

Title

Diversity and Blood Meal Analysis of Mosquitoes in Livestock area and Human Settlements in Yogyakarta, Indonesia

Member`s name and affiliations

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Aim

The objectives of this study are:

- 1.To understand the mosquito diversity in the pig farm area, human settlement near pig farm and human settlement without pig farm in semi-urban area in Indonesia.
- 2.To identify the blood meal of engorged mosquitoes collected from all sampling area.
- 3.To evaluate the risk of mosquito-borne diseases transmission in the human settlement near pig farm.

Methods

- a. Mosquito collection, identification and DNA extraction:

Mosquito was collected from the livestock, human settlement near pig farm and human settlement without pig farm using UV light trap in semi-urban area in Indonesia. The mosquito was identified using mosquito identification book from Ministry of Health Indonesia. All engorged mosquito were sorted and put in the 96% ethanol for preservation. DNA was extracted from all engorged mosquitoes using QIAGEN DNeasy Blood and Tissue Kit.

- b. Blood meal identification using NGS metabarcoding approach:

NGS Metabarcoding is a sensitive tool to identify the blood meal source of hematophagous insects. It can identify the degraded DNA blood and mix blood meal from a single mosquito while the conventional PCR tool could not identify it. All engorged mosquitoes were processed for library preparation. In this study, we used 12S rRNA and 16S rRNA gene markers specific for vertebrate to identify the blood meal of the mosquitoes.

Results

A total of 2,253 adult mosquitoes (1,663 females and 590 males) were collected from large pig farms, human settlements near pig farms, and human settlements without pig farms.

Table 1. Abundance of adult mosquito species collected from all sampling areas in Yogyakarta, Indonesia

Sex	No	Species	Large pig farms	Human settlement near pig farms	Human settlement without Pig farms	Vector for
<u>Female</u>						
	1	<i>Aedes aegypti</i>	29	181	123	Dengue Fever, Chikungunya, Zika
	2	<i>Aedes albopictus</i>	12	15	10	Dengue Fever, Chikungunya
	3	<i>Aedes poicilus</i>	18	2	1	Filariasis
	4	<i>Anopheles vagus</i>	241	19	2	Malaria
	5	<i>Anopheles aconitus</i>	28	1	0	Malaria
	6	<i>Anopheles barbirostris</i>	8	1	0	Malaria, Filariasis
	7	<i>Anopheles kochi</i>	1	0	0	Malaria
	8	<i>Anopheles longirostris</i>	1	0	0	Malaria
	9	<i>Anopheles maculatus</i>	2	0	0	Malaria
	10	<i>Anopheles sundaicus</i>	1	0	0	Malaria
	11	<i>Anopheles tessellatus</i>	1	0	0	Malaria
	12	<i>Armigeres subalbatus</i>	22	78	8	Filariasis rural bancroftian
	13	<i>Coquillitidia crassipes</i>	1	0	0	filariasis
	14	<i>Culex bitaeniorhynchus</i>	2	0	0	JEV
	15	<i>Culex fuscocephala</i>	1	3	0	JEV
	16	<i>Culex gelidus</i>	5	0	0	JEV
	17	<i>Culex hutchinsoni</i>	127	4	1	<u>No report</u>
	18	<i>Culex quinquefasciatus</i>	74	68	13	Filariasis
	19	<i>Culex tritaeniorhynchus</i>	381	11	5	JEV
	20	<i>Culex vishnui</i>	7	2	7	JEV
	21	<i>Ficalbia minima</i>	65	1	1	<u>No report</u>
	22	<i>Malaya genurostris</i>	2	0	0	-
	23	<i>Mansonia annulata</i>	1	1	0	Filariasis
	24	<i>Mansonia uniformis</i>	29	3	0	Filariasis
	25	<i>Mimomyia luzonensis</i>	26	1	0	<u>No report</u>
	26	<i>Uranotaenia atra</i>	10	6	0	<u>No report</u>
<u>Male</u>						
	1	<i>Aedes</i> spp.	32	85	101	-
	2	<i>Anopheles</i> spp.	116	36	10	-
	3	<i>Culex</i> spp.	72	73	17	-
	4	<i>Armigeres</i> spp.	1	38	6	-
	5	<i>Mansonia</i> spp.	3	0	0	-
Total			1319	629	305	

Nonmetric multidimensional scaling analysis showed that the community structure of mosquitoes in human settlements near pig farms and in human settlements without pig farms differed slightly (Fig. 1). The community structure of large pig farms was clearly different from that of human settlements near pig farms and human settlements without pig farms.

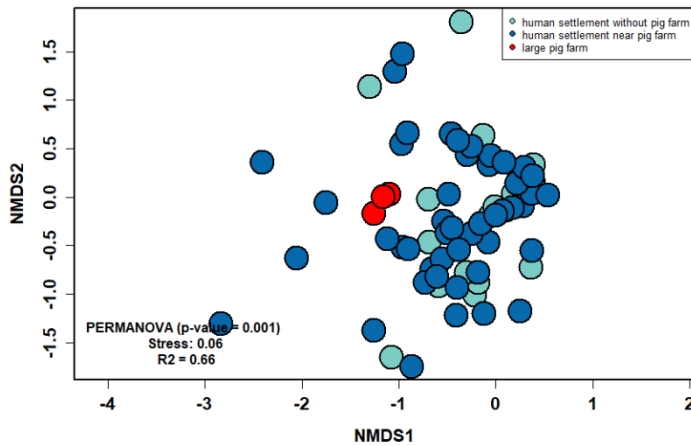


Fig. 1 Nonmetric multidimensional scaling plot of mosquito community showing similarity between pig farms, human settlements near pig farms, and human settlements without pig farms based on the Bray–Curtis dissimilarity matrix.

Community composition analysis of mosquitoes collected from human settlements near pig farms (Fig. 2) revealed the presence of animal-preferred mosquitoes that found in the pig farms (e.g., *Cx. tritaeniorhynchus*, *Cx.hutchinsoni*, *An. vagus*, *An. barbirostris*, *An. aconitus*) were also found in households near both large and small pig farms.

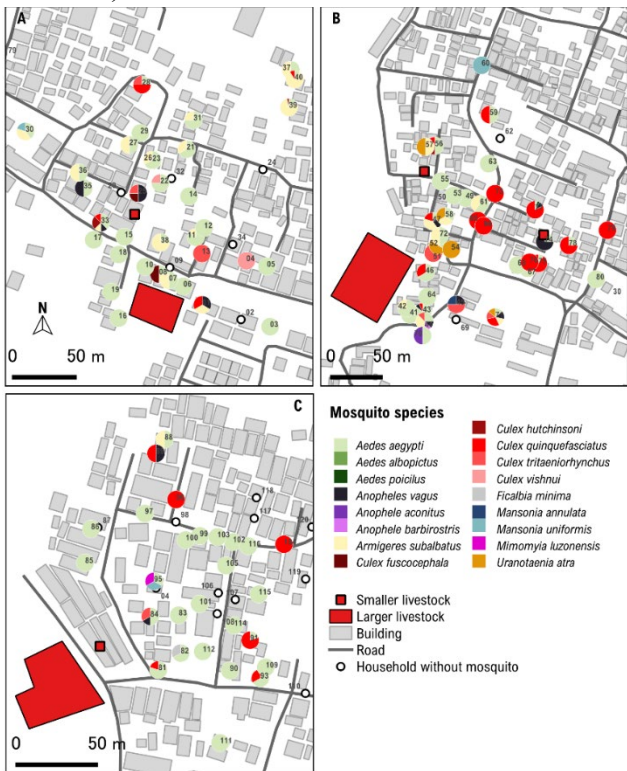


Fig. 2 The mosquito species distribution in human settlements near pig farms 1 (A), 2 (B), and 3 (C) in Ngestiharjo village, Yogyakarta.

Ae. aegypti, *An. vagus*, *An. aconitus*, *Ar. subalbatus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, and *Fi. minima* were observed to have multiple blood feeding on human and pig using 16S rRNA marker (Table 2) while only *Ae. aegypti*, *Ae. albopictus*, *An. vagus*, *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* have multiple blood feeding on human and pig using 12S rRNA (Table 3).

Table 2. Multiple blood feeding on human and pig using 16S rRNA

	<i>Aedes aegypti</i>	<i>Anopheles vagus</i>	<i>Anopheles aconitus</i>	<i>Armigeres subalbatus</i>	<i>Culex quinquefasciatus</i>	<i>Culex tritaeniorhynchus</i>	<i>Ficalbia minima</i>
caught in pig farm	2	8	1	0	7	4	1
caught in human settlement	5	2	0	1	5	0	0
total	7	10	1	1	12	4	1

Table 3. Multiple blood feeding on human and pig using 12S rRNA

	<i>Aedes aegypti</i>	<i>Aedes albopictus</i>	<i>Anopheles vagus</i>	<i>Culex quinquefasciatus</i>	<i>Culex tritaeniorhynchus</i>
caught in pig farm	3	1	4	3	2
caught in human settlement	5	0	2	6	0
total	8	1	6	9	2

The result of multiple blood feeding on human and pig could inform us that there is potential risk of mosquito-borne disease transmission such as Japanese encephalitis in which the pigs act as a host that amplify the Japanese encephalitis virus. The potential spillover of the virus from the pig to the human through the mosquito biting activity could be an important concern for the local government to relocate the pig farm from the human settlements.

Future Challenge

For the future perspective, the investigation on Japanese encephalitis virus detection needs to be conducted especially from the pig that presence in the pig farms. The challenge that may be faced is how the local government can communicate the result of our study to the society and provide a place to be used by the farmer to keep the pig far from their settlements due to the risk of Japanese encephalitis transmission.