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Population of *Aedes sp* in Highland of Wonosobo District and Its Competence as A Dengue Vector

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Abstract. The increased cases of dengue fever have occurred in the highland of Wonosobo District, and the epidemic taken place in 2009 had 59.3 cases per 100,000 populations. This study aimed to describe of vector competence of the mosquitoes as a dengue vector in the highland of Wonosobo District, Central Java Province. The serial laboratory work was done to measure of vector competence complementary with vector bionomic study. The samples were 20 villages, which were located at Wonosobo sub district. Every village was observed about 15-20 houses. The observed variables were vector competition, bionomic and transovarial infection level, and titer of virus on the mosquitoes after injection. Immunohistochemistry or IHC methods were used to identify transovarial infection status. The number of *Ae. aegypti* and *Ae. albopictus* were almost similar and both were found indoors or outdoors. Based on HI and OI index, the larvae density in the highland was enough high than standard of the program. Transovarial infection was found on *Ae. aegypti* and *Ae. albopictus*. Environment parameters such as temperature and relative humidity fulfilled the optimum requirement to support the vectors' life cycle. Transovarial infection has been proven, thus, it indicates that the local transmission has been occurred in this area. Titer of virus was also increasing after day per day. This indicate that the mosquitoes has the ability being vector. As used to do in other area, it is important to conduct breeding places elimination (PSN) indoors as well as outdoors, through active participation of the community in highland area.

Keywords: *Aedes* sp, mosquito, highland, transovarial infection

1. Introduction

Dengue Hemorrhagic Fever (DHF) is a disease caused by dengue virus, transmitted from human to human by *Aedes* mosquitoes (Ae.) sub-genus of Stegomyia. Dengue cases tend to increase and wider spread every year. WHO reports says that more than 40% of the world population are at risk of dengue fever. There was estimated 50 million new infections per year [1]. Intergovernmental Panel on Climate Change (IPCC) predict the incidence of Dengue Hemorrhagic fever (DHF) in Indonesia will increase in the following years [2].

Central Java is the DHF endemic Province since all of its districts (35 districts) reported dengue hemorrhagic case. In the year of 2009 the Incidence Rate (IR) was 5.79/10.000, and the Case Fatality

Rate (CFR) was 1.42%. Wonosobo, a highland district was showed the escalating of DHF incidence (CI was less than 1/10.000 during 2001-2008 and 5.7/10.000 in the year of 2009) and followed with the escalation of CFR (0.43% in the year of 2009 and 2.23 in the year of 2010). In the past, we used to argue that the case in highland area such as Wonosobo happened in others lower area (imported cases) since in highland circumstances *Aedes* spp was not competence to be DHF vector.

Increasing DHF cases in highland indicates the increase of DHF vector's density. There has been an environmental change such as temperature, rainfall, caused by global warming phenomena. Air temperature and humidity affect the viability of the vector [3]. These changes will also affect mosquitoes life cycle, especially the breeding of Aedes spp. The length period from egg to adult mosquito phase is getting shorter. Low temperature can suppress the development of larvae and eggs of Aedes spp [4, 5]. Changes in the environment will affect life of mosquitoes species on ecosystems and the pattern of viral vectors spread and thus can increase the incidence of dengue transmission.

At a temperature of 26^0C virus requires 25 days in mosquito body to get ready to be transmitted to humans (extrinsic period), conversely takes a relatively shorter, 10 days at a temperature of 30^0C. By then, life period could be the critical determinant of *Aedes* spp competency to be DHF vector in higland areas [6]. Increase the capacity of the mosquito to be vector is affected by the mosquito population density, cycle rate gonotropic, extrinsic incubation period as well as the readiness of the virus in the body of the vector for transmission. In [7] proves that the mosquito can live until 1 month in optimum air conditions. By taking into account the DHF virus extrinsic period, the environment changing and mosquitoes lifespan Aedes spp could be the potential vector for Dengue transmission in highland areas [8].

This study try to explore in which extend the *Aedes aegypti* in Wonosobo has competency as DHF vector.

2. Research Method

Entomology survey was done in Wonosobo sub district, the most populated and highest DHF incidence in Wonosobo district. involving 20 houses from 20 vilages. Ovitraps with specific attractant were placed indoor as well as outdoor [9]. Mosquito eggs were incubated to produce mosquito as research material. Mosquitos' age were 7 days used to further measurement. We detect susceptibility *Aedes* sp from dengue virus infection using immunohistochemical (IHC) technique. An laboratory study was applied to examine the mosquito competence as in vivo vectors with injected the mosquito with a virus of Den-2. Mosquito was identified the titer of virus load by using ELISA. Mosquito's age were used this study were 3 days and 10 days after reared from the eggs. Discriptive analysis was applied to explain to elaborate the data.

3. Result and discussion

3.1 Capacity of vector

The DHF incidence in Wonosobo district in the year of 2012 was 0,67/10.000. This incidence is higher than the year of 2006 (0,12/10.000) and in the year of 2008 (0,37/10.000). In 2009, DHF was increasing sharply, 5.79/10.000 population. Until in 2013, DHF still find in this district with incidence around 19,79/10.000 population.

Based on entomology survey, the *Aedes* sp larvae density are, House Index (HI)=8,8%, BI=1,70, Container I=4,65% and OI=5,39% and categorized as low density. Container as mosquito breeding place in household circumstances are batch tube, bucket, and used canned. The average number of egg in inner house ovitrap is 1,16 and 0,73 in outer house. The average number of larvae in village sample are OI 5,4%, and HI 8,8% (higher than standart of the program, 5%).

Mosquito eggs were reared and indentified. Table 1 shows the distribution of Aedes sp. *Aedes aegypti* was founded more inner house (78,4%) than *Aedes albopictus* (54,1%). We also found *Ae. anandalei* during survey using ovitrap.

Table 1. *Aedes* sp from ovitrap survey (rearing) and Mosquito Survey

Height (above sea level))	*Ae. Aegypti*		*Ae. albopictus*		*Ae. anandalei*		Sum	%
	f	%	f	%	f	%		
1. Rearing								
Indoor	58	78,4	15	20,3	0	0,0	74	66,7
Outdoor	9	24,3	20	54,1	8	21,6	37	33,3
2. Survey								
Indoor	34	77,3	10	22,7	0	0,0	0	0,0
Outdoor	1	11,1	8	88,9	0	0,0	0	0,0

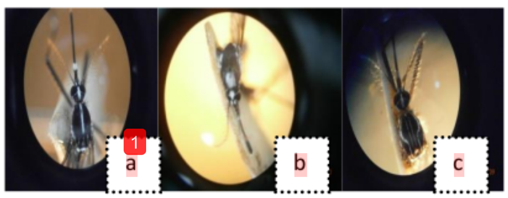

Figure 1. *Aedes* sp was identified in Wonosobo (a. *Ae. Albopictus*, b. *Ae. Anandalei*, c. *Ae. Aegypti*)

The quantity of larvae density and adult *Aedes* sp is linier. This figure indicating the mosquito's dynamic live circle was complete. Environment factors such as temperature, humidity in Wonosobo is adequate to support life circle process [10]. The original habitat of *Ae. albopictus* is forest and have adapted to live in the land use for agriculture surrounding human settlement [11, 12].

The existence of *Ae albopictus* population on the plateau suggests that this species is able to adapt to the environment in the highlands. Surveys both indoor and outdoor proves that there is a shift in the pattern of life from eco-type semi domestic to domestic.

Shifting Ae. albopictus from outside to inside human dwellings is perhap increase the chances of *Ae. albopictus* females in getting the blood [13, 14, 15]. In [15] stated that the shift *Ae. albopictus* into human dwellings can increase the lifespan, and increase its capacity as a vector, both in terms of vector-host contact as well as the density of the vector population. Changes in behavior patterns *Ae. albopictus* from domestic ecotypes into a semi-domestic, also reported in India [16].

Unlike the *Aedes* spp competition in the lowlands, especially in densely populated areas, population *Ae. aegypti* is also found outdoor. This indicates the behavior changing of *Ae. aegypti* to be peri-domestic, bionomics change of the house toward the outdoor [17].

3.2 Aedes sp susceptibility against Dengue virus as *Aedes* marker become vectors of dengue in the highland

Using IHC examination, we found susceptibility of *Aedes* sp in Wonosobo highland area, indicating its capability as DHF vector. Polymerase Chain Reaction (PCR) test failed to identify viral genetic marker. Transovarial infection confirmed the capability of *Aedes* sp as DHF vector in highland area. This phenomena demanding prompt program response to prevent endemic area in highland area. In [18] stated that Dengue virus was persistently ovarial transmitted until F7. We confirmed 7 positive dengue virus among 20 suspect DHF patients (35%) of Wonosobo District Hospital. DEN-1, DEN-2, DEN-3 and DEN-4 were identified moreover 2 patient were mixed infected [19]. Concerning in [20], the existence of four serotype through the year will increase the risk of viral transmission. Many researchers have proved transovarial transmission relating with various level of infection. Umniyati found 13,6-33,8% infection index from transovarial *Ae aegypti* taken wheel in Yogyakarta [21], whilst [7] found 38,5-70,2% from another place in Yogyakarta. Determinant factors of mosquito to become vector are mosquito susceptilbility, geographical variation and mosquito strain [23]. Some researchers stated that a genetic variation so called barrier *infection system* in the midgut, sexes, strain and species as determinant factors of vector susceptibility. Those physiologic barriers in the midgut are *Midgut Infection Barrier* (MIB) and *Midgut Escape Barrier* (MEB) [24], but, the molecular barrier mechanism remain unclear

In our research we found viral DEN-2 titer escalation in experiment group after three days injected compare with control group. In the tenth day, viral titer escalated indicating viral growth after invading physiologic barrier in mosquito body. Based on this finding, dengue hemorrhagic fever cases in Wonosobo plateau could be caused by local transmission as well as imported cases and prevention activities such as mosquito breeding places eradication are needed.

Table 2. Virus titer Den-2 in *Ae. aegypti* in ELISA plate.

	1	2	3	4	5	6	7
A	0,883	0,589	0,305	0,708	1,057	0,227	0,215
	A1	A2	A3	A4	A5	A6	A7
	Dilution 1:4	Dilution 1:10	Dilution 1:20	Incubation for 3 days	Incubation for 10 days	Control (negative)	Control (negative)

Description: A1-A3 is the dilution of the positive samples, A4-A5 mosquitoes injected in accordance with the developmental period of the virus in the mosquito's body, A6-A7: negative control.

4. Conclusion

The dengue incidence in Wonosobo highlands in 2013 amounted to 19.79 / 100,000 people. Increased incidence associated with environmental conditions that have met the requirements to support life optimum vector of dengue.

Competition *Ae. aegypti* and *Ae. albopictus* downloading a balanced approach and both types can be found either in the (indoor) and outside the home (out door). Found another species, namely *Ae. annandale* besides *Ae. aegypti* and *Ae. albopictus*. *Ae. aegypti* strain District of Wonosobo can be a vector of dengue based Den-2 virus multiplication in mosquitoes characterized by increased titers of the virus through the test in vivo,

Transovarial infection occurs in *Ae. aegypti* and *Ae. albopictus*. Transovarial evidence can make repeated epidemics, as happened in 2009.

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