

Resistance of Aedes Aegypti Mosquito on Cypermethrin and Cases of Dengue Hemorrhagic Fever and Overcrowding Population in Kediri City

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1 **Resistance of Aedes Aegypti Mosquito on Cypermethrin and Cases of Dengue Hemorrhagic Fever and Overcrowding Population in Kediri City**

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ABSTRACT

Based on the results of research on the Aedes aegypti mosquito resistance test in 2019 that the Kediri Regency area was declared resistant to malathion, for this reason, the Kediri District Health Office in the Dengue Hemorrhagic Fever vector control program for the past two years has switched from organophosphate group insecticides to synthetic pyrethroid (cypermethrin) insecticides. The purpose of this study was to determine the resistance status of Aedes aegypti mosquitoes to cypermethrin in Kediri Regency.

The type of research is True Experiment with posttest-only control group design. The Aedes aegypti mosquitoes used came from all sub-districts in Kediri Regency. The research sample was taken from the 3rd generation (F3) of adult Aedes aegypti mosquitoes which were exposed using impregnated paper with the active ingredient Cypermethrin 0.05% in a contact time of 30, 45 and 60 minutes. The data were analyzed to determine the resistance status referring to WHO, as well as to describe the population density with cases of dengue fever and dengue virus serotypes in Kediri Regency.

Kediri Regency until 2021 is not included in the densely populated (low) category. The area with the highest cases of Dengue Hemorrhagic Fever is in Pare District and the lowest case is in Semen and Kandangan Districts. Overall, the IR of Dengue Hemorrhagic Fever in Kediri District is 12.10 per 100,000 population and the CFR is 1.04%, with the serotype DEN-1, DEN. -2 and DEN-4. The average temperature and humidity of Aedes aegypti mosquito larvae for breeding are 27oC and 72%, respectively. Aedes aegypti mosquitoes from all sub-districts in Kediri Regency 100% died at 30 minutes after being exposed to impregnated paper with the active ingredient cypermethrin 0.05% and the sensitivity status was in the vulnerable category. It is recommended that the cypermethrin insecticide resistance status be tested every 2 – 3 years.

Keywords: Resistance, Aedes aegypti, Cypermethrin Insecticide, Dengue Hemorrhagic Fever, Population Density

Background

Until now, vector-borne diseases are still an important concern for health, diseases transmitted through intermediary animals (vectors), such as malaria, arboviruses such as dengue, chikungunya, Japanese B encephalitis (inflammation of the brain) and filariasis. The disease is still a problem for public health in Indonesia with high morbidity and mortality rates. The vector-borne disease is one of the environmental-based diseases that is influenced by physical, biological and socio-cultural environmental factors. These three factors will mutually influence the incidence of vector-borne diseases in their distribution areas

Given that the presence of vectors is influenced by the physical, biological and socio-cultural environment, control is not only the responsibility of the health sector but requires cross-sectoral and programmatic collaboration. Vector control methods can be done chemically or non-chemically, in reducing the growth of *Aedes aegypti* mosquitoes, it is necessary to control mosquito larvae before they become adult mosquitoes. Control of *Aedes aegypti* mosquito larvae can be done non-chemically to reduce chemical exposure so that resistance to any of the insecticides does not occur. Non-chemical control of larvae using predators, namely betta tail betta fish, which can eat 282 larvae per day, can be applied to a small bath, while *Pterophylumaltum* can eat 248 larvae per day and is applied to a large bath. (Apriliyah Joshua, 2015). In this regard, the basic concept of disease vector control uses the Integrated Vector Control (PVT) method, which is an approach that uses a combination of several vector control methods based on considerations of safety, rationality and effectiveness of its implementation and taking into account its sustainability. (Kemenkes RI, 2012). Vector control efforts carried out by the Kediri District Office since 2015 in preventing and tackling Dengue Hemorrhagic Fever by monitoring and fostering an Operational Working Group on Eradication of Mosquito Nests Plus 3 M (draining / cleaning, closing water places, and recycling used goods), training Jumantik, fishing, and Larvasidation and Fogging focus when there is a spread of cases that tend to increase. (Kediri, 2020).

Operational System Procedure (SOP) of dengue hemorrhagic fever vector control conducted in Kediri Regency is under guidelines for implementation and technical control of Dengue Hemorrhagic Fever vectors that after case reports, then followed up with Epidemiological Investigation activities or abbreviated as PE. If the results of the epidemiological investigation meet the criteria, case countermeasures are carried out with the principle of integrated control. Until now the most appropriate way to eradicate the dengue hemorrhagic fever vector (*Aedes aegypti* mosquito) is, by eradicating mosquito nests (PSN). Considering that these mosquitoes have been spread throughout the country both at home and in public places, eradication efforts are not only the duty of the government (health workers) but must be supported by the participation of the community. Limiting widespread disease transmission, preventing extraordinary events (KLB) and reducing the number of pain and death, the government also carries out vector eradication using insecticides (fogging focus) in areas found to be cases, if it meets the fumigation system based on the results of epidemiological investigations. (Kemenkes RI, 2011).

Kediri Regency is one of the areas in East Java where there are dengue fever sufferers every year. In 2017 there were 279 cases (IR= 17.71 per 100,000 population) with 7 deaths (CFR = 3.95%). The number of cases in 2018 was 486 cases (IR= 30.99 per 100,000 population) with a total of 9 deaths (CFR = 1.85%). In 2019 there were 1,382 cases (IR=

88.13 per 100,000 population) with 27 deaths (CFR= 1.95%). In 2020 there were 338 cases (IR= 21.55 per 100,000 population) with 7 deaths (CFR = 2.07%)(Kediri, 2020).

Results of research conducted Marlik (2018) That 4 (four) sub-districts are: Ngasem, Pare, Kandat and Kunjang districts are resistant to malathion 0.8%, tolerant at a concentration of 5% with exposure to 60 minutes. The mortality of *Aedes aegypti* mosquitoes exposed to Malathion was 0.8% at 5.42%, while 5% was 93.75%. After the vector was declared resistant to malathion, the Kediri District Health Office within the last two years switched to using pyrethroid synthetic insecticides in the Dengue Hemorrhagic Fever vector control program, for which researchers are interested in conducting further research testing *Aedes aegypti* mosquito resistance against cypermethrin in Kediri Regency. Resistance status is a state that indicates the level of ability of vector populations and disease-carrying animals to survive against a dose of pesticides that under normal circumstances can kill vector species and disease-carrying animals. The definition identifies that phenomena resistance occurs after vector populations and disease-carrying animals are exposed to pesticides.(Permenkes RI, 2017).

In addition to organophosphate insecticides of malathion type, there are also other types of insecticides, namely synthetic insecticides such as cypermethrin, alpha-cypermethrin and lambda cypermethrin. Research on the resistance of the *Aedes aegypti* mosquito to cypermethrin, using the Susceptibility method (Pradani, 2011). The results of the study proved that the *Aedes aegypti* mosquito is also resistant to cypermethrin insecticides. Research Sunaryo & Widiastuti (2018) It also proved that *Aedes aegypti* in North Sumatra Province and Jambi resistant against malathion 0.8%, cypermethrin 0.05% and lambda-cyhalothrin and tolerant to alpha-cypermethrin. Vector control of Dengue Hemorrhagic Fever Kediri Regency has been two years in a row using insecticides pyrethroid synthetic (cypermethrin), so the research team tried to conduct further research on the spread of *Aedes aegypti* mosquito resistance to cypermethrin synthesis group insecticides. The purpose of this study determines the status of *Aedes aegypti* mosquito resistance to cypermethrin in Kediri regency.

RESEARCH METHODS

This type of research is a True Experiment, with a posttest-only control group design experiment design. The study used *Aedes aegypti* mosquito test animals taken from 26 sub-districts in Kediri regency and then herded in entomology laboratories. Sensitivity test using the WHO's standard bioassay test susceptibility test uses impregnated paper made from active Cypermethrin 0.05% and control using ordinary HVS, with exposure lengths of 30 minutes, 45 minutes, 60 minutes and 24 hours.

Mosquito breeding work procedures

The bodies are bred to become mosquitoes (F1). Adult mosquitoes are bred and fed blood bait until they lay eggs, the eggs hatch into instar flicks 1, 2, 3, and 4. The flick changes pupae, when the pupae stage is transferred into the insectarium awaited until it becomes an adult mosquito (F2) and so on until it gets an adult mosquito (F3) that is used as a research test animal.

Adult mosquito resistance test *Aedes aegypti* WHO standard method susceptibility test using impregnated paper.

Prepare 3 tubes that are red speckled, each tube is inserted impregnated paper (paper that has been coated with cypermethrin concentration of 0.05%, then adult mosquito *Aedes aegypti* (F3) as many as 20 tails with the condition of mosquito stomach full of blood (fully fed). Describe with cypermethrin concentration of 0.05% for 15, 30, 45, and 60 minutes, while the control provided 2 green speckled test tubes and inserted paper containing no insecticide and inserted 20 mosquitoes with a full stomach condition (fully fed). After the test is completed, the mosquito is transferred into a paper cup and left to be stored for 24 hours, recording the temperature and humidity. During the storage of mosquitoes so as not to die should be given a wet towel during 24-hour storage, if the death control is between 5 - 20%, then the mortality treatment group should be corrected using Abbott's formula.

$$Correction\ of\ Death = \frac{\%Death\ treatment - \%Death\ control}{100 - \%Death\ control} \times 100$$

RESULTS AND DISCUSSIONS

Population Density and Number of Dengue Hemorrhagic Fever Cases in Kediri Regency

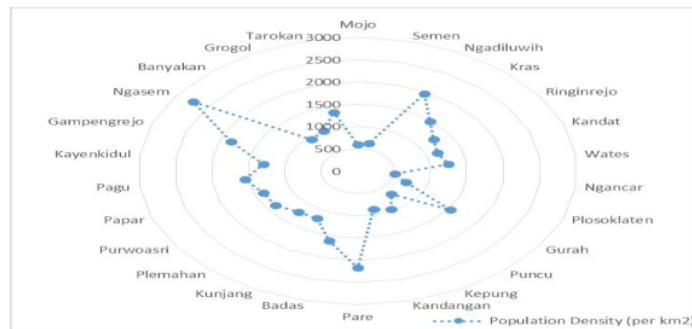


Figure 1 Population density in Kediri Regency in 2021
(Source : BPS Kabupaten Kediri, 2021)

Kediri Regency area is 1523.92 Km², with a total population of 1673,569 people. Mojo District is the most extensive subdistrict in Kediri Regency which is 141.5 km² and the narrowest district is Gampengrejo which is 18.36 km². Judging from the population of Pare District has the most populous population of 107,860 people and the least who has a population is gampengrejo district which is 33,998 people. Pare District has the highest population density of 2170.66 people / Km² and Ngancar District has the lowest population density of 510.27 people / Km² with an average of 1,098.20 people / Km². Badan Standarisasi Nasional (2004) All districts in Kediri regency population density included low category which is below 150 people / ha or 15,000 people // Km².

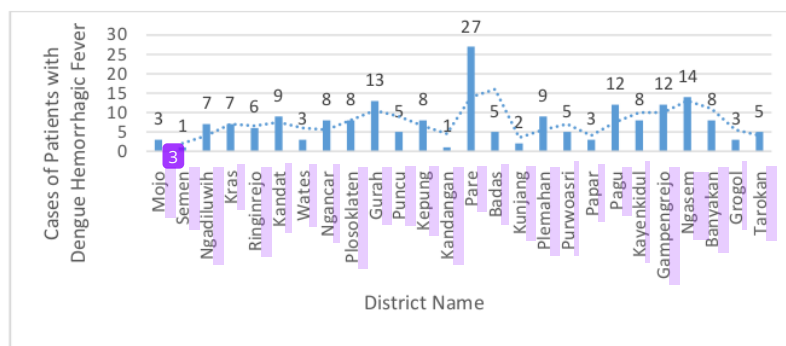


Figure 2

Number of Dengue Hemorrhagic Fever Cases in Kediri Regency In January - August 2021

(Dinkes Kediri Regency in 2021)

The number of dengue cases from January to August 2021 amounted to 192 cases and the Incidence Rate was 12.90. Pare District is the district that has the highest dengue hemorrhagic fever cases, which is 27 cases with an incidence rate of 71.02, while the lowest dengue cases are Semen District and Kandangan District.

The serotype test report conducted by BBTKL and PP in 2019 from all blood specimens of dengue hemorrhagic fever positive patients in Kediri Regency using the PCR method obtained the results of 7 (seven) specimens (+) with the identification of serotype DEN-1 as many as 3 specimens, DEN-2 as many as 2 specimens, DEN-4 as many as 2 specimens.

Percentage of Aedes aegypti Mosquito Deaths Against Cypermethrin Insecticides 0.05%

Aedes aegypti mosquitoes that are caught in laboratory rooms are conditioned for an average room temperature of 27oC, with an average humidity of 69%. The Florida city's Aedes aegypti mosquito is kept in a lab with mating releases at 280C ± 10C and 50 ± 10% RH (Alomar, 2022)The result of the death of Aedes aegypti mosquitoes after exposure to the insecticide cypermethrin 0.05% conventionally using impregnated paper made from active cypermethrin 0.05%, after exposure 30 minutes to 60 minutes and continued 1 day (24 hours) the percentage of Aedes aegypti mosquito deaths reached 100%. This indicates the sensitivity status of Aedes aegypti mosquitoes category susceptible to such insecticides. The Aedes aegypti mosquito control group was exposed without insecticides at the same time as the treatment group, the results of which obtained no death of Aedes aegypti mosquitoes.

The presence of Aedes aegypti with Dengue Hemorrhagic Fever Cases in Kediri Regency

The presence of Aedes aegypti mosquitoes taken in 26 (twenty-six) districts in the Kediri Regency shows that the level of vector density is still quite high. Topographically the area of Kediri Regency is mostly lowland and a small part is hills. Kediri Regency area is located between two mountains namely Mount Kelud and Mount Wilis, there are several sub-districts are mountainous areas that have higher plains, even from the results of data obtainedNurhaidah (2020)There are still many cases of Dengue Hemorrhagic Fever in the

environment, this shows that air temperatures in both lowland and high altitude areas do not know the presence of *Aedes aegypti* mosquitoes.

The presence of *Aedes aegypti* mosquitoes indicates that the area has a population of *Aedes aegypti* mosquitoes as vectors carrying the dengue virus. This is evidenced by the results of observations by surveying an area in Sendangguwo Village of Semarang City Indonesia. The air temperature where the growth of *Aedes aegypti* mosquitoes is very different. *Aedes aegypti* mosquitoes are found to be 51.2% in homes in areas that have an air temperature of 200C-300C, where theoretically *Aedes aegypti* mosquitoes can live well and thrive in these air temperatures, but from observations in homes in areas that have air temperatures < 200C and > 30 0C, *Aedes aegypti* mosquitoes are also found in people's homes with a percentage of 56.8%. (Novitasari & Sugiyanto 2015). The place where the *Aedes aegypti* mosquito is found also in Tulungrejo Village bumiaji district of Batu City Indonesia that the life behaviour of *Aedes aegypti* mosquitoes have undergone changes, which usually can only live in low datan areas, can now develop in high altitude areas where the height of the area above > 1,000 meters above sea level (above sea level) (Muhammad Haris Pamungkas, 2017).

Twenty-six subdistricts in Kediri Regency, both low and high-lying areas, have the presence of *Aedes aegypti* mosquitoes, indicating dengue hemorrhagic fever cases in the region will continue to exist and be sustainable. Dengue Hemorrhagic Fever cases in 2020 from 11 (eleven) subdistricts in Kediri Regency averaged 41.6 cases of dengue hemorrhagic fever. The largest 6 cases in the region are Wates, Pare and Kandat districts, with the number of cases above 80 cases of dengue fever. (Nurhaidah, Marlik, 2020). Dengue Hemorrhagic Fever cases in kediri regency are still relatively high, from January to August 2021 3 cases of Dengue Hemorrhagic Fever as many as 192 cases with a dengue hemorrhagic fever incidence rate (IR) of 12.10 per 100,000 population and cases that died until August 2021 as many as 2 cases, with a CFR of 1.04%. The CFR number obtained until that month is still above 1%, relatively high. The national target set by the CFR figure <1%.

Spread of dengue virus serotype in Kediri Regency

Dengue fever will occur by involving 3 factors, namely, agent, host and 7 environmental that support each other. Agent (cause) Dengue Hemorrhagic Fever disease is caused by the dengue virus. Dengue virus belongs to the arthropod-borne virus, genus *Flavivirus*, and family *Flaviviridae*. There are four serotypes of the dengue virus: DEN-1, DEN-2, DEN-3, DEN-4. It is transmitted through the bite of the *Aedes Aegyptus* and *albofictus* mosquitoes. The four serotypes have been widespread in Indonesia. Based on the results of examinations conducted by BBTKLPP Surabaya in 2019 using the PCR method of seven positive specimens of dengue hemorrhagic fever sufferers obtained the DEN-1 serotype, as many as 3. DEN-2 8 DEN-4 as many as 2. Based on the results of research conducted Ditjen P2P (2013) Dengue 3 is strongly associated with severe dengue cases and is the most widespread serotype followed by Dengue 2, Dengue 1 and Dengue 4. In kediri regency precisely DEN,3 which is very close to dengue hemorrhagic fever cases is not found.

Based on the serotypes found in kediri regency more serotype DEN,1 this occurs due to the serotype of dengue virus carried by the *Aedes aegypti* mosquito. Dengue virus is an arbovirus infection in humans, many occur in tropical countries and subtropics. Dengue

infection is a virus carried by the *Aedes aegypti* mosquito and transmitted to humans. This dengue virus can give symptoms of varying severity. The severity of Dengue Hemorrhagic Fever in children in Thailand from 1994 to 2006 found dengue infections serotype 2, DEN-2 and DEN-3 have a possible cause of Dengue Hemorrhagic Fever compared to DEN-4. (Fried, 2010). In Pakistan the dengue virus studied from 2007 to 2009 the most dominant serotypes were DEN-2 and DEN-3, of the two serotypes in Pakistan which were the most DAN-2 compared to DEN-3. (Fatima., 2011). The severity of dengue virus in Indonesia there are 4 serotypes of the virus, namely Dengue 1 (DEN-1), Dengue 2 (DEN-2), Dengue 3 (DEN-3) and Dengue 4 (DEN-4). Dengue virus detected in Kediri Regency in 2019 by BBTCL & P2 is DEN-1, DEN-2 and DEN-4, of the three serotypes that are most commonly found is Serotype DAN-1. Sukohar, 2014 Dengue virus spread in Indonesia that has a serotype virus that can cause severe cases is DEN with type 3 (Sukohar, 2014). Dengue virus that causes dengue fever in every country is the serotype DEN-2.

Overcrowding with Dengue Fever Cases

Population density in Kediri regency is included in the category of not dense, but cases of dengue hemorrhagic fever in the region are relatively high. Other areas in Indonesia, namely the Pare-Pare area are mostly also classified as not dense with a percentage of 77.3% of non-densely populated areas, but dengue hemorrhagic fever cases are relatively high. (Sahria Dari, Andi Nuddin, 2020). The assumption is that with the dense population, there are many containers in every house as a place to breed and breed mosquitoes, but with the passage of time and the development of advanced technology, with various human ways in controlling *Aedes aegypti* mosquitoes that allow bionomic changes of *Aedes aegypti* mosquitoes that were only able to live in clean water, can now live in puddles or dirty water. This is because the presence of support with a lot of vacant land triggers the presence of puddles or basins causing puddles. The results of the study that *Aedes aegypti* mosquitoes can live on dirty water such as lindi and hyacinth soaking water, with long life in dirty water for 17.6 days, showed that there are already bionomic changes of *Aedes aegypti* mosquitoes that can breed in dirty water. (Agustin, 2017). Population density in Kediri Regency in the category is not dense, but dengue hemorrhagic fever cases are relatively high. Cases of dengue fever have nothing to do with overcrowding. Most likely the bionomic changes of the *Aedes aegypti* mosquito are what the *Aedes aegypti* mosquitoes can survive, this is evidenced by the presence of mosquito larvae in the Kediri region that are spread both in low level and in the highlands.

***Aedes aegypti* mosquito susceptibility status to Cypermetrine**

The *Aedes aegypti* mosquito control program, which is carried out in the region in the control of vector Dengue hemorrhagic fever (DHF) by fogging/fumigation using malathion insecticides. Research Marlik (2018) *Aedes aegypti* mosquitoes from eleven sub-districts are resistant to themephos and adult mosquitoes are resistant to malathion insecticides. Nurmayanti (2020) larvae of *Aedes aegypti* *Aedes aegypti* mosquitoes in Kediri Regency resistant to temephos with a concentration of 0.04 mg / lt, reinforced again by Nurhaidah (2020) who conducted research in eleven sub-districts with the distribution of *Aedes aegypti* mosquito resistance status in the area, of the eleven sub-districts that are resistant to only 1

(one) area while others are vulnerable, to prove the presence of genes in *Aedes aegypti* mosquitoes that are tested resistant to insecticides organophosphate class can be done with PCR (Polymerase Chain Reaction) technique, if the *Aedes aegypti* mosquito is proven resistant to insecticides, Seen in the mosquito gene there is a band with a length of 250 bp, indicating VGSC is a gene that carries insecticide resistance (Yudhana, 2017). Most of Indonesia uses insecticides made from organophosphate. (Yudhana, 2017). Including Kediri Regency has long used organophosphat insecticides, this is also evidenced by the results of Marlik (2018) *Aedes aegypti* mosquitoes are declared resistant to malathion. Related to this, the Kediri district Health Office in order to control the Dengue Hemorrhagic Fever vector program switched to using chypermetric insecticides that have been carried out since the last 2 years. Insecticide chypermetrin has been used for 2 years, so it is necessary to test the resistance of *Aedes aegypti* mosquitoes to the insecticide.

Based on the results of research conducted that *Aedes aegypti* mosquitoes from 26 sub-districts in wilayakkediri districts are 100% vulnerable or die to cypermetrin. Chypermetrin is an insecticada that uses the synthetic chemical pyrethroid. This insecticide has not been used for a long time and the use of doses also meets the requirements or as recommended. This supports the susceptibility of *Aedes aegypti* mosquitoes to cypermetrin. Viewed in terms of vector control management that pre-maturation control and adult *Aedes aegypti* mosquitoes are different types of insecticide active ingredients, as well as technical control.(Permenkes RI, 2017).

Chypermetrin is an insecticada that uses the synthetic chemical pyrethroid. The target of insect organs attacked using insecticide chypermetrin is the nervous system in organisms, this indicates that this insecticide has an active substance of the type of neurotoxin, which is a toxic substance in the nerves. Insecticides chypermetrin are easily lost when exposed to water and the sun, the properties are more environmentally friendly, because it is easily degradable if in nature. Cypermetric insecticides are better and have very few negative effects on humans, but have toxic properties in insects, if insecticides are often displayed on insects will have a resistant impact on the insecticide. Mexico's Tapachula City has moved from pyrethroid insecticles to organophosphate insecticides. They used permethrin insecticidals in mosquito control for more than 5 years. Laboratory test results despite not using permethrin and deltametrin but *Ae aegypti* remain very resistant, the resistant ratio (RR) > 10 times (Solis-Santoyo, 2021). Insecticide chypermetrin used in Kediri Regency in *Aedes aegypti* mosquitoes is still susceptible or still sensitive to the insecticide, so that no insecticide needs to change the insecticada material used in vector control by fogging and needs to be tested for susceptibility every 2-3 years against the chypermetric insection used.

Conclusion

The population density in all sub-districts in Kediri Regency until 2021 belongs to the low category. The highest number of cases of EU Dengue Hemorrhagic Fever in the region is in Pare Subdistrict while dengue cases are lowest in semen and kandangan subdistricts, with an average IR of Dengue hemorrhagic fever of 12.10 per 100,000 population and CFR figure of 1.04%. The serotypes in the region are Serotypes DEN-1, DEN-2 and DEN-4. *Aedes aegypti* mosquito resistance test exposed to impregnated paper containing the active

ingredient cypermethrin 0.05% of all Districts of Kediri Regency 100% experienced death, showing sensitivity in the vulnerable status category

Suggestion

Vector control program chemically with fumigation methods with insecticide material Chypermetrin and application of 3 M plus is still implemented because it is an easy, cheap and safe control for the environment. Monitoring regularly by testing the status of resistance every 2-3 years

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