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DO WE SEE NATURAL *Wolbachia* IN FIELD-CAPTURED MOSQUITOS IN BANDUNG?

Savira Ekawardhani,[1] Imam Megantara[2], Riyadi,[2] Arifudin Achmad, [2] Hadyana Sukandar,[3] Lia Faridah [1]

[1]Division of Parasitology, Departmen of Biomedical Science, Faculty of medicine, Universitas Padjadjaran,
[2]Faculty of Medicine, Universitas Padjadjaran
[3]Department of Public Health, Faculty of Medicine, Universitas Padjadjaran
E-mail: savira@unpad.ac.id

Abstract

A new alternative in controlling mosquitos, the arbovirus vectors, has emerged in recent years and commonly called Wolbachia method. Currently, a large trial is still running since 2017 in Indonesia to assess the efficacy of Wolbachia-infected mosquito deployment in reducing dengue incidence in Yogyakarta. Awaiting the trial result before implementing the same method in Bandung, however we could not find any reports on the distribution of natural Wolbachia in urban mosquitos from Indonesia, especially for Culex sp and Aedes sp of Bandung. Therefore, field surveys were conducted at four locations (3 sub-districts) from Bandung City and its surrounding area to screen for the natural mosquito infection status of Wolbachia by PCR and sequencing method. We found that 83.3% of Bandung-captured Cx. quinquefasciatus were female, and 88.2% of those female were naturally infected with Wolbachia. The infection rate was lower for the male Culex, bringing down the total infection rate of Cx. quinquefasciatus to be around half of the captured mosquitos. However, the results from both female Ae. Aegypti of different locations revealed baffling very weak bands on the agarose gel. As for the infection status of Bandung's captured Ae. aegypti, it is worth to wait for our further results as well as reports from other studies. And thus, we opted to keep the inconclusive status of Wolbachia infection in Ae. aegypti from Bandung City.

Keywords: ***Aedes aegypti, arbovirus, Wolbachia***

1. Introduction

Around 1.8 billion of people in Asia-Pacific countries, like Indonesia, are at high risk for dengue and dengue hemorrhagic fever caused by the mosquito-borne

Dengue viruses (DENV), while globally almost half of the world's population are at the same high risk [1]. Bandung City of West Java still shows very high Case Fatality Rate for dengue [2]. Therefore, an elaborated effort is needed to control and prevent dengue incidence. Integrating new strategies for arbovirus vector management is a very important part of the said effort.

A new alternative in controlling mosquitos as the arbovirus vectors has emerged in recent years. Commonly called *Wolbachia* Method, this method utilizes the nature of *Wolbachia* strains capable of inducing cytoplasmic incompatibility (CI) in mosquitos, which results in the generation of unviable offspring when uninfected females mate with a *Wolbachia*-infected male [3]. Some studies also showed that *Wolbachia*-infected *Aedes aegypti* mosquitos were more resistant to disseminated dengue, Zika, chikungunya and yellow fever viruses [4,5,6].

The World Mosquito Program is an international research collaboration aiming on using *Wolbachia* in eliminating dengue transmission by its primary vector, *Ae. aegypti* [7]. The strategy heavily relies on public health intervention to spread the *Wolbachia*-infected mosquitos and thus, severely reducing the vectorial capacity of mosquito population to transmit dengue between humans. Indeed, there has been a trial since 2017 in Indonesia called AWED to assess the efficacy of *Wolbachia*-infected mosquito deployment in reducing dengue incidence in Yogyakarta [8]. The trial is still running and expected to be completed by December 2019. If the results are as expected, it would endorse the implementation of *Wolbachia* method elsewhere in the endemic regions, including Bandung City. However, it is also known that environmental conditions such as temperature, nutrition and pathogen infection modulate *Wolbachia* density in other insects [9], which in turn determines CI and viral blocking effects [10]. Consequently, geographic difference between two cities could also give some impacts on the strategy.

To date, there are no reports on the distribution of natural *Wolbachia* in wild mosquitos from Indonesia, especially for *Culex sp* and *Aedes sp* in Bandung City. Therefore, field surveys were conducted in this preliminary study to screen for the natural infection status of *Wolbachia* in mosquitos from Bandung City (and surrounding area). While the study is yet to be completed, this report is for raising a notion to a potential debate of whether some *Ae. aegypti* mosquitos in Bandung already harbor natural *Wolbachia*.

2. Study design

This is a descriptive study with purposive sampling. Field surveys were conducted to detect *Wolbachia* in field-collected mosquitos from various part of the city, in regard to previously reported dengue incidence data.

3. Material & Methods

Mosquitos: *Culex sp* and *Aedes sp* were collected using the modified commercial electric mosquito-traps supplemented with mosquito attractant, then the traps were put near or inside the houses. Mosquito collections were done simultaneously for three days in August 2018. We had a total of four trap locations in two different sub-districts of Bandung City (Sarijadi and Buah Batu, 1 location each) and in one of its surrounding area (Cimahi, two locations). Upon collection, the trapped mosquitos were directly placed in a microtubes and kept in -20°C freezer to kill them. Then, these mosquitos were identified under microscopes at Parasitology Laboratory, Faculty of Medicine, Universitas Padjadjaran by 2 different examiners. For DNA extraction, single mosquito was homogenized with a clean pestle and subjected to extraction according to the protocol of QIAamp® DNA Mini Kit (Qiagen™, Germany).

Detection of Wolbachia: PCR using the Master Mix 2x Kit (Invitrogen) was carried out with the temperature profile of 95°C for 3 min as initial denaturation, followed by 35 cycles of denaturation, annealing and elongation at 95°C, 55°C and 72°C respectively, and ended with 3 min of final elongation at 72°C and finishing at 20°C for 2 min. Primers used in this preliminary study were wsp81F and wsp691R as described by Zhou et al. (1998) [11]: wsp81F, 5'-TGG TCC AAT AAG TGA TGA AGA AAC-3'; wsp691R, 5'-AAA AAT TAA ACG CTA CTC CA-3'. A negative and positive control for the PCR assay were included in each run. The positive control was obtained by screening the adult *Drosophila sp* using PCR and sequencing of *wsp* gene to confirm that the amplified PCR product obtained was *Wolbachia*. All the positive PCR products were visualized under 1.5 per cent agarose gel electrophoresis.

Sequencing of Wolbachia : The positive PCR product was purified using QIAquick® Gel Extraction Kit (Qiagen, Germany) prior to DNA sequencing and then was outsourced for the sequencing process. Retrieved sequences were subjected to run in Basic Local Alignment Search Tool (BLAST®) available in the GenBank (http://blast.ncbi.nlm.nih.gov/).

4. Results

While the field surveys and mosquito collection are still running, a total of 32 mosquitos (30 male or female *Culex quinquefasciatus* and 2 female *Aedes aegypti*) were analyzed by the time of this report. The two samples of *Ae. aegypti* were from different location of Sarijadi, Buah Batu and Cimahi, respectively (Table 1). 83.3% of the captured *Culex quinquefasciatus* were female.

In total, 17 (53.1%) of tested *Culex quinquefasciatus* in this ongoing study were positive for *Wolbachia*. From the positive *Culex quinquefasciatus,* 15 mosquitos are female (88.2%). As for the *Ae. aegypti* female mosquitos, both samples were categorized as still inconclusive (Tabel 2).

Detection of *Wolbachia* in the *Culex quinquefasciatus* samples were straightforward of either being positive or negative as depicted in Figure 1, and with the clean negative control that excluded inter sample contamination possibility. However, the results from both female *Ae. Aegypti* of different locations revealed baffling very weak bands on the agarose gel and therefore assigned as inconclusive result.

Clean sequencing results on the positive mosquito samples were subjected to BLASTn search for finding the local similarity against deposited sequences in the database (NCBI database). As the bands from *Ae. aegypti* samples were too faint to purify, they were yet subjected to sequencing analysis.

Multiple alignment analysis (ClustalW) revealed 100% homology for all the positive samples from 4 different sampling locations. BLASTn results for the positive *Culex quinquefasciatus* showed that the highest hit with 100% similarity was indeed with *Wolbachia* endosymbiont of *Culex quinquefasciatus wsp* gene sequence (accession number LC276757, as depicted in Figure 2).

5. Discussions

Culex quinquefasciatus is a member of *Cx. pipiens* complex and is a known vector of St. Louis encephalitis and West Nile viruses in North America [12] and a secondary vector of JEV in China [13]. Recently, *Culex quinquefasciatus* was shown to be a potential vector of ZIKV capable of transmitting the virus in China [14] although later was contradicted by Main *et al.* (2018) [15], and therefore highlighted the importance of *Culex sp* monitoring in Bandung City and Indonesia as well, along with the corresponding debate.

In this study, we found that 83.3% of Bandung-captured *Cx. quinquefasciatus* were female, and 88.2% of those female *Cx. quinquefasciatus* were naturally infected with *Wolbachia*. The infection rate was lower for the male *Culex*, bringing down the total infection rate of *Cx. quinquefasciatus* to be around half of the captured mosquitos.

Natural *Wolbahia* infection for *Cx. quinquefasciatus* has been observed elsewhere with a similar or close percentage compared to our data, such as in Pakistan [16], and Iran for *Culex pipiens* [17]. It is yet to be investigated whether

the natural *Wolbachia* strain found in Bandung's mosquito capable of limiting arbovirus transmission or inducing the CI.

Conversely, our results on female *Ae. aegypti* were inconclusive in the way that we did not rule out the possibility yet of a natural infection of *Wolbachia*, based on the very recent (still un-peer reviewed) report from Carvajal *et al.*, 2018 [18], despite the previously common findings that *Ae. aegypti* were not naturally infected with *Wolbachia*. After their findings, Carvajal suggested that an improvement to the detection protocol could give positive results for *Wolbachia* in *Ae. aegypti*., which were previously inconclusive similar to our results. Therefore, upon finishing our extended field surveys on the larger area of Bandung City, we would implement the said improved detection protocol on our field-captured samples, as well as on our lab-reared standard *Ae. aegypti* (with and without pretreatment of the antibiotic tetracycline).

Interestingly, Baldini et al., (2014) [19] has described that improved methods of detection would likely lead to a more positive result, as has been shown by the detection of *Wolbachia* for the first time in Anopheles gambiae when using a high throughput sequencing of 16sRNA gene, and *Anopheles gambiae* has been said to be resistant to Wolbachia infection before. Hence, we opted to maintain the inconclusive status of Wolbachia infection status for Bandung *Ae. aegypti* until we obtain more results from the aforementioned strategy.

6. Conclusions

Overall, about half of the *Culex quinquefasciatus* in Bandung City captured in this study were positive for natural infection with the same strain of *Wolbachia*. As for the infection status of the capture *Ae. aegypti*, it is worth to wait for further results and reports from other studies, especially by Carjaval *et.al*, 2018 [18], and thus we concluded our findings on *Ae. aegypti* to be open for interpretation.

7. Limitation of the study

The samples were restricted only to 4 sampling locations and a very limited number of captured female *Ae aegypti.* Collection of *Ae. aegypti* from the field is yet to be finished, as well as the collection of lab-reared *Ae. aegypti* (cured and non-cured).

8. Conflict of Interest

All authors declared no conflict of interest in this study

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10. References

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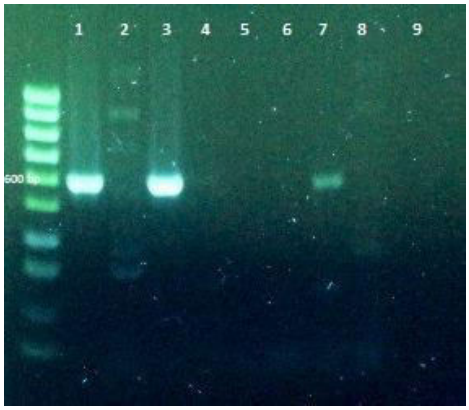
Table 1 Number of Catched Mosquitos

Location	Number of mosquitos
Buah Batu	(3 females, 1 male *Culex*)
Cimahi Location 1	(8 females and 3 males *Culex)*
Sarijadi	(10 females and 1 male *Culex,* 1 female *Ae. aegypti*)
Cimahi Location 2	(4 females *Culex,* 1 female *Ae. aegypti*)



Ladder 100 bp. Positive band is at 600 bp size.

1. *Culex* female Sarijadi (pos control)
2. ***Aedes* female Sarijadi (?)**
3. *Culex* female Cimahi Location 2 (pos)
4. *Culex* female Cimahi Location 2 (neg)
5. *Culex* female Cimahi Location 1 (neg)
6. *Culex* male Cimahi Location 1 (neg)
7. *Culex* male Sarijadi (pos)
8. ***Aedes* female Cimahi Location 2 (?)**
9. Negative control

Figure 1 Agarose Gel Electrophoresis of PCR Products

Table 2 Wolbachia Detection on Mosquitos

Location	Number of positive sample
Buah Batu	3 females, 1 male *Culex*
Cimahi Location 1	0
Sarijadi	10 females and 1 male *Culex*; 1 female *Ae. aegypti* (?)
Cimahi Location 2	2 females *Culex*, 1 female *Ae. Aegypti* (?)

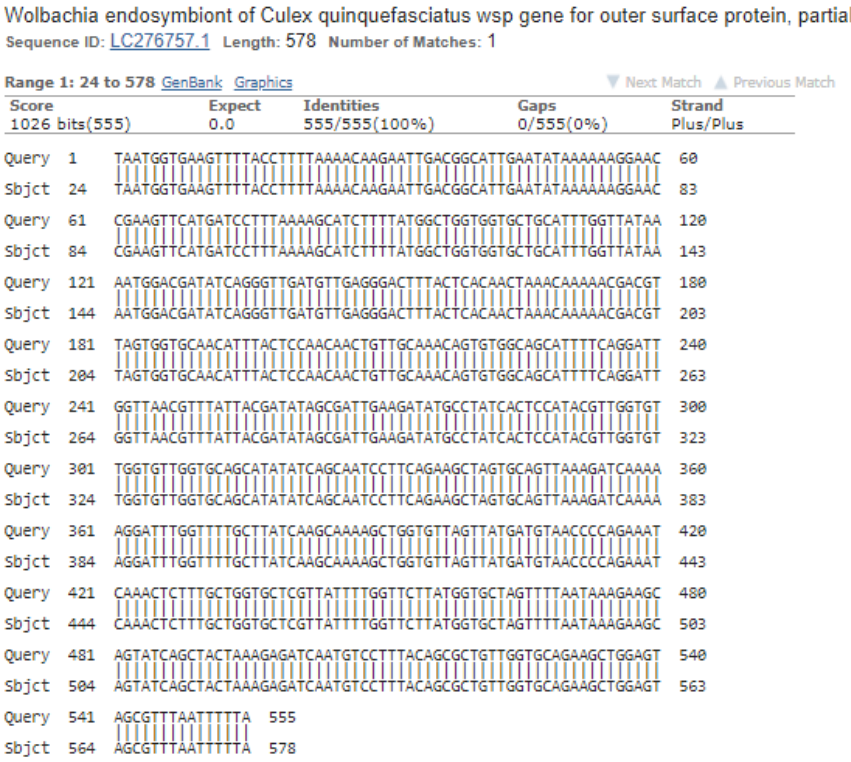


Figure 2 BLASTn Result of Positive Samples