is a critical determinant of vector capacity mainly in its potency to transmit dengue virus to the susceptible host.

The entomology survey result conducted by Martini *et al*, (2012) showed third quarter (66.7%) mosquitos' *parous rate* in Semarang in reproductive condition (ever laid eggs). It showed that most mosquitos was already in old age. Mosquito age determine the life of mosquito per day. Life chance of mosquitos is influenced by some factors including intrinsic factor like age and genetic, and extrinsic factor like nutrition, predator, and insecticide use (Fouque, 2006). The high

life chance of mosquito in a day would increase the chance of mosquito to feed blood mainly on DBD patients in the viremia phase. Mosquito would become invective and potentially spread virus (Freitas, 2007).

Breeding places of *Aedes sp*

The study observed 930 containers and 17,53% positively container containing of *Aedes sp* larvae. Type of container was classified to *Controlable Sites* and *Disposable Sites*. *Controlable sites* were container generally saving water for household need and they were able to control by the owner the houses. In this study, controlable sites that were inspected such as bathtub, bucket, crock, dispenser, drum, water tanks. Container in lavatory, aquarium, flower vase, pool, refrigerator, gutter, birdbath, jar mad from soil, jerry can, wash basin, pan coper bowl, drain trash, and bucket lid. Disposable Sites were goods that were not used again, generally, the owner put them around their house, its impact, it could become breeding place potentially when rainy season come. Disposable Sites were found in this study such as cans, midrib, sandals, tire, coconut shell, bamboo, tile, broken lamp and glass, sheeting. Proportion CS (95%) was more than DS (5%), also CS positive larvae (88,34%) was more than DS positive larvae (11,66%). Percentage of Disposable Sites (DS) were positive larvae mostly finding on cans (47,37%). Containers of disposable sites that was examined as many as 38 unit, but a half (50,0%) containers positive larvae.

Proportion of CS positive larvae most finding were bathtub (36,8%), bucket (19%), crock (13%), dispenser (9%), and drum (6,9%). That containers were used for saving the water and the owners were not provide the covers. Vector could lay their eggs easily. Beside, the owners did not brush the wall of the bath tub properly. The eggs of vector would hatch in a few days. While proportion DS positive larvae mostly found in cans (47,37%), midrib (15,79%), and sandals (10,53%). That phenomenon indicated that community was not throwing the garbage properly, and they let the garbage put around in their yard. Midrib that was examined positively containing larvae, and this described that community did not maintain cleanliness their environment from potentially breeding places.

Maya Index (MI) in Tembalang Sub-district

Almost the sample of houses were included in BRI2 (66,25%) dan HRI2 (89,17%). Low score of BRI described a few CS in the field dan had low risk to be breeding places of the vector. The high score of HRI showed that DS would be found many containers that was not used again available both indoor and outdoor. The other meaning was the house inspected was a unclean category. According to BRI and HRI, the residences' houses in Tembalang had a high risk as breeding places to vector, and DHF transmission could be transmitted easily. Daniz-Lozano (2002) revealed that number of CS and DS every house proportionate with score of BRI and HRI. The increasing of BRI score was more high risk of breeding place available, and increasing of HRI score was more unclean its areas inspected.

For determining of Maya Index could be arranged in matrix 3x3 based on BRI and HRI score (Table 2). The houses in high category both BRI and HRI were found 8 (3,3%) houses out of 240 inspected houses, but there were 55 (22,9%) houses had poor sanitation category. In other hand just 40 houses (16,7%) had low category, 145 houses (60,4%) were moderate category. Daniz-Lozano (2002) made classification according the matrix of the Maya Index table, namely: low if criteria of index were BRI1/HRI1, BRI1/HRI2, BRI2/HRI1; moderate were BRI1/HRI3, BRI2/HRI2, BRI3/HRI1; and high if it were BRI2/HRI3, BRI3/HRI2, BRI3/HRI3.

Table 2. Classification of *Maya Index* (MI) in Tembalang, 2014

Indicator	BRI 1	BRI 2	BRI 3
	(Low)	(Moderate)	(High)
HRI 1 (Low)	0 (Low)	0 (Low)	0 (moderate)
HRI 2 (Moderate)	40 (Low)	143 (Moderate)	31 (High)
HRI 3 (High)	2 (moderate)	16 (High)	8 (high)

The number of houses in mostly DS indicated that areas had poor sanitation, and had high risk to become breeding place to the vector (Daniz-lozano, 2002). A study in South Denpasar showed there was correlation *Maya Index* in high score with DHF incidence. The risk ratio of DHF incidence as many as 3,088 comparing the area having high score of *maya index* against low score of *maya index* (Purnama dan Baskoro, 2012). But, the study was done by Rahayu et al (2010) found that there was no correlation DHF incidence and *maya index* in Sawahan Sub district.

Conclusion

Vector density in Tembalang sub-district Semarang City was very high based on vector HI=21.17%, CI=50%, dan BI=13.8. It showed that Tembalang area having high transmission of DHF. *Maya index*, which indicates hygiene and sanitation, showed 23% houses in Tembalang Subdistrict had poor sanitation. This mean that breeding places of vector was abundant in this area. There were two species of DHF vector, *Aedes albopictus* and *Ae. aegypti*, and proportion of *Ae. albopictus* was half of *Ae. aegypti*. *Ae. aegypti* from Sambiroto village contained virus Den-1 positively. To control the vector population and decrease DHF cases, it is important to conduct breeding places elimination (*pemberantasan sarang nyamuk/PSN*), both indoors and outdoors, through active participation of the community.

References

- Abednego HM. 1996. Menggerakkan masyarakat dalam pemberantasan sarang nyamuk demam berdarah dengue (PSN-DBD): Petunjuk bagi kader dan tokoh masyarakat pada pencegahan penyakit demam berdarah dengue. Jakarta : Ditjen P2M dan PLP Depkes RI.
- Anderson JR, and Rico-Hesse R. 2006. *Aedes aegypti* Vectorial Capacity is Determined by Infecting Genotype of Virus Dengue. Am J Med Hyg 75(5): 886-893
- Capinera JL. 2008. Encyclopedia of Entomology. 2 nd. Vol 1-4. Springer: Leipzig. 4346pp.
- Chen CD, Nazni WA, Lee HL, Seleena B, Mohd Masri S, and Chiang YF. 2006. *Mixed breeding of Aedes aegypti (L.) and Aedes albopictus Skuse in four dengue endemic areas in Kuala Lumpur and Selangor, Malaysia*. Trop. Biomed. 23 (2); p. 224-227.

- Cook PE, Hugo LE, Iturbe-Ormaetxe, I, Williams CR, Chenoweth, SF, Ritchie SA, Ryan PA, Kay BK, and O'Neill S. 2006. The Use of Transcriptional Profiles to Predict Adult Mosquito Age under Field Conditions. *PNAS*. 103 (48) : 18060–18065
- Focks DA. 2003. A Review of Entomological Sampling Methods and Indicators for Dengue Vectors. Special Programme for Research and Training in Tropical Diseases. WHO : Geneva.
- Freitas RM-D, Codeco CT, and Lourenco-De-Oliveira R. 2007. Daily Survival Rates and Dispersal of *Aedes aegypti* Females in Rio De Janeiro, Brazil. *Am. J. Trop. Med. Hyg.* 76 (4): 659-665.
- Hamady D, Saifur, R.G.M., Hassan, A.A., Che Salma, M. R., Boots, M., Satho, T., Jaal, Z., and Bakar, S.A. 2010. Indoor-Breeding of *Aedes albopictus* in Northern Peninsular Malaysia and Its Potential Epidemiological Implication. *PloS One*. 5(7): 1-9.
- Joshi V, Morruya DT, and Sharma RC. 2002. Persistence of Dengue 3 Virus through Transovarial Transmission Passage in Successive Generation of *Ae. aegypti* Mosquito. *Am J Trop Med Hyg* 67 (2) : 158-161.
- Kementerian Kesehatan RI. 2011. Modul Pengendalian Demam Berdarah Dengue. Jakarta: Kemenkes RI.
- Lozano, R.D., Rodriguez, M.H., Avila, M.H. 2002. Gender-related Family Head Schooling and *Aedes aegypti* Larval Breeding Risk in Southern Mexico in *Salud. Publica de Mexico*. 44: 237-242
- Mardihusodo, S.J. 2005. Manajemen Pengendalian Vektor Demam Dengue. Dalam Simposium Dengue Control Up date. Yogyakarta : Pusat Kedokteran Tropis UGM.
- Martini. 2014. Model Transmisi Demam Berdarah Dengue di Dataran Tinggi Kabupaten Wonosobo Provinsi Jawa Tengah (Kajian Bionomik Vektor dan Lingkungan). Disertasi. Unair, Surabaya.
- Mc Michael, A.J., Champbell-Lendrum, D.H., Corvalan, C.F., Ebi, K.L, Githeko, A.K., Scheraga, J.D., and Woodward, A. 2003. Climate Change and Human Health Risk and Response. Geneva : WHO
- Nur Aida H, Abu Hassan A, Nurita AT, Salmah MR, Norasmah B. 2008. *Population analysis of Aedes albopictus (Skuse) (Diptera: Culicidae) under uncontrolled laboratory conditions*. *Trop Biomed* 25: 117–125
- Rao BB. 2010. Larval Habitats of *Aedes albopictus* (Skuse) in Rural Areas of Calicut, Kerala, India. *J Vector Borne Dis*. 47:175-7.
- Purnama SG, Baskoro T. 2012. *Maya Index dan Kepadatan Larva Aedes aegypti Terhadap Infeksi Dengue*. *Makara Kesehatan*. 16 (2): 57-64
- Rahayu M, Baskoro T, Wahyudi B. 2010. Studi Kohort Kejadian Penyakit Demam Berdarah Dengue. *Berita Kedokteran Masyarakat*. 26 (4): 163-170.
- Regis, L., Souza W.V., Furtado, A.F., Fonseca, C.D., Silveira, Jr PJ., Melo-Santos, M.A., Carvalho, M.S., Monteiro, A.M. 2009. An Entomological Surveillance System Based on Open Spatial Information for Participative Dengue Control. *Annals of the Brazilian Academy of Sciences*. 81(4): 655-662
- Rozilawati H, Zairi J, and Adanan CR. 2007. Seasonal abundance of *Aedes albopictus* in selected urban and suburban areas in Penang, Malaysia. *Trop Biomed* 24: 83–94.
- Soegijanto, S. 2006. *Demam Berdarah Dengue.. Surabaya: Airlangga University Press*.
- Santoso B.A. 2008. Hubungan Pengetahuan Sikap dan Perilaku (PSP) Masyarakat Terhadap Vektor DBD di Kota Palembang Provinsi Sumatera Selatan. *J. Ekol. Kes*. 7:732-9.
- Suroso, T. Hadinegoro, S.R., Wuryadi, S., Simanjuntak, G., Umar, A.I, Ritoyo, P. Dj., Kusriyatuti, R., Ali Izhar, AR. 2003. Pencegahan dan penanggulangan penyakit demam dengue dan demam berdarah dengue. Jakarta: Depkes RI.
- Suryo Suminar, D.R. 2008. Penentuan tingkat kerentanan wilayah terhadap perkembangbiakan nyamuk *Aedes aegypti* dan *Aedes albopictus* dengan penginderaan jauh dan system informasi geografis. Prosiding seminar nasional sains dan teknologi-II. Universitas Lampung;

2008. Di akses pada tanggal 16 Februari 2010. Di unduh pada <http://lemlit.unila.ac.id/file/arsip%202009/SATEK%202008/VERSI%20PDF/bidang%204/IV-13.pdf>
- Umniyati, SR. 2009. Teknik Imunositokimia dengan antibodi monoklonal DSSC7 untuk kajian patogenesis infeksi dan penularan transovarial virus dengue serta surveilansi virologis vektor dengue. Disertasi. Jogjakarta : Pasca Sarjana UGM
- Uzcategui, N. Y., Camacho, D., Comach, G., de Uzcategui, R. Cuello, Colmes, E. C., Gould, E. A. 2001. Molecular epidemiology of dengue type 2 virus in Venezuela: evidence for in situ virus evolution and recombination. *J. General Virol.* 82 : 2945–2953.
- Widayani, Prima. Pemanfaatan Data Pengideraan Jauh dan Sistim Informasi Geografi (GIS) untuk Zonasi Daerah rawan Demam Berdarah Dengue. Workshop Spasial and Temporal Analysis of Malaria Epidemiological Data. Yogyakarta : Fakultas Geografi UGM: 2007.
- Wongkoon S, Jaroensutasinee M, Jaroensutasinee K, Preechaporn W, Chumkiew S. 2006. Larval Occurrence and Climatic Factors Affecting DHF Incidence in Samui Islands, Thailand. *Inter. J. Biol. Life Sci.* 2 (2): 107-112.