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LARVAL MOSQUITO (DIPTERA: CULICIDAE) AT DUSUN IV GOSOMA VILLAGE, NORTH HALMAHERA, INDONESIA: A SURVEY MOSQUITO LARVAE BASED ON MOSQUITO BREEDING SITES.

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ABSTRACT

North Halmahera Region is not categorized in the list of higher regions of dengue fever in Indonesia. However, in the last few years, the occurrence of fever dengue in this region tend to increase. To get basic information in order to find the proper strategy for dengue fever control, a survey of mosquito larvae in the Dusun IV Gosoma village, North Halmahera have done with the purpose was to described the density of mosquito's larvae (larval index) and the type of mosquito larvae in the different environment conditions based on mosquito breeding sites. The research was cross-sectional designed and it was held in July until August 2018 at Dusun IV Gosoma Village, Tobelo District, North Halmahera region. The mosquito larvae were checked at the containers as a potential breeding sites using single larvae methods. The larvae were identified in the Integrated Science laboratory, University of Halmahera. The identified larvae were used to calculated the Container Index (CI), House Index (HI) and Larvae Free Index (LFI). Temperature, moisture, light intensity and pH were measured directly in sites. A survey of mosquito larvae at 35 of houses which has 100 of containers. Only two types of mosquito were found, Aedes aegypti (91, 67%) and Culex pipiens pipiens (8,33%). Larvae index at Dusun IV Gosoma village was calculated, HI, CI and LFI were 37.14%, 22% and 62.86% respectively. These indicating a potential risk for dengue fever transmission in that region. The average of temperature, moisture, light intensity in the location of Ae. Aegypti were 29.75 ^{o}C, 77.50% and 1890 lux, respectively. While the average of temperature and pH within the positive containers of Ae. aegypti were 27.5 ^{o}C and 6.93 respectively. The average of temperature, moisture, light intensity in the location of Cx. pipiens pipiens found were 30,75^{o}C, 75,50% and 1734,5 lux respectively. While the average of temperature and pH within the positive containers Cx. pipiens pipiens were 28 ^{o}C and 7,5 respectively. The types of mosquitoes was found at the Dusun IV Gosoma Village were Ae. aegypti and Cx.pipiens pipiens but Ae.aegypti mosquito was predominantly with a high density that make it potentially affect the spread of DHF.

Keywords: *Aedes aegypti, Culex pipiens pipiens, DHF, larvae index, north halmahera*

LARVA NYAMUK (DIPTERA: CULICIDAE) DI WILAYAH DUSUN IV DESA GOSOMA, HALMAHERA UTARA, INDONESIA: SUATU SURVEI LARVA NYAMUK BERDASARKAN TEMPAT PERINDUKAN

ABSTRAK

Kabupaten Halmahera Utara tidak termasuk dalam daftar 10 kabupaten yang tinggi penyakit demam berdarah di Indonesia. Namun dalam beberapa tahun belakangan, jumlah kasus demam berdarah di wilayah Halmahera Utara cenderung meningkat. Untuk mendapatkan informasi dasar yang bermanfaat dalam menyusun strategi pengendalian vektor, telah dilakukan survei larva nyamuk di wilayah Dusun IV Desa Gosoma, Halmahera Utara. Tujuan dari penelitian ini adalah untuk mengetahui kepadatan larva (indeks larva) dan jenis larva nyamuk pada beragam lingkungan berdasarkan tempat perkembangbiakan. Penelitian ini dirancang secara *cross sectional*, dilaksanakan pada bulan Juli dan Agustus 2018 di wilayah Dusun IV Desa Gosoma, Kecamatan Tobelo, Kabupaten Halmahera Utara. Larva nyamuk diperiksa pada setiap kontainer yang potensial sebagai tempat perindukan dengan cara *single larva methods*. Identifikasi larva

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dilakukan di laboratorium MIPA terpadu Universitas Halmahera. Hasil identifikasi digunakan untuk menghitung Container Index (CI), House Index (HI) dan Larvae Free Index (LFI). Pengukuran suhu, kelembaban udara, intensitas cahaya dan pH dilakukan pada saat pemeriksaan pada lingkungan ditemukan larva. Survei larva nyamuk pada 35 rumah terdapat 100 kontainer yang diperiksa teridentifikasi hanya *Aedes aegypti* 91, 67% dan *Culex pipiens pipiens* 8,33%. Hasil perhitungan indeks larva wilayah Dusun IV Desa Gosoma HI 37,14%, CI 22% dan LFI 62,86%. Ini menunjukkan tingginya risiko penularan penyakit demam berdarah. Purata suhu, kelembaban udara dan intensitas cahaya di lingkungan ditemukan larva *Ae. aegypti* berturut-turut adalah 29,75 ^{o}C, 77,50% dan 1890 lux. Purata suhu 27,5 ^{o}C dan pH 6,93 terdeteksi pada kontainer positif *Ae. aegypti*. Purata suhu, kelembaban udara dan intensitas cahaya di lingkungan ditemukan larva *Cx. pipiens pipiens* adalah 30,75 ^{o}C, 75,50% dan 1734,5 lux. Purata suhu dan pH air pada kontainer yang positif larva *Cx. pipiens pipiens* masing-masing adalah 28 ^{o}C dan 7,5. Jenis nyamuk yang ditemukan di wilayah Dusun IV Desa Gosoma adalah *Ae. aegypti* dan *Cx. pipiens pipiens*. Nyamuk *Ae. aegypti* lebih dominan dan memiliki densitas yang cukup tinggi sehingga dapat berpotensi terhadap penyebaran DBD.

Kata Kunci: *Aedes aegypti, Culex pipiens pipiens,* DBD, indeks larva, halmahera utara

INTRODUCTION

Mosquitoes (*Order Diptera: Family Culicidae*), classified into two subfamilies *Anophelinae* and *Culicinae*, are cosmopolitan insects.[1]This mosquito group is the centre of worldwide medical entomology research because of their essential as vectors of dangerous diseases such as malaria, dengue, yellow fever, encephalitis, lymphatic filariasis and chikungunya.[2]

Mosquitoes breeding sites are an essential part of the mosquito's life cycle. The larvae of *Aedes* generally found on puddle-pool of clean water and does not flow. Mosquitoes larvae are also found in the bathtub, barrels of water, cans and bottles, coconut shell, wreckage cars and in the holes of the tree that contains water.[3] The research reported by Greif *et al*[2] in Cebu Philippines explained that *Aedes aegypti* was spread throughout in the sampling areas, this species has a very wide range of habitat tolerance in both urban and rural areas but it was not found for *Culex pipiens quinquefasciatus* in the regions of Toledo and Naga. Good environments sanitation in the areas could be the reason of the absence of *Culex pipiens quinquefasciatus* because the ideal habitat for this type of mosquito breeding usually in a dirty and polluted water.[2]

Dengue Hemorrhagic Fever (DHF) is an infection caused by the dengue virus and transmitted through the mosquito *Aedes aegypti* and *Aedes albopictus* as the primary vectors. Recent information of DHF over the world have summarized by WHO, shown that the prevalence of DHF in Asia Pacific nearly 75% of the burden of dengue in the world, between 2004 and 2010. Also reported Indonesia as the second country with the largest case of DHF among 30 countries endemic regions.[4]

Indonesia has 34 provinces, among those provinces, West Java, East Java and Central Java are the provinces with the largest cases of DHF compared to other provinces. In 2017 the cases number recorded in West Java, East Java and Central Java provinces were 10,016 cases, 7,838 cases and 7,400 cases respectively.[4]

North Maluku province has the lowest cases number of DHF with 37 cases recorded. Also the number of Incidence Rate (IR) DHF in North Maluku is the lowest among other provinces with a value of 3.06 per 100,000 population in 2017.[4] North Halmahera Regency is not included in the list of 10 area which is high disease DHF in Indonesia. Statistical data shown that in 2017, DHF incidence in North Halmahera was not categorized in the 10 most types of diseases in North Halmahera.[5] However in last two years, the occurrence of fever dengue in this region tends to increase.

Although DHF is in the low category but based on entomology and distribution of DHF vectors in North Maluku especially in Ternate city, Tidore Kepulauan, and East

Halmahera Regency were in the category of risk areas refer to experiencing transmission and development of DHF in 2015.[6]

Surveillance of *Ae. aegypti* is essential in determining the distribution, population density, major larval habitats, and spatial and temporal risk factors related to dengue transmission, and levels of insecticide susceptibility or resistance.[7] Those all information (data) are important in order to prioritize areas and seasons for vector control. Also will enable the selection and use of the most appropriate vector control tools, and monitor their effectiveness. For practical reasons, the larval survey method is more commonly used rather than egg or adult collections. Indices used to assess the levels of *Ae. aegypti* infestations include House Index (HI): percentage of houses infested with larvae and/or pupae; Container Index (CI): percentage of water-holding containers infested with larvae or pupae; Breateau Index (BI): number of positive containers per 100 houses inspected. Andother surveillance on *Ae. aegypti* density such as adult surveys and Pupal Index (PI): number of pupae per house.[7]

Dusun Gosoma is the one of village in North Halmahera with high populated. Recent scientific information related the type, density of mosquitoesand transmitting dangerous diseases such as malaria, filariasis, DHF and other diseases in that area is still poor understood. It is important to provide basic data and information in order useful in vector control strategies in this area. The purpose of this research was to describe the density of mosquito's larvae (larval index) and the type of mosquito larvae in the different environment conditions based on mosquito breeding sites at Dusun IV Gosoma village, North Halmahera.

METHOD

This type of research was descriptive with a *cross-sectional* design carried out in July and August 2018 at Dusun IV Gosoma Village, Tobelo District, North Halmahera Regency (see Figure 1). Larval surveys were only carried out once in 35 houses, both indoor and outdoor the house. The Sampling of larvae using *single larvae methods*[3]at various mosquito breeding sites. Each container that can hold water is examined for the presence of larvae, and the type of container is recorded. Data of the larvae survey, various larval indices were obtained, for *House Index* (HI), *Container Index* (CI) and *Larvae Free Index* (LFI) were calculated using the bellow formula as described by WHO SEARO[7] and Suryanegara *et al* [8]:

$$\text{HI} = \frac{Number\ of\ house\ infested\ larvae}{Number\ of\ house\ inspected} \text{x } 100\%$$

$$\text{CI} = \frac{Number\ of\ positive\ containers}{Number\ of\ containers\ inspected} \text{x } 100\%$$

$$\text{LFI} = \frac{\text{Number of house uninfested larvae}}{\text{Number of house inspected}} \text{x } 100\%$$

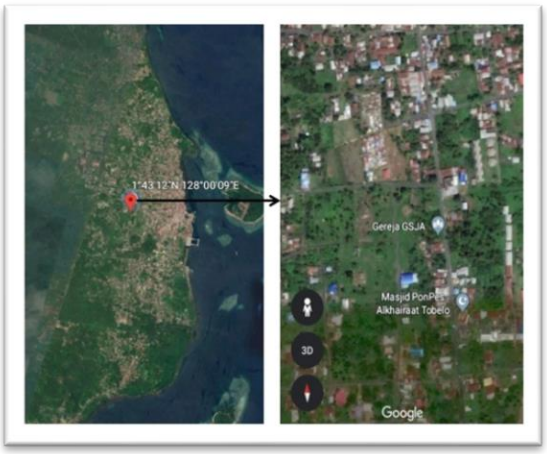
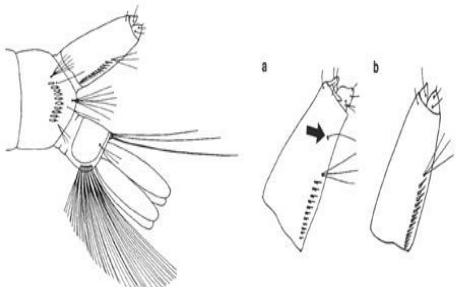


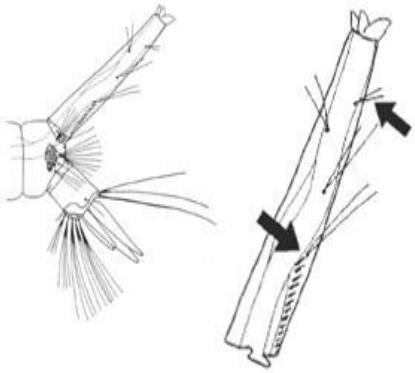
Figure 1.
Dusun IV Gosoma Village as the research area
(Source map: https://earth.google.com/web/)

Identification of mosquito larvae was made in the Integrated Science laboratory, University of Halmahera. Microscopic examination to identify the level of mosquito species based on morphological characteristics (head, thorax, and abdominal segments) in three and four instar larvae. The taxonomic key for genera *Aedes* and *Culex* according to Becker *et al*.[9]



• Additional *tuft* siphonal (1-S), single seta inserted laterally at 1/3 apical of siphon (*Ae. Cretinus*)
• There are no lateral additional (*Ae. Aegypti*) (see Figure 2)

Figure 2. Siphon: (a) *Ae. cretinus*; (b) *Ae. aegypti*



• *Tuft basalmost* is usually inserted close to the last *pecten tooth* (*Cx. p. Pipiens*) (see Figure 3)

Figure 3. Siphon: *Cx. p. pipiens*

The measurement of the light intensity, temperature, and humidity were carried out during the larvae examination. The containers infested with larvae were measured water temperature and pH. Air temperature and humidity were measured using Thermo-Hygrometer Alla France trademark. The light intensity was measured using the Light Meter (Lux Meter) Lutron LX-100 trademark. Digital pH meter Hanna trademark was used for measuring acidity or basicity of water.

RESULTS

Morphological microscope examined show that there were different in the siphon and antenna between *Ae. aegypti*and *Cx pipiens pipiens* (See Figure 4). Generally, size of siphon and the type of antenna for *Ae.aegypti* is large and short; and have a unbranched antenna, respectively while for *Cx. pipiens pipiens* is longer and have a lighter color; and have a branched antenna. Based on that it was found a total of 24 mosquito larvae were identified, consist of predominantly *Ae. aegypti* amounted to 22 larvae (91.67%) and another two larvae of *Cx. pipiens pipiens* (8.33%).

Examination of mosquito larvae was carried out in breeding sites at Dusun IV Gosoma Village at 35 houses with 100 containers. The results showed that from the 35 houses inspected, only 13 houses were found to be a positive for *Ae. aegypti* larvae with HI value of 37.14%. While, from 100 containers inspected, 22 containers were positive of *Ae. aegypti* larvae with CI value of

22%. Interestingly, mosquito larvae were found in outdoor containers nearly five timemore than indoor,with CI values of 32.73% and 8.89% respectively, as shown in Table 1.

Table 1.
Density of *Aedes aegypti* larvae at Dusun IV Gosoma Village

Object	Amount			Larvae Index (%)		
	Inspected	Positive Larvae	Negative Larvae	HI	CI	LFI
Houses	35	13	22	37,14	-	62,86
Containers	100	22	78	-	22	-
*Indoor	45	4	41	-	8,89	-
* Outdoor	55	18	37	-	32,73	-

Table 2.
Distribution of CI value for each water container infested of *Aedes aegypti* larvae at indoor and outdoor

Type of containers	Indoor			Outdoor		
	Inspected	Positive Larvae	CI (%)	Inspected	Positive Larvae	CI (%)
Bathtub	35	3	8,57	0	0	0
Refrigerator	3	0	0	0	0	0
Dispenser	2	0	0	0	0	0
Water bucket	3	1	33,33	0	0	0
Rain barrel	2	0	0	8	7	87,5
Water cup	0	0	0	15	3	20
Pandanus leaves	0	0	0	1	0	0
Former bucket	0	0	0	20	6	30
Cans	0	0	0	10	1	10
Plastic bottle	0	0	0	1	1	100
Amount	45	4	8,89	55	18	32,73

Table 2 shown, indoor positive containers of *Ae. aegypti* larvae were found at water bucket and bathtub with the CI values of 33.33% and 8.57% respectively, while for outdoor positive containers were found at plastic bottles, rain barrel, former bucket, water cup, and cans, with CI numbers of 100%, 87.5%, 30%, 20%, and 10% respectively.

Environment temperature, humidity and light intensity that *Ae. aegypti* larvae founded were 29.75^{o}C, 77.50%, and 1890 lux, respectively. The average temperature and pH of water in the *Ae.aegypti* larvae positive containers were 27.5^{o}C and 6.93 respectively. While the average temperature, humidity and light intensity in the environment that *Cx. pipienspipiens* larvae founded were 30.75^{o}C, 75.50%, and 1734.5 lux respectively. The average temperature and pH of the water in the container are favorable for *Cx. pipiens pipiens* larvae were 28^{o}C and 7.5, respectively.

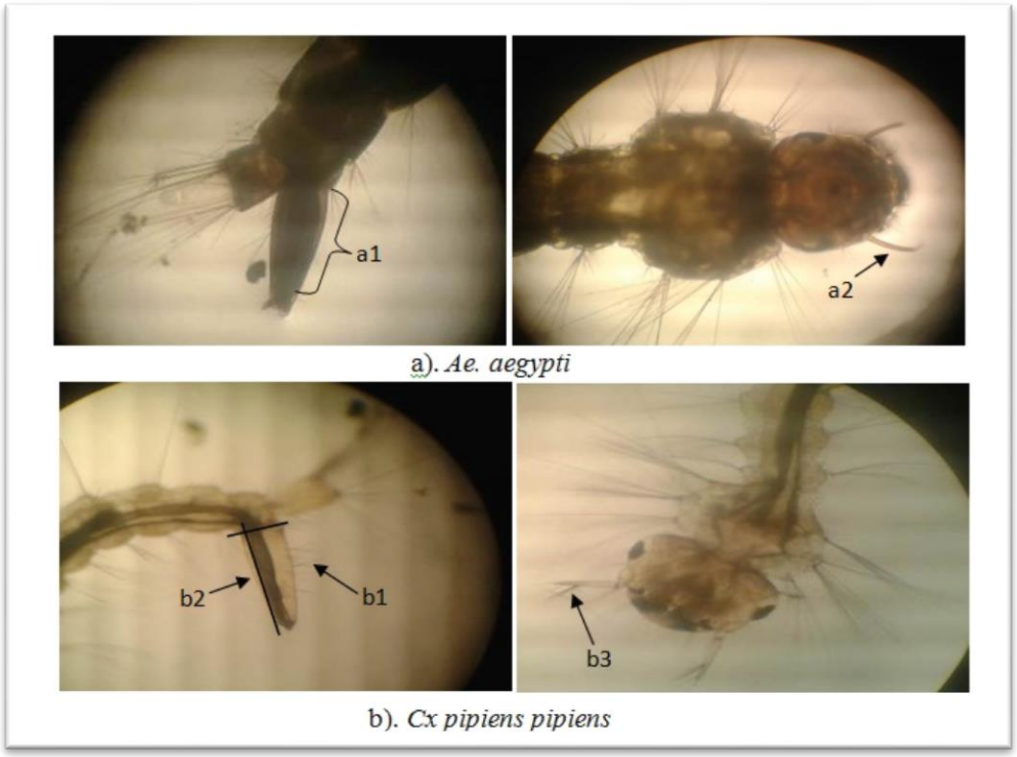


Figure 4.
Mosquito larvae identified at Dusun IV Gosoma Village. a) *Ae. aegypti*: (a1) short and large size of siphon; (a2) the antenna on the head is unbranched, b). *Cx pipiens pipiens*: (b1) have tuft on *siphon* more than 1 pair; (b2) the ratio width and length of siphon is approximately 1: 3; (b3) have a branched antenna.

DISCUSSION

The density of mosquito larvae at Dusun IV Gosoma Village was obtained respectively HI= 37.14%, CI = 22% (CI = *indoor* 8.89%; CI *outdoor* = 32.73%), and LFI = 62.86%. The criteria of the high-risk region for the transmission of DHF if the values of HI ≥ 10%, CI ≥ 5% and LFI≤ 95%.[3] The HI value obtained at 37.14% showed the high spread of *Ae aegypti* mosquitoes in people's houses. This indicating the potentially the occurrence transmission DFH.[10] The HI value in this study has similarities with the previous research conducted at several Puskesmas service areas in North Maluku. For instance, the HI value including Ternate City (Siko 34%; Kalumpang 37% and Kalumata 35%), Tidore Islands Regency (Soasio 40%; Tomalau 35% and Ome 38 %), and East Halmahera Regency (Getoli 35%; Buli 30% and Soasangaji 34%)[6] ranged between 34% – 40%. Research in other Eastern Indonesia regions such as Papua including Biak Numfor Regency was 83.5%, Merauke Regency 40%, and Sarmi Regency 64%.[11] The ideal HI value set by government programs was <5%.

The CI and LFI values at Dusun IV Gosoma Village were 22% and 62.86%, respectively. A similar study was reported by Kinansi *et al*[11] in endemic areas in Papua that has the CI values above 10% and LFI value under 95% including Biak Numfor Regency (47% and 17%), Merauke Regency (19% and 60%) and Sarmi Regency (60% and 36%). Perwitasari *et al*[6] reported that the North Maluku region had an average values of CI and LFI that were not in accordance with the ideal standards of government programs including Ternate City (29.76% and 64.66%), Tidore Islands Regency (29.3% and 62.33%), and East Halmahera Regency (29.53% and 67%).

According to the National Institute of Communicable Diseases cited by Ramadhani and Astuty,[12] Windyaraini *et al*[13] that the criteria of the high-risk region for the transmission of DHF if the values of HI ≥ 10%, CI ≥ 5% and also Nadifah *et al*[3] add 1 parameter as standard namely LFI ≤ 95 %. Thus, the Dusun IV Gosoma Village can be categorized as an area that potentially disease transmission of DHF.

The environmental sanitation became important aspects of this research. It was found that the CI value obtained was high at the outdoor. Observations showed that there was a lot of plastic wastes (inorganic) in surround people's houses such as water cups, buckets, cans and plastic bottles that are potential as breeding places for mosquitoes. Research on the relationship between environmental sanitation with vector density and the incidence of DHF has been widely reported.[14,15,16] For instance, a study reported by Purba in Sangatta Utara showed that there was a relationship between action, clean water supply, and waste management with a population density of *Ae. aegypti* mosquitoes (HI, CI, BI, and PI, at the ρ value 0.05).[14] According to Apriyani *et al*[15] that not proper management of solid waste treatment has a risk of 3.73 times greater exposure to DHF compared to good management. Therefore, public awareness of environmental sanitation are needed in order to minimize the dengue spread.[16] Mosquito Nest Eradication (MNE) may be a solution in preventing dengue mosquito breeding which can be done through 3M Plus hygiene movements.[17]

The mosquito larvae were found predominantly of *Ae. aegypti.* In General, the mosquito is well-known as the primary vector of dengue fever (yellow fever) and chikungunya. Additionally, it could be also a secondary vector of human filariasis. *Ae. aegypti* has an important role in animal health including as the primary vector of dirofilaria in dogs and has been implicated in the transmission of hemorrhagic septicemia in buffaloes, fowl-pox and *enzootic* hepatitis which cause abortion in sheep and cattle.[1]

The Group of *Culex pipiens* is well-known consist of several species, subspecies, forms, races, physiological variants or biotypes including *Cx. pipiens pipiens* Linnaeus, *Cx. p. pipiens* biotype *molestus* Forskal, *Cx. p. quinquefasciatus* Say, *Cx. p. pallens* Coquillett, *Cx. restuans* Theobald and *Cx. torrentium* Martini in the Holarctic as well as two Australian members, *Cx.australicus* Dobrotworsky and Drummond, *Cx. globocoxitus* Dobrotworsky.[9] There was genus *Culex* mosquitoes found in Indonesia including West Timor region: *Cx. quinquefasciatus, Cx. bitaeniorrhynchus, Cx. gelidus, Cx. pseudovishnui, Cx, Cx. Sitens, Cx. vishui*. In the area of Bengkalis Riau was found *Cx. quinquefasciatus, Cx. Fuscocephalus,Cx. Hutchinson* and *Cx. gelidus*.[18] Soekirno[19] have found 7 species of Culexmosquito including *Cx. fuscanus, Cx. gelidus, Cx. Balifaxi, Cx. pallidothorax, Cx. quinquefasciatus, Cx. tritaeniorhynchus and Cx. Vishnui* in Sidangoli. In this study was indentified using characteristic of siphon larvae and head as described by Becker *et al*[9] and it was correlated as *Cx p.pipens*.

This study according to Sholichah[18] some diseases that can be transmitted through mosquito bites of *Culex* sp including lymphatic filariasis (elephantiasis), Japanese Encephalitis (JE), *St. Louis Encephalitis* and *West Nile Virus* (WNS). Related to this research, *Cx. pipiens pipiens* is known as major vector in the transmission of a number of disease-causing pathogens in humans, livestock and wildlife including viruses (e.g. West Nile Virus), protozoan (e.g. avian malaria) and metazoan parasites (e.g. filarial worms).[20]

Environmental indicators such as temperature, humidity, pH and light intensity describes the ideal conditions for mosquito breeding. According to Ariati and Musadad,[21] the optimum temperature and humidity for breeding mosquitoes ranged between 24°C to

28°C and 70% to 90% respectively. Hodijah and colleagues research reported that *Aedes* spp. were found at temperatures ranging between 28-31°C, humidity 60%-75% and light intensity 85Lux-260 lux.[22] Research on the characteristics of the breeding habitat of *Anopheles* sp in Bula District, Eastern Seram Regency acquired the pH ranges from 7 to 7,5.[23] Neutral pH range obtained on measurements of water quality of the river San Isidro Philippines, habitatof *Culex annulirostris*.[24] This results showed an average temperature and pH on positive containersof *Ae. aegypti* (27.5°C and 6.93) and *Cx. pipiens pipiens* (28^{o}C and 7.5) larvae were fits with the required of the mosquito to the cycle of life. Similarly in humidity and water pH were found in both types of mosquitoes were in the optimal level to support for breeding. Light intensity was obtained in this research more than 1000 luxthis could be affected by getting direct sunlight exposure during the day.

Climate change factors contributing to the increase in temperature, precipitation and humidity. It may affect on the pattern of infectious diseases such as viruses, bacteria or parasites and whose vectors.[25] Temperature effects on maturity and replication of organisms, including vector. Rising temperatures can accelerate the incubation period of the disease and it extends disease transmission. Rising precipitation will be increased mosquito breeding places of *Ae. aegypti*. High-intensity rainfall in a long time will reduce the breeding places of *Ae. aegypti* while low-intensity rainfall in a long time will increase the number of places to breed.[25] In high humidity condition will make the mosquitoes become active and more often aggressive (bite). Overall, there was a significant relationship between environmental factor such air humidity and the density of *Anopheles*, *Ae.aegypti* and *Culex pipiens* mosquitoes.[25,26,27]

CONCLUSION AND RECOMMENDATION

The mosquitoes larvae founded in containers at the Dusun IV Gosoma Village were dominated by *Ae. aegypti* (91.67%) and followed *Cx. pipiens pipiens* (8.33%). The values of HI (37.14%), CI (22%), and LFI (62.86%) were exceeded the ideal standard issued by National Institute of Communicable Diseases. This indicating the high risk potential for dengue transmission at dusun IV Gosoma Village. In general, environmental factors such as temperature, moisture and pH at the region were favorable for development of vectors (*Ae.aegypti* and *Cx. pipiens pipiens*).

The advice of this research as follows:

1. The community should more care about environmental sanitation and carrying out intensive Mosquito Nest Eradication (MNE) through 3M Plus hygiene movements to circular letter from the Ministry of Health No. PM.01.11/Menkes/591/2016 dated 8 November 2016.[28] The steps are undertaken monitoring mosquito larvae and PSN (Pemberantasan Sarang Nyamuk) 3M Plus in every home regularly to eradicate mosquito breeding sites will includes:
    a) Drain the areas where are often used as water reservoirs such as bathtubs, buckets of water, the shelter of a drinking water tank, water storage in the refrigerator, and dispensers;
    b) Sealed water reservoir such as a drum/barrel of water, a jug of water and others; and
    c) Recover or recycle used items that can hold water such as plastic bottles, cans, old tires because become the potential breeding for Aedes mosquito.

The meaning of "Plus" at 3M Plus is everything prevention of mosquito bites, such as:

a) Sow or drip of larvicides in shelters that are difficult to clean,
b) Use insect repellent or mosquito,
c) Use mosquito nets while sleeping,
d) Maintain wiggler predator fish,
e) Plant mosquito repellent plants,
f) Adjust the lighting and ventilation in the house,
g) Avoid the habit of hanging clothes in the home can be a place of rest mosquitoes, and
h) Start to use shower water for a bath, to reduce bath.

2. The impact of climate change and global warming on vector breeding disease can be a concern in advanced research.

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