Prevalence of Dengue Virus Transovarial Transmission and DHF Incidence Rate in Grogol Sub-district of Sukoharjo District

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** ABSTRACT **

Environmental changes in physical, biological, and social aspects have enabled the occurrence of DHF cases in Sukoharjo. Such changes may affect virulence of dengue virus, shorten extrinsic periods and increase vector dengue capacity. This study aimed to determine the spread of dengue virus transovarial transmission and the incidence rate of DHF in Grogol Sub-district of Sukoharjo District. This observational analytic study used a cross-sectional approach conducted in 2016. The population was Ae. aegypti mosquitoes and DHF patients (based on laboratory diagnosis with serologic examination, i.e., IgG, IgM and/or NS1 indicating positive DHF). Data analysis used Chi-square, Multiple Linear Regression, and spatially weighted regression with GeoDa and SatScan. The study found virDEN-3 transovarial transmission in Grogol Sub-district of Sukoharjo District and the spread of virDEN-3 transovarial transmission following the high spread of Ovitrap Index. The DHF incidences (cases) tended to cluster at a radius of 100-200 m from a region found the positive virDEN-3 transovarial transmission. The statistical tests showed a relationship between the virDEN-3 transovarial transmission and the DHF incidence with a p-value of <0.05. Spatially, the incidence of DHF in this area followed a certain spatial distribution pattern, with a clustering of dengue cases centered on the coordinates of -7.588240 S, 110.809450 E with a radius of 2.00 km.

** INTRODUCTION **

Dengue hemorrhagic fever (DHF), an infectious disease transmitted from one person to another, is caused by dengue virus carried by the Aedes (Ae.) mosquito of the subgenus Stegomyia. From the subgenus, Ae. aegypti is the most important epidemic vector, while Ae. albopictus is the secondary one. The dengue virus belongs to the genus...
Flavivirus of the family Flaviviridae, and is a single stranded RNA nucleotide virus. Based on its serotype, the dengue virus is divided into DEN-1, DEN-2, DEN-3 and DEN-4. The dengue virus results in varying clinical manifestations from flu or Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), to Dengue Shock Syndrome (DSS), which is sometimes accompanied by convulsions due to encephalopathy.

The increasing number of dengue fever cases and the widespread of areas reporting the incidence of the cases in Indonesia are due to very complex and varied factors. DHF is a disease closely related to the environment; thus, changes in one or several environments can lead to changes in the life cycle of mosquitoes, which will ultimately affect the incidence of DHF in the region. In this last period, the growing phenomenon due to the evidence of transovarial transmission can put more health concerns in Indonesia. Halstead in 1990 did actually review the vertical transmission mechanism but the proof of transovarial transmission in Indonesia was conducted by Mardihusodo (2007) and Tribuwono et al (2006), with the later showing transovarial transmission proven in Semarang of Central Java.

Initial surveys conducted on 4-14 April 2015 in Langenharjo and Telukan villages, Grogol sub-district, Sukoharjo District, done with epidemiological inquiry, show some important preliminary data, i.e., slum settlements, various factory buildings, numerous counters supporting mosquito larvae, dense population, and lack of awareness of the importance of environmental hygiene. Data on the number of DHF cases per sub-district at Grogol Public Health Center in 2012 showed 36 cases and no casualties. In 2013, the number of cases increased to 233 cases with IR by 0.20% and CFR mortality by 0.002%. For 2014, the number of cases decreased to 152 cases but the number of people who died became four, with IR by 0.13% and CFR by 2.6%. To date, efforts have been made to prevent the increase and spread of the disease through prevention programs.

Based on the aforementioned data, environmental changes in terms of physical, biological, and social had possibly occurred. Environmental changes that occur in Sukoharjo District are, among others, the increase in air temperature, humidity, rainfall, reduced vegetation due to the use of agricultural land/plantations for settlements, and increasing population density and population mobility. Such changes may affect virulence of dengue virus, shorten extrinsic periods, and increase the capacity of dengue vectors.

**RESEARCH METHOD**

This analytic observational study used a cross-sectional study design conducted in Grogol Sub-district of Sukoharjo District in 2016 and at the Parasitology Laboratory of the Faculty of Medicine of Universitas Gadjah Mada (FM-UGM) of Yogyakarta. The population of the study was Ae.aegypti mosquito of egg origin, the result of colonization at the Parasitology Laboratory of FM-UGM, while the area population was the entire villages in the Grogol Sub-district and all the dengue fever cases (laboratory diagnosis with serology, i.e., IgM and/or DHF NS1 positive). The sample of the study was the Ae.aegypti mosquito from the eggs as the result of colonization at the Parasitology Laboratory of FM-UGM, taken from each village in DHF epidemic and sporadic areas and all cases of DHF (DHF positive laboratory diagnosis result with serologic examination, i.e., IgG, IgM, and/or NS1). This research has been approved by the Research Ethics Committee of FM-UGM.

The collection of Ae.aegypti mosquito eggs used an ovitrap labeled according to location outside/inside the house. The colonization of mosquitoes at the Parasitology Laboratory of FM-UGM from egg to adult was done to obtain adult mosquitoes which were then processed for virDen detection. RT PCR was employed for dengue virus examination. Spatial mapping of dengue hemorrhagic cases found during the study used a GPS device and the data were then analyzed with GIS software (SaTScan, GeoDa and Arc GIS) based on field surveys such as vector breeding sites and environmental factors. Furthermore, the breeding sites were made buffer in each area representing the mosquito sources. Buffer zone is useful to assess the level of vulnerability of the emergence of mosquitoes to infect humans.

The results of descriptive analysis were presented in the form of a narrated table. Bivariate analysis was done by chi-square test to determine whether there was a correlation between an independent variable, i.e., prevalence of DEN3 transovarial transmission and a dependent variable, i.e., DHF incidence. Spatially weighted regression analysis was done by using GeoDa to determine whether there was an influence between an independent variable, i.e., DEN3 transovarial transmission prevalence and a dependent variable, i.e., DHF incidence. Lastly, multivariate analysis was done using multiple linear regression test to determine whether there was an influence between the climatology indicators (temperature, humidity and rainfall) and the incidence rate.
RESULT AND DISCUSSION

Based on the results of the electrophoresis of RT PCR Serotyping Dengue examination from 12 samples, there were seven DEN-3 virus positive samples, i.e., the Village of Pondok, Sanggrahan, Cemani, Grogol, Telukan, Langenharjo, and Gedangan. Grogol Sub-district is the most endemic area of DHF in Sukoharjo District. The number of DHF cases per village in Grogol Sub-District can be seen in Table 1.

Table 1. The number of DHF cases in Grogol Sub-district of Sukoharjo District

<table>
<thead>
<tr>
<th>No</th>
<th>Village</th>
<th>2015</th>
<th>%</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Langenharjo</td>
<td>20</td>
<td>13.2</td>
<td>7</td>
<td>9.3</td>
</tr>
<tr>
<td>2</td>
<td>Parangjoro</td>
<td>6</td>
<td>3.9</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>Pandeyan</td>
<td>6</td>
<td>3.9</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>Kadokan</td>
<td>5</td>
<td>3.3</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>5</td>
<td>Pondok</td>
<td>4</td>
<td>2.6</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>6</td>
<td>Grogol</td>
<td>16</td>
<td>10.5</td>
<td>9</td>
<td>12.0</td>
</tr>
<tr>
<td>7</td>
<td>Madegondo</td>
<td>10</td>
<td>6.6</td>
<td>6</td>
<td>8.0</td>
</tr>
<tr>
<td>8</td>
<td>Telukan</td>
<td>24</td>
<td>15.8</td>
<td>8</td>
<td>10.7</td>
</tr>
<tr>
<td>9</td>
<td>Kwarasan</td>
<td>7</td>
<td>4.6</td>
<td>7</td>
<td>9.3</td>
</tr>
<tr>
<td>10</td>
<td>Gedangan</td>
<td>6</td>
<td>3.9</td>
<td>7</td>
<td>9.3</td>
</tr>
<tr>
<td>11</td>
<td>Manang</td>
<td>3</td>
<td>2.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>12</td>
<td>Sanggrahan</td>
<td>14</td>
<td>9.2</td>
<td>7</td>
<td>9.3</td>
</tr>
<tr>
<td>13</td>
<td>Banaran</td>
<td>5</td>
<td>3.3</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>14</td>
<td>Cemani</td>
<td>26</td>
<td>17.1</td>
<td>12</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>152</td>
<td>100</td>
<td>75</td>
<td>100</td>
</tr>
</tbody>
</table>

The nearest neighbor analysis (NNA) in this study was to find out whether the spreading pattern of DHF was uniform, random or clustered. This analysis used data of distance between one case coordinate point and another. The pattern consisted of 3 categories, i.e., cluster if the value is less than 0, random if the value is 1, and uniform if the value is 2.5.12

Buffer for the range of virDEN3 transovarial transmission against the incidence of DHF was done to see whether there was any clustering of cases around the positive region of virDEN3 transovarial transmission. Figure 8 shows case groupings on the buffer of virDEN3 transovarial transmission. Nearest neighbor analysis (NNA) toward the 100 m buffer found a tendency of clustering cases at a radius of 100-200 m.

Based on Figure 1, in Grogol Sub-district there were seven villages that were positive virDEN3 transovarial transmission. In addition, based on the buffer analysis, at a radius of 200 meters from virDEN3 transovarial transmission, 32 cases were found. However, with the same radius and buffer analysis, 5 villages with negative virDEN3 transovarial transmission showed only 6 cases found.

From the 200 meter buffer analysis, the number of cases in the areas (villages) with positive DEN3 transovarial transmission had a higher percentage than those with negative one. In the positive areas, there were four cases in Pondok Village (100%), six cases in Gedangan (100%), eight cases in Sanggrahan (100%), ten cases in Cemani (83.33%), six cases in Telukan (75%), six cases in Grogol (66.67%), and three cases in Langenharjo (42.86%). For the areas with negative transmission, two cases were found in Parangjoro (66.7%), two cases were in Pandeyan (66.7%), one case was in Kadokan (50%), and six cases were in Kwarasan (85.7%).

The chi-square test showed an X² value of 6.395 with a p-value of 0.011; thus, there was a significant correlation between DEN-3 transovarial transmission and DHF incidence. In other words, the higher the proportion of DEN-3 transovarial transmission, the more DHF incidences in Grogol Sub-district of Sukoharjo District. The Odd Ratio (OR) calculation showed an OR value of 2.219; thus, the areas with positive DEN-3 transovarial transmission had a 2.219 time greater risk of contracting DHF than those with negative transmission.

Spatially weighted regression (spatial error model) analysis with GeoDa was intended to find out the suitability of Transovarial
Transmission Map (result of RT PCR electrophoresis of Serotyping Dengue 3) with the incidence of DHF in Grogol District where the two items of mapping were stacked. The result of analysis showed a z value of 5.606017 and a p of 0.000 (p < 0.05); thus, there was a significant relationship between transovarial transmission and DHF incidence in Grogol District. It could also be said that the incidence rate of DHF was related to virDen 3 transovarial transmissions.

The Likelihood Ratio Test showed the p value of 0.0027306 (p < 0.05); thus, the occurrence of DHF followed a certain spatial spread pattern. In other words, the incidence of DHF was influenced by the factors in the area/spatial distribution where the dengue patients lived. Further analysis used Poisson Model, Purely Spatial based on village unit analysis (aggregate data) and the use of population variable. The result showed one cluster as the Most Likely Cluster which happened from 1 January 2016 to 31 December 2016 centered on coordinate of -7.588240 S, 110.809450 E at a radius of 2.00 km, i.e., in RW 21 of Cemani Village adjacent to RW 6 of Sanggrahan Village.

Discussion

This study proves the existence of transovarial transmission in a number of dengue endemic villages. The transovarial examination shows the vertical transmission occurred from female mosquitoes to their eggs. Transovarial transmission causes an area to continue to be endemic because cases will always exist and not be interrupted. DHF persistently transmitted transovarially will increase in frequency until the seventh generation. This condition might be caused by decreased rainfall in 2016,
while in 2015 rain frequently fell throughout the year. Climatic factors affect the number of dengue cases, i.e., the temperature and relative humidity during the rainy season supporting the propagation of dengue virus. Another study conducted in Malaysia to prove the virDEN transovarial transmission in its role for epidemiology Dengue concluded that virDEN transovarial transmission in Ae. aegypti plays a role in improving and maintaining Dengue epidemic, while the presence of virDEN transovarial transmission in Ae. albopictus provides clues to the presence of dengue cases within the period of 7-41 days.

This study shows that there is a significant relationship between transovarial transmission and DHF incidence in Grogol Sub-District of Sukoharjo District (p = 0.000, p <0.05). In other words, the incidence of DHF is related to virDEN-3 transovarial transmission. The presence of dengue virus transovarial infection in Ae. aegypti mosquitoes shows that mosquitoes play an important role as viral transmitters and as a defense for the dengue epidemic. This is the cause dengue endemic to occur in the area and DHF cases always increase in the rainy season. The dengue virus (virDEN-3) in the mosquito’s body can be transmitted at any time. Spatial analysis using Geoda shows that spatially the occurrence of DHF in Grogol Sub-district follows certain a spatial spread pattern, meaning the DHF incidence is influenced by factors that exist in the area/ space with the distribution of dengue cases. Grogol Sub-district has a temperature and humidity level of 70% which is ideal for viruses both in eggs and in mosquito bodies.

Eggs obtained from Ae. aegypti mosquitoes in intravenous (parenteral) DEN-3 viral infections when it is incubated at several weeks of the incubation period at room temperature, the percentage of transovarial transmission increases. This suggests that at room temperature, the virus has the opportunity to multiply during embryonic time in the egg. This study also shows that during the dry season when mosquitoes get a chance to suck the blood of viraemia host and lay their eggs in an appropriate environment, some eggs can live through inter-epidemic periods of life, and initiate the viral life cycle of human-mosquito-human. Transovarial transmission can last up to the 7th generation of mosquitoes with virDEN parenteral infection. Transovarial transmission is assumed to be an important aspect in maintaining virDEN during inter-epidemic in environment.

**CONCLUSION**

In this study, virDEN-3 transovarial transmission is correlated to DHF incidence, in which spatially the incidence follows a certain spatial spread pattern. The factors playing a role in virDen transmission or dengue cases distribution include temperature, humidity, and rainfall ideally.

**REFERENCE**


