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# Resistance status of *aedes sp* strain from high land in Central Java, Indonesia, as an indicator of increasing vector's capacity of dengue hemorrhagic fever

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## Abstract

**Context:** The increased cases of dengue hemorrhagic fever (DHF) have been reported in the highland of Wonosobo District. Vector-control program are used for prevention and control of dengue cases. Vector's profile toward insecticide efficacy is important which need to be elucidated. **Aims:** The aim of this study was to describe the resistance status of the vector against malathion insecticide. **Settings and Design:** A cross-sectional survey was done in the highland area, Wonosobo sub-district, Indonesia. **Methods and Material:** The study included a sample of 37 villages, which were located at Wonosobo sub-district. At every village, we observed 15 houses. The observed variables were mosquito bionomic, and their resistance status was based on the biochemical and molecular parameters. To detect resistance status, we used ELISA equipment. **Statistical analysis used:** Data were collected by entomology students and were analyzed using descriptive statistics. **Results:** The study results showed that the population of *Aedes sp* in the Wonosobo District was higher than the standard control of dengue program. Vector indexes were HI (14.75%), CI (6.8%), BI (15.6%), and OI (11.3%). We identified that vectors have already developed resistant to organophosphate insecticide, as many as 50% out of the total sample tested. **Conclusions:** This finding indicated that *Aedes* sp strain in the Wonosobo District highland has competence to be dengue vectors. Therefore, it is important to use another type of insecticide such as pyrethroid.

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## Full Text

### Introduction

Dengue hemorrhagic fever (DHF) is a communicable disease caused by dengue virus and transmitted from person to person through Aedes (Ae) from subgenus Stegomyia. There has been constant increase in the incidence and geographical expansion of DHF. According to the World Health Organization (WHO), 50 million new cases occur annually and more than 40% of world population are at risk.[1] Subsequently, in 2008, according to the Indonesian National report, the incidence rate (IR) was 58.94/100.000 with a case fatality rate (CFR) of 0.86%.[2] Although, in comparison to the 2007, there was a decrease in the incidence of DHF this time, it is still high compared with national target of about 20/100.000 per year. As predicted by Intergovernmental Panel on Climate Change (IPCC), there would be a substantial increase in the DHF incidence in Indonesia in the following years.

Central Java Province is one of the DHF endemic areas in Indonesia. According to the Health Agency in Central Java, there were 18,877 DHF cases in 2009. The disease IR was 57.9/100.000, and CFR was 1.42%. In that period, all districts and cities (35 districts/cities) in Central Java province either in highland or lowland reported DHF cases. From 2001 to 2008, Wonosobo District reported the IR less than 10/100.000 people. The following year, the IR of DHF in Wonosobo was 59.7/100.000 people and CFR was 0.43%, whereas in 2010, the IR was 22.3/100.000 people. In 2009, the DHF infection spread in all districts in Central Java Province including plateau areas such as Banjarnegara and Wonosobo. This fact indicates that the assumption that dengue cases of occurred in Wonosobo are imported cases can no longer be maintained. The possibility that the local mosquitoes have been changed and have capacity to be vectors need to be considered. The environmental factors such as abiotic environment (global warming), temperature, rainfall, and humidity, the use of certain pesticide, and human activities may affect vector's bionomic. Michael et al. stated that human activities affected the tropical forest and the higher gas emission in air; hence, the earth temperature increases about 1–3.5°C. The change in the environment component will impact the species on the ecosystem and the transmission pattern of vector and viruses.[3]

Dengue prevention activities in Indonesia have been done. An un adequate result happened due to the various causes such as lack of staff capability in applying vector control technology, vector surveillance system and managerial. Factors responsible for the failure of DHF prevention in Indonesia are scarcity of skilled healthcare units, lack of the development in science and technology, and no standards for vector surveillance system, technical practice, and vector control management.[4],[5]

The Wonosobo District, located in Central Java Province in Indonesia, is a highland that lies between 270 and 2250 masl. Wonosobo District is mountainous area. The district large is 98.468,11 ha. The land use changed compared to the previous year. There is an increase in the land use by buildings, yards, pools, farms, and so on.[6] According to the Wonosobo Health agency, the geographic expansion of DHF patients distribution reported from the Wonosobo Health agency is large, and all the health services around the district reported the cases (either dengue fever or DHF). In 2009, a total of 179 cases (IR = 29/100.000) were reported by Wonosobo Health Centre which is located in the 757 m masl. Thus, it indicates that increased cases of dengue transmission have occurred in this highland region. In the past, we used to think that topography of Wonosobo and environment parameters such as air and temperature affect the capability of mosquito as the DHF vector.[7],[8] Similar to other cultivation places, the farmers in Wonosobo used pesticides to secure their crops. Massive application of certain insecticide can implicate the increase of vector resistance against that insecticide.[9] The research report in 1985 showed that the vector Anopheles aconitus is resistance against DDT and dieldrin in Central Java Province.[10] Marcombe et al. stated some factors causing the resistance, such as agriculture practice, vector control intervention, and the urbanization. Mosquito resistance to the pesticide allows mosquitoes to survive longer and develop its capacity as a vector of dengue.[11]

### Subject and Methods

This was observational study based on the cross-sectional survey approach. This research prior with the spot survey in the study location to get the Aedes mosquito strain Highland of Wonosobo District. Mosquitoes in the highland of Wonosobo District are subject of this study. The mosquitoes were obtained by placing the ovitrap (mosquito egg trap) in the village with the new DHF case. The ovitraps were placed indoor and outdoor of 15 houses. The measured variables in this research are the type of Aedes sp found in the highland area of Wonosobo

District, the trapped mosquitoes types, the mosquito density, and the resistance status based on the biochemist and biomolecular tests.

In the biochemical test, the mosquitoes were homogenized individually in 100 μL buffer phosphate (pH 7.4). A 50 μL aliquot from every homogenate was placed in the microplate and then 50 μL substrate α-naphthyl acetate was added. In every hole of the microplate, 50 μL coupling agent was added as a color indicator. The microplate was incubated for 10 minutes in the room temperature. The color intensity produced by every hole was measured for the optical density (OD) value using a micro-assay reader at a wavelength of 450 nm and then a comparison with OD value from negative control is made.

Data were collected by the researcher and the entomologist. To warrant the equal perception in this research, there was a prior coordination between the researcher and the team. The collected data were analyzed descriptively as shown in [Table 1] and [Figure 1].{Table 1}{Figure 1}

## Results

Previously, people believed that DHF in Wonosobo district were occurred because import cases or not local transmission. Not all sub-districts reported the DHF case. Wonosobo sub-district reported the highest DHF cases in 2014–2015 with a DHF IR of 26.72/100.000. It was followed by Leksono sub-district with a DHF IR of 25.33/100.000. On the other hand, the other 13 sub-districts had IR below 10/100.000. However, the highest IR in the Wonosobo District is in Wonosobo Barat village, 150.98/100.000 people, followed by Sambek village, 78.17/100.000 people.

The collected larvae density during survey in 38 villages is described in the [Table 1], and the variables used to measure larvae populations were house index (HI), Breteau index (BI), container index (CI), and ovitrap index (OI). The density of mosquitoes is quite high. It was showed that the HI was 14.75%, CI 7%, BI 15.16%, and OI 11.3%. The rearing result from the larvae and ovitrap survey that had been identified showed the presence of Ae. aegypti and Ae. albopictus [Figure 1]. In the previous survey (in 2014), the presence of Ae. Annadale was identified.

Based on the observation of the mosquito breeding place, it can be concluded that bathroom container had the highest larvae concentration (60.5%) from all the positive larvae container types. Bamboo tube, used can, broken glasses/jar, used tire, flower pot/vase, water dispenser, fridge, and water-filled ponds also proved to be mosquito breeding paces. The dispenser is a container with almost 5% larvae. In the laboratory, larvae from the field were reared to be adult mosquito. Then, mosquito sent to advanced laboratory of Vector Research Centre to be done biochemical resistance test. We tested 90 adult mosquitos' samples that collected from 13 villages in Wonosobo sub district. It showed that almost of that collected from 13 villages in Wonosobo showed that almost half of the mosquito samples were categorised high resistance against insecticide malathion.

This resistance was tested quantitatively using the Elisa reader or visually by measuring the color change. The resistant character of Aedes sp against organophosphate. The escalation of esterase enzyme as the Aides' enzyme defense toward organophosphate is the indicator of Aides resistancy investigations, the mosquitoes residency status is described in [Table 1]. Almost all the tested mosquitoes were collected from Mlipak, Leksono, and Gunung Talang (90%). There are very limited mosquitoes found that are susceptible to the organophosphate insecticide, excluding Kalianget village (50%).

## Discussion

The increased presence of resistance enzyme indicates the occurrence of the main mechanism on the resistance against organophosphate.[12] All regions in Wonosobo showed that the resistance has occurred in this district. In 2009, the outbreak of DHF was four times higher than the previous year. Since then, the DHF case reports are decreasing, and the number is steady now, with approximately 25–50 DHF cases reported every year. The evidence that the transmission occurred was proved in a previous study. The use of malathion had stopped for the last 3 years. Nowadays, a pyrethroid-type insecticide is used in the control program.

Even though insecticide use had been replaced by pyrethroid in the last 3 years, the mosquito population which is resistance against malathion still exists. The insecticide use for a long period of time to the same target can give the selection pressure of population and push the Aedes sp population faster to become resistant.[13],[14] The weak mosquito population decreased allowing the resistant mosquito population to live and breed.

A number of studies reported the occurrence of resistance. Shinta et al.[15] reported the Ae. aegypti population in five areas in Jakarta, and Bogor city in Indonesia is resistant against malathion 0.8%. Research in Mampang Prapatan and Tanjung Priok also showed the same result.[12] In Purbalingga, Kendal, Grobogan, and Semarang, there were also resistance cases against malathion.[16] Many provinces in Indonesia have reported the resistance of Aedes sp.

Malathion is the insecticide within organophosphate classification. Based on their function, esterases are classified into the hydrolase enzyme group. That enzyme catalyses the reaction of aliphatic hydrolase compound, an aromatic ester, choline ester, and organophosphate. Enzyme could break the insecticide resistant process for the organophosphate group.[17] The resistance mechanism happened because it blocked the insecticide compound to reach its target by enzymatic activities.

The characteristic difference of general resistant in every mosquito population in the villages described nonspecific esterase enzyme expression that was formed inside every individual mosquito. The increase in esterase enzyme on resistant strain was caused by the presence of gene amplification that encode the esterase enzyme (esterase α-2 and esterase α-2), and hence, it causes the general expression percentage enhancement. Selection pressure from the domestic insecticide (pyrethroid) that is used by people enables the nonspecific esterase enzyme enhancement from mosquito population.[18] The resistance mechanism occurs because of the change in the insecticide work target (gene mutation), which causes the increase in detoxification enzyme activity, such as esterase, oxidase, or glutathione-s-transferase blocking that insecticide action.[19]

## Conclusions

The mosquito density in Wonosobo District is higher than the standard of DHF control due to the HI (14.75%), CI (6.8%), BI (15.6), and OI (11.30%). Fifty percent of the Aedes sp samples collected in Wonosobo District were biochemically tested positive resistant.

The mosquito's capability of being a DHF vector in Wonosobo convinces the belief that HDF transmission occurs even though Wonosobo is a highland area in Central Java Province.

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Ethical considerations

This research was done in 2015. We got permission from local government to carry out the research in his area. Ethical clearance got from committee ethic of medicine Faculty of Airlangga University.

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Conflicts of interest

There are no conflicts of interest.

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