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PRAIMAGO DENSITY OF AEDES SP AND ITS RELATIONSHIP WITH THE INCIDENCE OF DHF IN KUPANG CITY

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ABSTRACT
Mosquito density may represent the bionomic of Aedes sp and the risk of spreading the disease. The Indonesian Ministry of Health has been using ABJ, HI, CI, BI and Pupa Index (PI) to calculate the density of Aedes sp praimago and assess the risk of dengue transmission in an area, so it is necessary to look for an index that describes the individual risk of dengue transmission. The research objective was to analyze the relationship between praimago density of Aedes sp on the incidence of dengue fever in Kupang City, where in Kupang City. The research was conducted in 25 urban villages. Samples were taken from 20 people per sub-district obtained by cluster sampling, namely the center point is the DHF sufferer, then the nearest house was searched within a radius of 100 meters until a total of 20 houses were reached or 500 houses for Kupang City. The independent variables were larvae / pupa density (HP, PC, CPP, LH, PH, LCIH, PCIH, LPCIH, PPCIH, LPH, PPH) and egg density (TE, EDH, OIH), while the dependent variable was the incidence of DHF. Praimago density was determined by conducting an entomological survey in the form of a survey of eggs and larvae of Aedes sp. The data were processed to see the density of larvae and eggs per house, then statistically analyzed using independent t test, Chi Square, and Pearson Correlation. The study found that there was a relationship between larvae / pupa density (HP, PC, CPP, LH, LCIH, LPCIH, LPH) and egg density (TE, EDH, OIH) with the incidence of DHF. It concluded that the density of egg density and larvae / pupa density in a house can be used to estimate the risk of dengue fever from family members in the house.

Keywords: aedes sp; dengue hemorrhagic fever; praimago density

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INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a vector-borne disease caused by the Dengue virus. DHF is still a major problem in tropical and subtropical areas, including Indonesia. The burden of disease, high mortality, poverty and social burden are the impacts that can be caused by DHF (Ali, Asha, & Aneesh, 2014). East Nusa Tenggara Province (NTT) is an endemic area for dengue fever, and Kupang City as the capital of NTT Province has had the highest DHF Incidence Rate (IR) in NTT from year to year and is even higher than the national figure. Prevention and control of DHF are always carried out in Kupang City (Dinkes Kota Kupang, 2016), but DBD is always present with a high incidence rate too. The high number of DHF cases in Kupang City and the discovery of DHF cases in both the dry and rainy seasons indicate that the risk of DHF transmission in Kupang City is high. Mosquito density can describe the bionomics of Aedes sp and the risk of disease spread. It has been proven that the presence of larvae is related to the incidence of DHF with an Odds Ratio (OR) 5.8 (Sari & Darnoto, 2012).

The Indonesian Ministry of Health has so far only used ABJ, HI, CI, BI and Pupa Index (PI) to calculate the density of Aedes sp preimago and the risk of a region to dengue fever transmission. It is necessary to develop other larval and pupal indices such as Pupal per Container Index (PCI), Pupal Per Person (PPP), Egg Density Index (EDI), larval per container index (LCI), and Larval Density Index (LDI) (Bhat & Krishnamoorthy, 2014; Focks, Brenner, Hayes, & Daniels, 2000; Weeraratne, Perera, Mansoor, & Karunaratne, 2013; Yotopranoto, 2013; Zeidler, Acosta, Barreto, & Cordeiro, 2008) which index has been developed in several countries to see the risk of dengue fever transmission. It is also necessary to look for an index that describes the individual risk of dengue fever transmission. Another reason for searching for larval/pupal index based on individual risk is because mosquitoes will not fly and bite people who are outside their flight range, so people who live near mosquito breeding places will be at greater risk of being bitten and infected with the Dengue virus. It is hoped that later a larval index will be found that influences the incidence of DHF in individuals.

Several indicators of pre-imago density based on individual risk that will be studied are House Positivity (HP), Positive Container (PC), Container Positive Pupa (CPP), Larva in House (LH), Pupa in House (PH), Larva per Container Index in House (LCIH), Pupa per Container Index in House (PCIH), Larva per Positive Container Index in House (LPCIH), Pupa per Positive Container Index in House (PPCIH), Larva per Person in House (LPH), Pupa per Person in House (PPH), Total Eggs (TE), Eggs Density in House (EDH), Ovitrap Index in House (OIH). The purpose of the study was to analyze the relationship between larval density (HP, PC, CPP, LH, PH, LCIH, PCIH, LPCIH, PPCIH, LPH, and PPH) and egg density (TE, EDH, and OIH) to the incidence of DHF in Kupang City.

METHOD

This observational analytical study was conducted with a case control design. The study was conducted in 25 sub-districts in Kupang City with case samples of DHF patients and control samples of non-DHF patients who lived around DHF patients within a radius of 100 meters in the same sub-district. The sample size was 20 houses per sub-district or 500 houses from 12 sub-districts in the dry season and 13 sub-districts in the dry season. The locations and samples used in the dry and rainy seasons were different because the locations of the cases at the time of the study were also different. Samples were taken using cluster sampling because DHF cases were few and spread across several sub-districts. This study used an individual unit of analysis because individuals are able to represent conditions in society. The independent variables are larval density (HP, PC, CPP, LH, PH, LCIH, PCIH, LPCIH, PPCIH, LPH, and PPH) and egg density (TE, EDH, and OIH), while the dependent variable is the incidence of DHF. The density of preimago can be determined by conducting an entomological survey in the form of an Aedes sp. egg and larval survey. The tools needed are ovitrap, ovistrip, scoop, pipette, gamadotic (a tool for taking larvae/pupae in large containers), flashlight, small bottle/plastic, label paper, stationery and examination form.

The larvae/pupa survey was conducted in the same house as the egg collection site. All larvae in each surveyed house were taken using a pipette, scoop or gamadotic and then placed in a small bottle or plastic and labeled, then taken to the laboratory to be identified using an identification key. The data was processed to see the density of larvae and eggs per house. The processed data was then analyzed statistically, namely bivariate analysis using frequency distribution, independent t-test, Chi Square, and Pearson Correlation. Research ethics were obtained from the FKM UNAIR ethics commission No: 521-KEPK in 2016.

RESULT

This study found that House Positivity (HP) in the dry season was significantly related to the incidence of DHF (OR 9.059) or people with houses that were positive for larvae/pupae were at risk of contracting DHF 9.059 times compared to those whose houses were not positive for larvae/pupae, as shown in Table 1.

Table 1.
Relationship between House Positivity (HP) with Dengue Fever Incidence in Kupang City

HP	DHF	Non DHF	Total	P Value	OR (95% C.I.)
Positive	27 (73%)	201 (43,4%)	228 (45,6%)	0,001	3,519 (1,665 – 7,439)
Negative	10 (27%)	262 (56,6%)	272 (54,4%)		

Table 2.
Relationship between Positive Container with Dengue Fever Incident in Kupang City

Variable	Status	Sample (n)	Mean PC	S.D.	Min - Max	P Value
PC	DHF	37 (7,4%)	0,84	0.727	0 - 4	0,012
	Non DHF	463 (92,6%)	0,52	0.726	0 – 5	
CPP	DHF	37 (7,4%)	0.68	0.669	0 – 3	0,001
	Non DHF	463 (92,6%)	0.34	0.588	0 – 4	
LH	DHF	37 (7,4%)	204,11	328,574	0 – 1221	0,009
	Non DHF	463 (92,6%)	54,83	114,390	0 – 1305	
PH	DHF	37 (7,4%)	10,24	20,597	0 - 102	0,195
	Non DHF	463 (92,6%)	6,06	18,717	0 - 203	
LCIH	DHF	37 (7,4%)	113,949	205,083	0,0 - 1042	0,027
	Non DHF	463 (92,6%)	35,720	67,713	0,0 - 595	
PCIH	DHF	37 (7,4%)	5,886	12,255	0,0 - 51	0,533
	Non DHF	463 (92,6%)	4,290	15,153	0,0 - 203	
LPCIH	DHF	37 (7,4%)	169,022	265,332	0,0 - 1042	0,007
	Non DHF	463 (92,6%)	45,044	77,470	0,0 – 595	
PPCIH	DHF	37 (7,4%)	9,468	19,816	0,0 – 102	0,145
	Non DHF	463 (92,6%)	5,221	16,792	0,0 – 203	
LPH	DHF	37 (7,4%)	41,635	79,946	0,0 – 407,0	0,036
	Non DHF	463 (92,6%)	12,844	26,279	0,0 – 326,3	
PPH	DHF	37 (7,4%)	1,989	4,560	0,0 – 24,5	0,485
	Non DHF	463 (92,6%)	1,444	4,570	0,0 – 40,6	
TE	DHF	37 (7,4%)	31.30	30.054	0 – 108	0,010
	Non DHF	463 (92,6%)	17.60	23.849	0 – 160	
EDH	DHF	37 (7,4%)	7,846	7,5174	0,0 - 27	0,010
	Non DHF	463 (92,6%)	4,419	5,970	0,0 - 40	
OIH	DHF	37 (7,4%)	45.946	28.573	0,0 - 100	0,000
	Non DHF	463 (92,6%)	25.918	23.331	0,0 – 100	

The number of TPAs that were positive for larvae (Positive Container/PC) in this study ranged from 0-5 in houses without DHF sufferers and 0-4 in houses with DHF sufferers, as shown in Table 2. This study also found many TPAs that were positive for pupae (Container Positive Pupa/CPP), namely a maximum of 3 TPAs in houses with DHF sufferers and a maximum of 4 TPAs in houses without DHF sufferers. The number of larvae and pupae in the observed houses or Larva in House (LH) was 32,937 larvae, and statistically there was a difference in the number of larvae in houses of DHF sufferers and non-sufferers. Pupa in

House (PH) or the number of pupae per house ranged from 0 - 203 pupae in non-DHF sufferers and only 0 - 102 in DHF sufferers, and it turned out that PH was not related to the incidence of DHF.

The number of larvae per TPA in each house or Larva Container Index in House (LCIH) ranges from 0.0 – 1042, and there is a relationship between LCIH and the incidence of DHF. The highest number of pupae per TPA (PCIH) in the surveyed houses was 203 pupae and this was found in houses without DHF sufferers. Based on the t-test, it turns out that it is not related to the incidence of DHF. The highest number of larvae per positive TPA (LPCIH) was 1,042 and based on the t-test, a significant relationship was found between LPCIH and the incidence of DHF. This study also found that overall the average number of pupae per positive TPA (PPCIH) in DHF patients was 9,468, while in non-DHF patients it was 5,221. Statistically, there was no relationship between PPCIH and the incidence of DHF ($p>0.05$). This study found that the number of larvae per household (LPH) in the rainy season was higher than in the dry season. The relationship between LPH and the incidence of DHF was found to be significant in this study. The number of pupae per family member or Pupa per Person in House (PPH) was found to be not significantly related to the incidence of DHF ($p>0.05$). The number of eggs or Total Eggs (TE) in DHF patients was found to be more than non-DHF patients, and statistically there was a significant relationship between TE and the incidence of DHF. While the highest egg density (Eggs Density in House/EDH) was 40 and EDH was found to be related to the incidence of DHF. The percentage of ovitraps that were positive for eggs (Ovitrap Index in House/OIH) varied between 0.0-100 and there was a significant relationship between OIH and the incidence of DHF.

DISCUSSION

Determinan kejadian vector borne The determinants of vector borne diseases are humans, the environment, agents and vectors, so if the vector in this case Aedes sp larvae is in the house, the possibility of dengue fever will be greater than in a house where Aedes sp larvae are not found. The presence of Aedes sp larvae which will later become adult Aedes sp mosquitoes plays a role in the transmission of dengue fever, because dengue fever can only be transmitted through Aedes sp mosquitoes, of which there are 3 known species in Indonesia, namely Ae. aegypti, Ae. albopictus and Ae. scutellaris (Ditjen PP & PL, 2013).

The presence of larvae in the house (HP) is associated with the incidence of DHF with $p \leq 0.05$ and OR 3.519, which means that houses where larvae are found positive have family members at risk of contracting DHF 3.519 times higher than houses where larvae are negative. This is in accordance with previous studies in Bandarlampung and Kolaka which found a relationship between the presence of larvae in landfills and the incidence of DHF ($p \leq 0.05$),10 and research in Medan found a relationship between the presence of Ae. aegypti larvae and the incidence of DHF ($p \leq 0.05$) (Parida, Dharma, & Hasan, 2013). The number of TPAs that are positive for larvae (PC) and the number of TPAs that are positive for pupae are also related to the incidence of DHF ($p \leq 0.05$). The average PC and CPP are higher in DHF patients than in non-DHF patients, this shows that the more TPAs that are positive for larvae and positive for pupae, the greater the possibility of finding adult mosquitoes, so the greater the risk of contracting DHF.

Previous studies have rarely looked for a relationship between PC and CPP with DHF incidence, but it has been reported that there is a relationship between the number of TPA and the presence of Ae. aegypti larvae ($p \leq 0.05$).(Parida et al., 2013). Research in Thailand reported that the average number of containers positive for Aedes larvae per house ranged from 0.01 to 0.17 (Wongkoon, Jaroensutasinee, Jaroensutasinee, Preechaporn, & Chumkiew, 2007), while in India it ranges from 0.00 - 6.69 (Bhat & Krishnamoorthy, 2014). Container positivity in India is lower than the Positive Container in this study which ranges from 0-5 TPA because container positivity in the previous study was calculated by dividing the number of positive TPA divided by the number of TPA observed multiplied by 100 and this was calculated based on the type of TPA, while the positive container in the study in Kupang City was the total number of TPAs that were positive for larvae, pupae or both and this was calculated per house.

The number of larvae in each house (LH) in this study in Kupang City was found to be related to the incidence of DHF ($p \leq 0.05$), while the number of pupae in each house (PH) was not related to the incidence of DHF ($p > 0.05$). The number of mosquitoes is an important factor that influences the intensity of transmission of vector borne diseases including DHF. The number of larvae can describe the density of adult mosquitoes so that it can also describe the risk of DHF. The more larvae there are, the more adult mosquitoes there are and the greater the risk of DHF transmission. On average, larvae in this study in Kupang City were more in DHF patients, this shows that the risk of DHF in Kupang City is greater in people whose homes are found to have more larvae.

The number of larvae per number of landfills in each house (LCIH) in this study in Kupang City showed a relationship with the incidence of DHF ($p \leq 0.05$), in contrast to the number of pupae per number of landfills (PCIH) which did not show any relationship with the incidence of DHF ($p > 0.05$). This shows that the density of larvae can better describe the density of adult mosquitoes as a whole and also the risk of DHF transmission compared to the density of pupae, which in this study found a very small number of pupae compared to the number of larvae, namely 3,185 pupae (9.7%) of a total of 32,937 larvae and pupae. Research in India has reported that the PCI value is 0.10 and this was carried out in an area (Bhat & Krishnamoorthy, 2014), whereas in this study PCI for individuals or homes in patients was 5,886 and in non-DHF patients was 4,290.

The LCIH figure is almost the same as CI, the difference is that CI only compares the number of positive TPA larvae per number of TPA observed, while LCIH not only compares how many TPA are positive but also calculates the number of larvae from all positive TPA per all TPA observed. The benefit of knowing LCIH is to estimate the density of larvae based on the number of TPA, LCIH 113.95 in DHF patients means the density of larvae for each TPA in DHF patients is 113.95, while LCIH 35.72 in non-DHF patients means the density of larvae for each TPA in non-DHF patients is 35.72. The higher the density of larvae in the TPA, the higher the risk of DHF.

The number of larvae per number of positive larval landfills (LPCIH) is associated with the incidence of dengue fever ($p \leq 0.05$), while the number of pupae per number of positive larval landfills (PPCIH) is not associated with the incidence of dengue fever ($p > 0.05$). There are still few studies on the number of larvae or pupae per number of positive landfills, especially those related to the incidence of dengue fever. Research in India found that the number of pupae per positive landfill for a region was 2.43,(Bhat & Krishnamoorthy, 2014) and that is lower compared to this study which found the number of pupae per positive TPA was 9.47 in DHF sufferers and 5.22 in non-DHF sufferers.

The LPCIH figure in the study in Kupang City shows that larval density is related to the incidence of DHF, namely the greater the number of larvae per TPA that are positive for larvae and pupae, the higher the risk of DHF. The PPCIH figure is not related to the incidence of DHF in the study in Kupang City, however, the PPCIH in DHF sufferers is higher than in non-DHF sufferers, which means that the greater the number of pupae in the TPA, the greater the risk of DHF. The LPCIH index is more important than LCIH because LCIH only calculates larval density based on the number of TPA, while LPCIH calculates larval density based on positive TPA. In addition, not all TPA will be positive for larvae, so even though there are many TPA but if they are always cleaned then there will be no larvae in the TPA, whereas if there are many TPA that are positive for larvae then the risk of transmission will be greater. Therefore, DHF control is not carried out to reduce the number of TPA but to eradicate mosquito nests, namely by always maintaining the cleanliness of the TPA so that it does not become a breeding ground for the pre-imago stage of mosquitoes.

The number of larvae per family member (LPH) in this study in Kupang City was related to the incidence of DHF ($p \leq 0.05$). This shows that LPH can describe the risk of family members to contact with mosquitoes and contract the Dengue virus through mosquito bites. The average LPH in DHF patients was 41.64, while in non-sufferers it was only 12.84, which means that the greater the LPH, the greater the risk of contact with mosquitoes and contracting the Dengue virus through mosquito bites. The number of pupae per family member (PPH) in DHF patients (1,989) was greater than in non-DHF patients (1,444), but it was not related to the incidence of DHF ($p > 0.05$). This shows that the comparison of the number of pupae with the number of family members cannot describe the risk of family members to contact mosquitoes and contract the Dengue virus through mosquito bites. Pupae per person have also been reported in Puerto Rico 4.48, and in Mexico 2.84 (Focks, 2003) and this figure is greater than the figure in Kupang City.

The pupal index is considered better than the larval or egg index for several reasons, namely: its development stage is closer to that of adult mosquitoes so that counting the number of pupae can more accurately describe the density of adult mosquitoes than counting larvae or eggs; it is cheaper and quicker to do if the aim is to see the species of adult mosquitoes; and pupal mortality is low and it has been proven that the number of pupae is related to the number of adult mosquitoes (Focks, 2003). In contrast to the explanation, it turns out that in this study the number of pupae and eggs is not related to the incidence of DHF because the larvae that have become pupae are very few, besides that it is possible that the existing pupae have become adult mosquitoes so that they cannot describe the density of adult mosquitoes, and also cannot describe the risk of DHF based on pupa density.

With the incidence of DHF with ($p \leq 0.05$), as well as egg density (EDH) and ovitrap index (OI) are associated with the incidence of DHF ($p \leq 0.05$). This is different from previous research in Brazil which found that egg positivity or the presence of eggs was not associated with the risk of DHF incidence (Dibo, Chierotti, Ferrari, Mendonca, & Neto, 2008). Research on EDH has been rarely conducted previously, but egg density has been reported in Brazil ranging from 0 – 59.9 and ovitrap index ranging from 0 – 28.1% (Cecílio et al., 2015). The egg density in Brazil is almost the same as the research in Kupang City which ranges from 0 - 40, while the ovitrap index is higher in Kupang City, namely 0 - 100% or there are houses where all ovitraps do not have mosquito eggs but there are also houses where all ovitraps are positive for mosquito eggs.

Both ED, EDH and OIH are greater in DHF patients compared to non-DHF patients. This shows that the greater the number of eggs and the higher the density of eggs per ovitrap, the higher the risk of DHF, and the lower the number of eggs and the density of eggs per ovitrap, the lower the risk of DHF. In addition, statistically related to DHF, so here it is necessary to eliminate mosquito breeding places, namely cleaning or eliminating water reservoirs that have the potential to be mosquito egg-laying places. Seeing the importance of the role of larval density in the incidence of DHF, the community is expected to actively participate in eradicating DHF mosquitoes in their homes and in their areas with 3M plus. 3M plus activities that can be carried out include always cleaning the TPA at least once a week by scrubbing the inner walls of small TPAs, or controlling the presence of larvae by collecting larvae at least once a week. Providing abate, providing fish that eat larvae for large TPAs or for areas with insufficient water availability, including closing all TPAs tightly so that there are no gaps for mosquitoes to enter the TPA. Reusing large unused TPAs for trash bins or flower planting places so that they do not become breeding grounds for mosquitoes.

In addition, the government needs to conduct socialization to the community about the importance of DHF disease surveillance carried out starting from the individual and household scope. The community needs to conduct a home survey and at the same time carry out independent interventions in each house so that the existing TPA does not become positive for larvae or pupae, and the larvae or pupae do not become adult mosquitoes. It is also necessary to distribute survey tools in each Neighborhood Association (RT) to facilitate the implementation of independent surveys by the local community. The HI, CI and BI larval indices that are commonly used so far still have many shortcomings, namely these indices fail to provide adequate data per region or per person and factors known to be related to the level of dengue transmission. The CI index is considered the weakest larval index because this index only describes the proportion of positive TPA and does not consider the number of TPA in an area, per house. The HI index may be better but HI does not describe the number of positive TPA per house. The BI index is better than CI and HI because it combines information on TPA and houses (Dibo et al., 2008).

Most of the indices and also DHF control measures are based on mosquito population suppression and not on mosquito population eradication, for example HI and CI are obtained only by surveying houses and TPA that are positive for larvae and appealing to the community to routinely clean the TPA and not taking concrete action to eliminate the larvae and pupae. Officers sometimes distribute temephos (abate) directly to houses that are positive for larvae and pupae but do not directly control the use of abate in the field, in addition, long-term use of temephos can also cause resistance in larvae if not given in the right dose. Based on these reasons, this study created a new praimago index that aims to obtain a better index to describe the risk of DHF incidents. In addition, the new praimago index in this study also aims to eradicate the larval population because to obtain this index, all existing larvae and pupae must be taken so that this includes concrete steps to eradicate DHF vectors. The action of cleaning larvae and TPA is certainly not the task and responsibility of health workers, but this can be done together with the community when officers conduct household surveys. There are two advantages to finding indicators in this study, namely officers can carry out promotive and preventive activities, officers obtain an index of larvae and pupae that can be used to predict the risk of dengue fever, and at the same time provide counseling to the community to always clean the TPA routinely, while the community also has a direct benefit, namely that all larvae and TPA are cleaned without having to dispose of the existing water.

CONCLUSION

This study concluded that vector density, namely the density of pre-imago in a house, can be used to estimate the risk of dengue fever from family members living in the house. The density of pre-imago that is related to the incidence of dengue fever is the density of larvae including HP, PC, CPP, LH, LCIH, LPCIH, and LPH, while the density of eggs includes: TE, EDH and OIH. The community is expected to actively participate in eradicating dengue mosquitoes in their homes and in their areas with 3M plus, including by always cleaning the TPA at least once a week for small TPA or providing abate, providing fish that eat larvae, and closing all TPA tightly for houses with large TPA and with limited water availability so that it is not possible to drain once a week. In addition, it can also be done by reusing large TPA that are no longer used to. In addition, the government needs to conduct socialization to the community about the importance of dengue fever surveillance which is carried out starting from the individual and household scope. The community needs to conduct a home survey and at the same time carry out independent interventions in each house so that the existing TPA does not become positive for larvae or pupae, and the larvae or pupae do not become adult mosquitoes. It is also necessary to distribute survey tools in each Neighborhood Association (RT) to facilitate the implementation of independent surveys by the local community.

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