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Ovitrap survey and serotype identification of dengue virus on *Aedes sp* mosquito in Potorono, Banguntapan, Bantul, Indonesia

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Abstract

Dengue fever is caused by DENV transmitted by *Aedes sp*. Ovitrap is used to survey the density of *Aedes sp* population. The aim of the study was to survey the adult p of *Aedes sp* and to identify DENV serotype. Ovitrap were used to collect the eggs of *Aedes sp* in indoor and outdoor of randomly selected houses. Total of 100 ovitraps was provided in 4 areas, 95 ovitraps were collected after nine days. Dengue virus serotypes were determined by RT-PCR. Ovitrap index in RT 7, RT 13, RT 1, and RT 3 were 42,9%, 64%, 54.2%, and 20%, respectively. The results of RT-PCR in 4 areas showed negative DENV 1-4. Ovitrap index in 4 areas of Potorono, Banguntapan, Bantul is high with the most abundant mosquitoes was *Aedes aegypti*. Surveillance of adult *Aedes sp* mosquitos is necessary for vector management control of DENV transmission.

Keywords: *Aedes sp*, ovitraps, ovitrap index, DENV serotype

1. Introduction

Aedes aegypti and *Aedes albopictus* are important vectors in the transmission of dengue virus (DENV 1-4), mosquito-borne pathogens, the cause of dengue fever in sub-tropical and tropical countries [1, 2]. Banguntapan is one of three districts with the most dengue fever cases in Bantul [3]. There were variations of serotype of DEN-V found in Indonesia. The most serotype in Surabaya and Yogyakarta was DENV-3, 62.8% and 76.4% respectively [4, 5]. The shift of serotype dominance has occurred in Surabaya, previously from DENV-2 to DENV-1 and now DENV-3, possibly due to host mobility, virus transport and geographical factors [4]. Mosquito surveillance is needed as part of vector management that involves community participation, personal protection, and control of mosquitoes [1]. Surveillance density of *Aedes sp* is important to determine the factors that influence dengue virus transmission. The most commonly used indicator for vector surveillance is adult survey using ovitrap to estimate adult density [6]. The installation of black ovitrap to survey the presence of *Aedes sp*. mosquitos is considered effective to capture gravid mosquitoes for oviposition. Examination using RT PCR on *Aedes sp* was able to determine the serotype of DENV [7, 8]. RT-PCR examination to identify DENV serotype both in human blood samples and from *Aedes aegypti* mosquito samples is considered to be successful [9]. Data regarding DENV serotype distribution is needed in order to determine the pattern of the disease which will be used to predict the epidemic status of an area [8, 4]. Data collecting from this study was using to manage integrated vector control. The aims of this study were to survey adult population density of *Aedes sp* and to identify DEN virus serotype, in Potorono, Banguntapan, Bantul, Indonesia.

2. Materials and Methods

2.1 Study area

The study was conducted in the area of Potorono, Banguntapan, Bantul, Indonesia. The highest and lowest temperature was recorded 37 °C and 24 °C, respectively. The population density was 2670 live/km² [10].

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2.2 Methods

This study was an observational descriptive study with a cross-sectional design to determine ovitrap index and serotype of *Aedes sp.* Based on the data from Banguntapan 1 primary care, there were 4 selected areas/neighborhood wards, normally called RT (Rukun Tetangga). Two areas were RT 7 and RT 13 which had no cases in the recent years, and the other 2 areas were RT 1 and RT 3 which had more cases in 1 year. Ovitrap were provided indoor and outdoor in each area 25 ovitraps in Potorono, Banguntapan Bantul during January-March 2016.

2.3 Ovitrap preparation

Oviposition trap (ovitrap) consists of 600 ml plastic bottles, with upper portions were cut and made into size 6.5 cm in diameter and 1 cm in height. The outer part of the bottles was painted black¹¹ and labeled with a number and area code, RT 7, RT 13, RT 1, and RT 3. Then the bottles were filled with water as much as $\frac{3}{4}$ volume. The container includes partially

coated filter paper exposed to the water as a breeding place. Ovitrap installation both indoor and outdoor were checked after 9 days. The eggs were collected and counted (Figure 1). Collected eggs were taken to Parasitology laboratory of, Universitas Islam Indonesia. Coated filter paper that contained eggs were inserted to transparent plastic glasses topped with mosquito net, sugar solution supplied ad libitum was placed in the top as a nutrition source. After 1 weeks, the eggs changed to larva and adult mosquitoes.

2.4 Species identification

Species identification of adult mosquitoes based on its morphology in the cephalothorax was done using mosquito aspirator. Adult mosquitoes were frozen for 5 minutes and inserted into microtube fit to each area. Adult mosquitoes in the microtube were sent to Parasitology laboratory of Medical Faculty of Universitas Gadjah Mada, using icebox to identify virus serotype using RT-PCR examination.

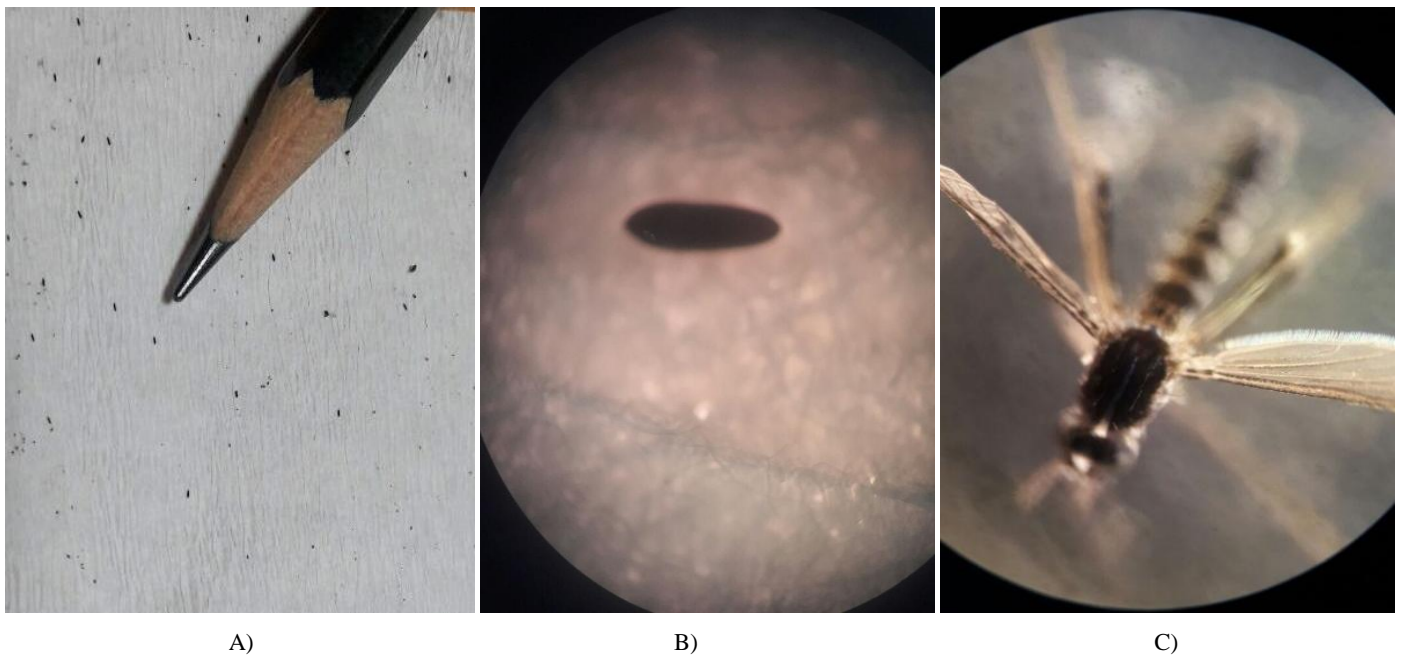


Fig 1: A) Counting eggs from ovitrap. B) Microscopic picture eggs of *Aedes sp.* from ovitrap. C) Identification of adult mosquito morphology from cephal.

2.5 RT PCR

Aedes sp thorax was grouped into 4 areas and then extracted using QIAmp®. After that, a two-step RT-PCR method was used with a primer of Lanciotti specifics primer^[12] to identify

virus serotype. The results of PCR products were electrophoresed on 2% agarose gel with 0.5 x TBE buffer and analyzed by marker 100 bp DNA ladder.

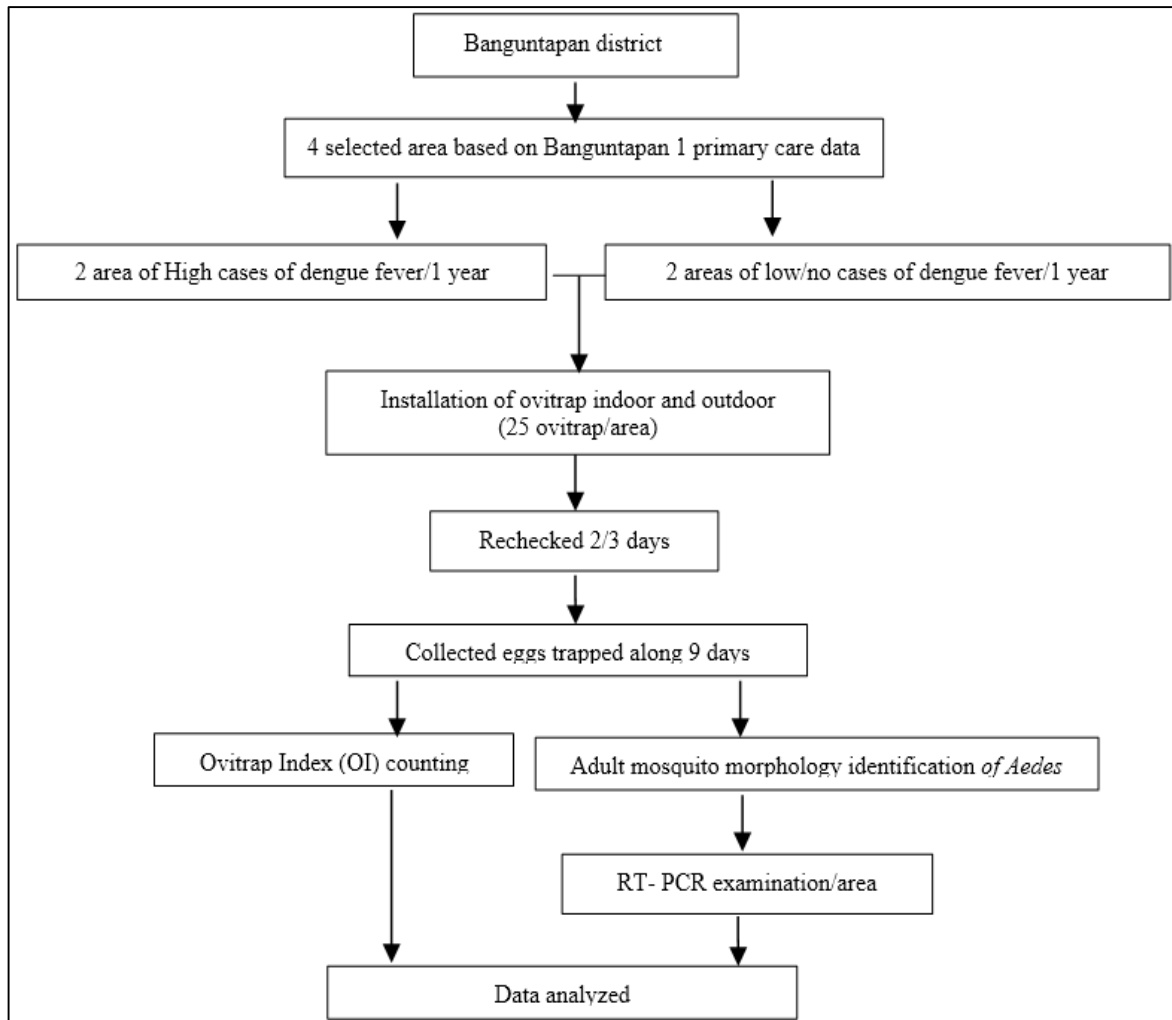


Fig 2: Sample identification scheme.

2.6 Data Analysis

Data was analyzed as ovitrap index and mosquito density was enumerated by the formula:

$$\text{Ovitrap Index} = \frac{\text{No. of Aedes-positive ovitrap}}{\text{No. of ovitrap collected}} \times 100\% \text{ [13]}$$

2.7 Ethics Approval

Ethical approval for the study was obtained from the Ethical Review Board of Faculty of Medicine, Universitas Islam Indonesia No. 11/Ka.Kom.Et/70.KE/XI/2016.

3. Results and Discussion

3.1 Ovitrap index and mosquito density

Total of 100 ovitrap were provided in 4 areas of Potorono, Banguntapan, Bantul. Two areas (RT 7 and RT 13) had no cases last year, while the other 2 areas (RT 1 and RT 03) had a lot of cases within the last 1 year. Each area was installed with 25 ovitrap, both in indoor and outdoor. The total amount of ovitrap collected after 9 days were 95 ovitrap. There were five ovitrap that could not be collected because some was lost or the water inside was spilled (Table 1).

Table 1: Ovitrap index and mosquito density in 4 selected areas of Potorono, Banguntapan, Bantul (2016).

Location	Total ovitrap collected	Total ovitrap with eggs inside	Total egg	Ovitrap Index (OI) %	Mosquitos density
RT with no cases					
- RT 7	21	9	157	42.9	7
- RT 13	25	16	394	64	16
RT with cases					
- RT 01	24	13	539	54.2	22
- RT 03	25	5	167	20	7

In present study, ovitrap index in RT 7, RT 13, RT 1, and RT 3 were 42.9%, 64%, 54.2%, and 20%, respectively. The level of ovitrap index in 4 areas was high. This outcome is different from the results of the previous year, in which Banguntapan had an ovitrap index of 38.18% [5]. Based on the classification

of index level, RT 7, RT 13, RT 1 were level 4. The action to be taken in level 4 including private pest control and using larvacides to control mosquito problem [13].

Table 1 shows the total density of adult mosquito in RT 7, RT 13, RT 1, and RT 3 were 7, 16, 22, and 7 respectively. Based

on the results of observation and ovitrap index measurement as well as egg density in 4 areas, the RT 7 and RT 13, RT 1 and RT 03. Some of the eggs collected from the ovitraps were intact, while some were not. Integrated vector control should be based on the data about local vector bioecology, the dynamics of disease transmission, ecosystem, and local community behavior (evidence-based) [14]. In this study, the ovitrap installations were done in March 2017 which is known as the rainy season. The increase of Dengue hemorrhagic fever is affected by the presence of water container, environmental sanitation, and vector density of *Aedes sp* [15]. Vector density was influenced by temperature, population density, and container indoor and outdoor [16]. When *Aedes sp* live in an environment where humidity was less than 60%, its lifespan will decrease which will prevent the migration of DEN virus from its stomach to salivary gland [17].

3.2 Morphology identification adult *Aedes sp* mosquitos

Table 2 shows the results of identification of adult mosquito species from rearing results found that both in the area with no cases and with dengue cases obtained the most mosquito species result is *Aedes aegypti* each of 74.7% and 58.2%. Previous research in the endemic area in Mojokerto also showed that most adult mosquitoes obtained from ovitrap method are *Aedes aegypti* species 76.88% [18]. Kelurahan Potorono Banguntapan Bantul is a region with high population density and experiencing a change towards the urban areas. Rice fields have been widely used for housing. Based on location and population density, as previous research shows urban areas have the most *Aedes aegypti* mosquito species [18, 19, 20]. In contrast to the results of previous studies in Singapore, which is a country with high density and is an urban area, the most commonly encountered adult mosquitoes are *Aedes albopictus* 2.15% compared with *Aedes aegypti* only 1.33% [7]. The distribution of mosquito species was determined by the altitude of an area, temperature, and local vegetation [21], human presence, and water reservoirs [22].

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Table 2: Identification of adult mosquitoes based on its morphology in Potorono, Banguntapan, Bantul (2016).

Location	<i>Aedes aegypti</i>	<i>Aedes albopictus</i>	Total
RT with no cases			
- RT 7	73	40	113
- RT 13	134	30	164
Total	207 (74.7%)	70 (25.3%)	277
RT with cases			
- RT 01	177	112	289
- RT 03	39	43	82
Total	216 (58.2%)	155 (41.8)	371

3.3 Serotype DEN virus identification using RT-PCR

Figure 2 showed the identification of serotype DEN virus using RT PCR. The RT PCR used in this study utilizes a primer which was based on Lanciotti with DENV-1, DENV-2, DENV-3, and DENV-4 are 482 bp, 119 bp, 290 bp, dan 389 bp respectively [12]. The DEN virus examined included DEN-1-4 with DENV-3 positive control. This is based on previous research that the DENV serotype in Bantul is dominated by DENV-3 [5]. Generally, the 4 areas showed a negative result for DEN-1, DEN-2, DEN-3, and DEN-4. In a previous study in Banguntapan it was found that of 10

mosquitoes, only 3 were DEN virus-infected with an infection index of 30% [5]. Similar results were also obtained in endemic areas in Bogor by bait method of people inside the house. The mosquitoes obtained were treated with RT-PCR and the result was negative for DENV-1 [23]. Routine serotype DENV examination can be used to predict the possibility of outbreak [5].

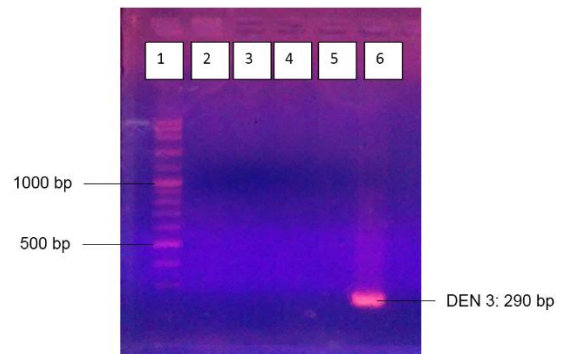


Fig 2: The result of RT-PCR using Lanciotti primers to detect serotype of Dengue virus in adult mosquitoes from 4 areas in Potorono, Banguntapan, Bantul on agarose gel 2%. Line 1: Marker 100 bp DNA ladder: line 2: RT 1 negative; line 3: RT 3: negative; line 4: RT 7: negative; line 5: negative; line 6: positive control DEN

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The DEN virus in *Aedes aegypti* mosquitoes in Singapore shows different serotypes from both *Aedes aegypti* and *Aedes albopictus* species [7]. The presence of DEN virus is not the only cause of dengue fever cases. High population mobility is also suspected to be the cause of dengue hemorrhagic cases [24]. A previous study in RS DR. Soetomo, Surabaya showed that 65.7% of DHF patients were within school-age [25]. Activity from morning till afternoon is at school. *Aedes sp* mosquitoes have peak suck inside the house/indoor at 10.00-11.00 [23]. Sub Banguntapan is one of 3 sub-districts in Bantul which has a high dengue fever case [3]. The high population and geographically close to the city of Yogyakarta, which is a dengue endemic area of dengue becomes the possible cause of dengue fever cases in Banguntapan sub-district. Implementation of mosquito-nest eradication has been routinely done. Thus, it is highly probable that the residents of Banguntapan affected by dengue fever are not from the region but from the workplace or school that mostly in Yogyakarta City. Transmission of DEN virus is transmitted during school activity because most of the morning time is spent in school. So in this study cases of dengue fever can still occur despite the presence of DENV negative.

4. Conclusion

The ovitrap index of 4 areas selected was high, in level 4. The results of identification of both areas showed that the most commonly found species was *Aedes aegypti*, which was found as much as 58.2% in areas with high case number (RT 1 and RT 3), and 74.7% in areas without case (RT 7 and RT 13) of sub-district Potorono, Banguntapan, Bantul. The results of serotype identification of DENV in both areas showed a negative result. DENV mosquito and serotype surveys are needed to observe the fluctuation of mosquito's bio-ecological changes in predicting outbreaks. The implementation of mosquito-nest eradication should be done until the smallest area is RT.

5. Conflict of interest

All author declare no conflict of interest

6. Acknowledgment

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