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RESEARCH ARTICLE

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Mosquito Community in Primate Captivity (*Tarsius* sp.) and its Potential as Transmitters of Zoonotic Mosquito-Borne Diseases

Sarasvathi Cecile[1*], Upik Kesumawati Hadi[1], Uus Saepulohi[2], Silmi Mariya[2], Huda Shalahudin Darusman[1,2]

[1)]Faculty of Veterinary Medicine Bogor Agricultural University (IPB University), Jl. Agatis Raya, Kampus IPB Darmaga, Bogor, 16680, Indonesia
[2)]Primate Research Center, Institute of Research and Community Services, IPB University, Jalan Lodaya II No. 5, Bogor, Indonesia

Abstract
By means of conservation, ectoparasites monitoring and surveillance especially mosquitoes in primate captivity become important. Mosquito is one of the ectoparasites which acts as a vector of various types of zoonotic diseases such as Dengue, Zika, Japanese encephalitis, Rift Valley fever, filariasis, and others. This study was aimed to determine the diversity of mosquito species, their fluctuations as well as the potential of mosquitoes as a Dengue virus (DENV) vector around the tarsier captivity in the animal conservation laboratory IPB Primate Research Center (IPB PRC). Mosquitoes were collected from February to April 2020 using light traps and sweep net every two hours from 06.00 pm to 06.00 am. Detection of the presence of Dengue virus (DENV) is carried out using Reverse Transcriptase Real-time Polymerase Chain Reaction (RT-qPCR) against *Aedes albopictus* and *Armigeres subalbatus*. The results showed that there were 4 species of mosquitoes caught around the tarsier captivity namely *Aedes albopictus*, *Culex quinquefasciatus*, *Armigeres subalbatus*, and *Armigeres foliatus*. The dominant mosquito species were *Armigeres subalbatus* (62.11%) and *Aedes albopictus* (41.61%). Detection of Dengue virus (DENV) serotypes 1, 2, 3, 4 in *Aedes albopictus* and *Armigeres subalbatus* gave negative results. The presence of mosquitoes that have the potential to carry zoonotic disease around the tarsier captivity in IPB PRC shows the potential for Mosquito-Borne Diseases to both tarsiers and human.

Key words: Dengue, Mosquito, Non-Human Primate, Tarsier, Vector, Zoonotic

1. Introduction

Increasing population growth and development activities for various uses can cause damage to ecosystems such as forests. Forest destruction can increase the potential for disease transmission from wild animals to humans through vectors. One of the vectors that can transmit zoonotic diseases is mosquitoes. Some mosquito-borne diseases involve wild animals such as primates as reservoirs. In this study, the molecular detection of viruses was done from mosquitoes collected around the tarsier captivity in IPB PRC.

From an evolutionary point of view, Indonesia has various primate species, both primitive and modern primates. One of the primitive primates in Indonesia is tarsier (Supriatna *et al.* 2001). Tarsiers are primates of the Tarsius genus and the Tarsiidae family, the only families that still exist today from the Tarsiiformes order. Tarsiers are primitive Indonesian primates scattered on Sumatra, Kalimantan, Sulawesi, and several other small islands.

By means of conservation, monitoring, and surveillance of ectoparasites, especially mosquitoes in primate captivity, is essential. The presence of mosquitoes in primate breeding environments, especially relatively close to human, can allow the transmission of zoonotic diseases from mosquitoes called mosquito-borne disease. Primate breeding facilities owned by the IPB Primate Research Center (IPB PRC) can be a model or illustration of

*Coresponding author
Email Address : sarasvathicecile@gmail.com

interactions between wildlife and human activities. The interaction of wild animals with humans in their surroundings can occur directly or indirectly. Zoonotic disease transmission through vectors such as mosquitoes is an example of indirect interaction between wildlife and humans. Mosquitoes are one of the arthropods that can potentially to spread zoonotic diseases in the form of viruses or other pathogens.

Zoonotic diseases caused by the mosquito-borne virus often occur in tropical countries, including Indonesia. One of the viruses that can be transmitted from primates to humans through mosquitoes is the dengue virus (DENV). This virus is transmitted by female Aedes mosquitoes to humans through mosquito saliva. *Aedes aegypti* is known as the main vector of the dengue virus transmission. Apart from *Aedes aegypti*, Aedes mosquito species that also can be vectors for this virus transmission are *Aedes albopictus*, *Aedes polynesiensis*, *Aedes scutellaris* (Seema and Jain 2005). Despite the replication of DENV in primates, they do not show any clinical symptoms of the disease that resemble in human infection (Zompi *et al.* 2012). There have not been any reports of DENV replication in primitive primates such as tarsiers. However, several other primate species have been reported to be natural reservoirs and can be used as animal models for the dengue virus.

The environment around the tarsier captivity in the IPB Primate Research Center is quite humid and is surrounded by shrubs and trees and relatively dark. Apart from being aimed at creating an environment closer to compatibility with nocturnal animals, this environmental condition supports the breeding ground for mosquitoes that allows disease transmission from primates to humans through mosquito vectors. Tarsier ex-situ site at PRC IPB is located near the housing area. It makes studies on species diversity, density fluctuations in mosquito communities, and their potential as disease vectors need to be carried out to estimate the potential spillover of mosquito-borne disease from Nonhuman Primates (NHP) to humans. The study may be ideally conducted in the area where NHP and human are at the reach of the mosquito environment.

2. Materials and Methods

2.1. Mosquitoes Collection

Adult mosquitoes collected to know the diversity and fluctuation were observed at night around the IPB PRC tarsier captivity 12 times from February to April. Collections were done using a sweep net for 25 minutes at 5 points every 2 hours over 12 hours (18.00-06.00 GMT+7) to determine the fluctuation in mosquito density and using three light traps installed about 1.5 meters above ground level starting from 18.00- 06.00 (GMT+7). The mosquitoes that were collected were put to death by putting them in a plastic box that had been given chloroformed cotton. The mosquitoes' corpses were pinned and identified. The results of the collected mosquitoes will be used to calculate relative abundance, frequency, species dominance, and the fluctuations of mosquito activity at night.

2.2. Mosquito Identification

The mosquito was attached to the tip of the triangular paper, where the needle was attached with clear nail polish as an adhesive. Attachment to triangular paper is carried out on the lateral part of the thorax to make the entire side of the mosquito be seen to be identified. Observations were carried out under a stereo microscope and then identified with the Aedes Mosquito Identification Key (Departemen Kesehatan, 2008a), the Armigeres Mosquito Identification Key, and the Culex Mosquito Identification Key (Departemen Kesehatan, 2008b). The identification includes the species and sex of the mosquitoes.

2.3. Mosquito Collection to Detect Mosquito-Borne Disease Agent

Japanese Encephalitis, Zika, Yellow Fever, and Dengue are some of the zoonotic diseases that can be transmitted by primate to human through mosquitoes. Along with the high disease incidence rate of the Dengue outbreak in Indonesia in early 2020, this study aimed to test mosquito samples against dengue virus primer. Two species of mosquitoes tested for dengue virus infection were *Aedes albopictus* and *Armigeres subalbatus*. The *Aedes albopictus* mosquito is one of the mosquitoes that is the main vector of the dengue virus, but testing of the dengue virus against the *Armigeres subalbatus* mosquito was meant for exploratory purposes. Mosquito collection was carried out by sweep net from 06.00-10.00 WIB one day before being tested using Reverse Transcriptase Real-time Polymerase Chain Reaction (RT-qPCR). Each species of collected mosquitoes were about 25-30 females put into two different vials and then placed in the freezer at -20 ºC.

2.4. RNA Extraction from Mosquito Sample

The mosquitoes that have been stored in the freezer for one day are then removed and crushed with a mortar using 3 mL of Viral Transport Medium (VTM) solution and then put into an Eppendorf tube. The samples were then processed for RNA extraction according to Zymo Research's Total RNA Purification by Quick-RNA MiniPrep method.

2.5. Dengue Virus serotype 1, 2, 3, 4 Analysis

Dengue virus identification in *Aedes albopictus* and *Armigeres subalbatus* mosquitoes was analyzed by using the Reverse Transcriptase Real-time Polymerase Chain Reaction (RT-qPCR) technique. The process of viral RNA amplification using the RT-qPCR technique in this study was carried out based on the manual protocol of the Sensifast Probe Lo-ROX One-Step RT PCR Kit Bioline (Meridian Bioscience, Cincinnati, Ohio USA).

RNA molecules that have been extracted were tested against four serotypes of Dengue virus, namely DEN-1, DEN-2, DEN-3, and DEN-4. The RT-qPCR master mix used consisted of 0.6 µl of DEN forward primer, 0.6 µl reverse primer, 0.6 µl DEN probe, 0.2µl reverse transcriptase enzyme, 0.4µl RNAse Inhibitor, Sensifast One-Step Probe Lo-Rox 10 µl, and RNA 8 prints. µl. The primary sequences used are provided in Table 1. The Real-time PCR program was carried out with the following steps: reverse transcription at 45 ºC for 10 minutes, enzyme activation at 95 ºC for 2 minutes, denaturation at 95 ºC for 15 seconds, and primary attachment at 60 ºC for 20 seconds. The process of denaturation to primary attachment was repeated for 40 cycles.

2.6. Data Analysis

Data analysis was performed using Microsoft Excel 2019 software calculations (Microsoft Corporation One Microsoft Redmond, USA) to determine fluctuations in mosquito activity at night and relative abundance, frequency, and species dominance measured using the following calculations:

Relative abundance is the ratio of the number of individual vector species to the total number of vector species obtained and expressed in percent.

$$\text{Relative Abundance}(\%) = \frac{\text{Number of specific species caught x 100\%}}{\text{Number of all species caught in total}}$$

The frequency of caught mosquitoes is calculated based on the ratio of the number of mosquito catches of a particular species to the total number of catches.

$$\text{Frequency} = \frac{\text{Frequency of particular species caught in each collection}}{\text{Total number of catches}}$$

The dominance rate is calculated based on the multiplication result of the relative abundance and the frequency of the mosquitoes being caught by that species in one capture time.

Table 1. Primary nucleotide bases and probes sequences

Testing	Primer/Probe	Nucleotide sequences (5'-3')	Primary adhesion temperature (°C)
DENV 1	DENV1_F	CAATGGATGACAACAGAAGAYATG	56.6
	DENV1_R	TCCATCCATGGGTTTTCCTCTAT	59.5
	DENV1_P	TCAGTGTGGAATAGGGTTT	70.0
DENV2	DENV2_F	GCAGAAACACAACATGGAACRATAGT	56.6
	DENV2_R	TGATGTAGCTGTCTCCRAATGG	59.8
	DENV2_P	TCAACATAGAAGCAGAACC	68.0
DENV3	DENV3_F	ATGGAATGTGTGGGAGGTGG	59.1
	DENV3_R	GGCTTTCTATCCARTAGCCCATG	59.8
	DENV3_P	TATGGCTGAAACTCCGAG	68.0
DENV4	DENV4_F	GCAGATCTCTGGAAAAATGAACCA	60.4
	DENV4_R	GAGAATCTCTTCACCAACCCYTG	59.8

Dominance Rate = Relative Abundance x Frequency

3. Results

The various types of mosquitoes caught by light traps in IPB PRC consisted of four species, namely *Aedes albopictus*, *Culex quinquefasciatus*, *Armigeres subalbatus*, and *Armigeres foliatus*. With the sweep net method, only three species were found: *Aedes albopictus*, *Culex quinquefasciatus*, and *Armigeres subalbatus*.

Aedes albopictus has morphological characteristics in the form of an elongated white patch on the mesonotum or middle of the back (median stripe) (Hadi and Koesharto 2006). The hind leg tibia has a white bracelet. The proboscis is shorter than the femur of the mosquito's forefoot. On the pleura, there are white scales forming white irregular spots. There is a large collection of white scales between the supra alar feathers above the wing roots, which characterizes this mosquito (Figure 1A). *Armigeres subalbatus* is a mosquito with large size. It is characterized by a black and white striped ventral abdomen and a long proboscis that curves downward at the tip (Figure 1B). *Armigeres foliatus* is also a mosquito with large size and has a proboscis that is long and curves down at the ends. The mosquito's ventral abdomen side is white (Figure 1C). *Culex quinquefasciatus* has morphological characteristics on its proboscis, which does not have a white band. The integument and pleuron are evenly pale brown. The abdominal pinch has a narrow basal band (Figure 1D).

Based on the results, the mosquitoes found

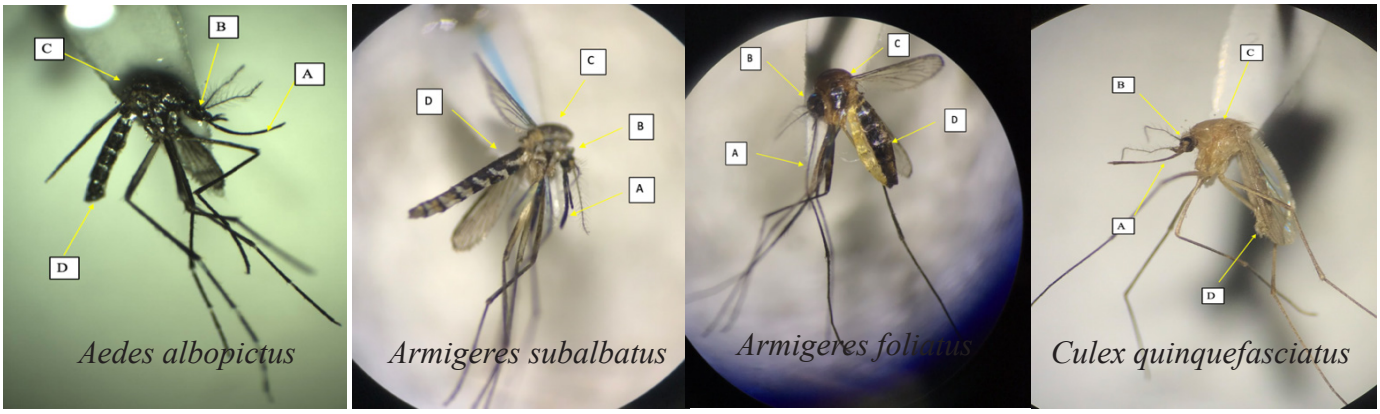

Figure 1. The variety of mosquitoes found around the tarsier captivity at PRC IPB in February-April 2020 (A. proboscis, B. head, C. thorax, D. abdomen)

in the laboratory for conservation animals of IPB PRC were quite diverse. This is due to the humid environment of the cage and there are many trees and shrubs that can be an undesignated place for mosquitoes to rest and provide a dark atmosphere that mosquitoes preferred. Around the captivity, puddles, and trenches are also found, which can be potential breeding grounds for mosquitoes. According to Hadi and Koesharto (2006), mosquitoes need water such as lakes, irrigation canals, rock water, septic tanks, and sewers as a medium for breeding and development of pre-adult mosquitoes.

The four species of mosquitoes obtained from the collection around the tarsier captivity were also found in a collection conducted by Andini (2008) around the Bornean Orangutan (*Pongo pygmaeus*) captivity in several ex-situ facilities such as Taman Safari Indonesia, Bandung Zoo, Cikananga Wildlife Center, and Ragunan Zoological Park. It shows that the four types of mosquitoes tend to be found in such kinds of habitats as forests or plantations and near animals.

Based on the data obtained during the surveillance period for twelve times from February to April 2020, the total number of mosquitoes caught using light traps was 45 individuals and 322 individuals using sweep nets. Based on the data seen in Table 2 and Table 3, the highest number of species found by both sweep net and light trap was *Armigeres subalbatus* and followed by *Aedes albopictus*. This is likely due to the preferred environment for the two species to breed. According to Harbach (2008), the breeding habitat for pre-adult *Armigeres subalbatus* is dirty water or has high organic content, such as water in rock holes, trees, bamboo, and embankments. The Armigeres mosquito can also be found in forest areas or shady areas. Besides that, it can also be found in puddles of groundwater and shrubs with humid and shady environmental conditions.

This habitat is also preferred by the *Aedes albopictus* mosquito, which WHO stated in 2005 that *Aedes albopictus* breeds in tree holes and drums, this is also supported by the behavior of the Armigeres mosquito, which according to Astuti and Marina (2009) is zoophilic-anthropophilic and exophagic. The *Aedes albopictus* mosquito has also been found to suck the blood of pigs (Ponlawat and Harrington, 2005), rabbits, rats, dogs, cows, deer, turtles, birds, and cats (Niebylski *et al.* 1994). Culex mosquitoes are also found by both methods of capture. This mosquito is nocturnal, which means it is actively biting at night. Culex mosquito feeding behaviour is between 7 pm to 4 am, with peak bites from 12 pm to 2 am (Sukendra and Shidqon, 2016). Only 15 *Culex quinquefasciatus* mosquitoes were caught

Table 2. Density and percentage of each species of mosquito caught by light traps

Surveillance (date)	Number of mosquitoes caught			Number of mosquitoes (%)	Average of mosquitoes
	Aedes albopictus (%)	*Culex quinquefasciatus* (%)	*Armigeres subalbatus* (%)		
1 (01/02/20)	14 (48.35)	1 (3.45)	14 (48.27)	29 (100)	7,25 ± 7,80
2 (03/02/20)	14 (43.75)	0 (0.0)	18 (59.37)	32 (100)	8,00 ± 9,38
3 (10/02/20)	19 (52.77)	3 (8,33)	14 (38.88)	36 (100)	9,00 ± 8,91
4 (13/02/20)	6 (33.33)	1 (5.26)	11 (61.11)	18 (100)	4,50 ± 5,06
5 (17/02/20)	4 (28.61)	0 (0.0)	10 (71.43)	14 (100)	3,50 ± 4,73
6 (20/02/20)	8 (40)	0 (0.0)	12 (60)	20 (100)	5,00 ± 6
7 (02/03/20)	14 (29.87)	2 (4.25)	31 (65.96)	47 (100)	11,75 ± 14,24
8 (05/03/20)	6 (50)	0 (0.0)	6 (50)	12 (100)	3,00 ± 3,46
9 (07/03/20)	18 (35.34)	1 (1.96)	32 (62.75)	51 (100)	12,75 ± 15,26
10 (21/02/20)	8 (40)	0 (0.0)	12 (60)	20 (100)	5,00 ± 6
11 (02/02/20)	7 (21.21)	1 (3.03)	25 (75.75)	33 (100)	8,25 ± 11,58
12 (03/04/20)	16 (51.61)	0 (0.0)	15 (48.41)	31 (100)	7,75 ± 8,96
Amount (%)	**134 (39)**	**9 (2.6)**	**200 (58.4)**	**343**	
Average	**11.17 ± 5.20**	**0.75 ± 0.97**	**16.67 ± 8.33**		

Table 3. Density and percentage of each species of mosquito caught by sweep nets

Surveillance (date)	Number of mosquitoes caught				Number of Mosquito (%)	Average of Mosquito
	Aedes albopictus (%)	*Culex quinquefasciatus* (%)	*Armigeres subalbatus* (%)	*Armigeres foliatus* (%)		
1 (01/02/20)	0 (0.0)	1 (20)	4 (80)	0 (0.0)	5 (100)	1,25 ± 1,89
2 (03/02/20)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0,5 ± 1
3 (10/02/20)	1 (11.11)	2 (22.22)	5 (55.55)	1 (11.11)	9 (100)	2,25 ± 1,89
4 (13/02/20)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	1 (100)	0,25 ± 0,50
5 (17/02/20)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (100)	0
6 (20/02/20)	1 (20)	0 (0.0)	4 (80)	0 (0.0)	5 (100)	1,25 ± 1,89
7 (02/03/20)	3 (60)	0 (0.0)	2 (40)	0 (0.0)	5 (100)	1,25 ± 1,50
8 (05/03/20)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (100)	0
9 (07/03/20)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (100)	0
10 (21/02/20)	0 (0.0)	0 (0.0)	6 (100)	0 (0.0)	6 (100)	1,5 ± 3
11 (02/02/20)	0 (0.0)	0 (0.0)	5 (100)	0 (0.0)	5 (100)	1,25 ± 2,5
12 (03/04/20)	1 (14.35)	1 (14.35)	5 (71.43)	0 (0.0)	7 (100)	1,75 ± 2,22
Amount (%)	**6 (13.3)**	**6 (13.3)**	**32 (71)**	**1 (2.4)**	**45**	
Average	**0.92 ± 0.90**	**0.92 ± 0.80**	**4.92 ± 2.39**	**0.15 ± 0.29**		

using both methods of capture. This is likely caused by the behavior of the *Culex quinquefasciatus* that preferred living inside the house. Not only biting humans, female *Culex quinquefasciatus* mosquitoes also bite several types of animals, including frogs, pigs, horses, cows, sheep, dogs, and rabbits (Bhattacharya and Basu, 2016). This underpins the possibility that these three species are interested in sucking the blood of tarsiers in IPB PRC.

Relative abundance, frequency, and dominance rates of mosquitoes caught by light traps and sweep nets are presented in Table 4 and Table 5. *Armigeres subalbatus* mosquito was the most common with the highest relative abundance, namely 71.11% by the light trap method and 62.11% by the method. This species was also found regularly during the collection with the highest dominance value, 47.41% using the light trap method and 62.11% using the sweep net method. The Aedes albopictus also had a high relative abundance and dominance rate using the sweep net method, 41.61%.

With the light trap method, the Aedes albopictus and *Culex quinquefasciatus* had the same relative abundance and dominance rates, which are 13.33% and 4.44%. However, the *Culex quinquefasciatus* had the lowest relative abundance and dominance rate among the three species in the sweep net collection method, which are 2.80% and 1.40%. This might be caused by the limited breeding area and the behavior of the female mosquito that prefers human blood or is anthropophilic. The *Culex quinquefasciatus* also prefers doing activities and resting indoors, while the *Armigeres foliatus* has the lowest relative abundance and dominance rates, which are 2.22% and 0.19%.

An overview of the density of mosquitoes every two hours is presented in Figure 2. Of the three species caught, *Aedes albopictus* and *Armigeres subalbatus* mosquitoes are both always found in each collection period, regardless of the small amount. This could be caused by the Armigeres mosquito, which likes both animal and human blood (zoophilic-anthropophilic). This mosquito is also an exophagic mosquito, that prefers to do activities and rest outside rather than inside the house (Astuti and Marina, 2009). Different from the Aedes albopictus which according to (Soegijanto, 2006) is exophilic. Based on the data presented in Figure 2, the peak activity of the captured *Armigeres subalbatus* mosquito occurred at 6 pm to 8 am WIB while the peak activity of Aedes albopictus occurred at 4 am to 6 am (GMT+7).

Table 4. Relative abundance, frequency, and dominance rate of mosquitoes caught by light traps

Species	Relative Abundance (%)	Frequency	Dominance Rate (%)
Aedes albopictus	13.33	0.33	4.44
Culex quinquefasciatus	13.33	0.33	4.44
Armigeres subalbatus	71.11	0.67	47.41
Armigeres foliatus	2.22	0.08	0.19

Table 5. Relative abundance, frequency, and dominance rate of mosquitoes caught by sweep net

Species	Relative Abundance (%)	Frequency	Dominance Rate (%)
Aedes albopictus	41.61	1	41.61
Culex quinquefasciatus	2.80	0.5	1.40
Armigeres subalbatus	62.11	1	62.11

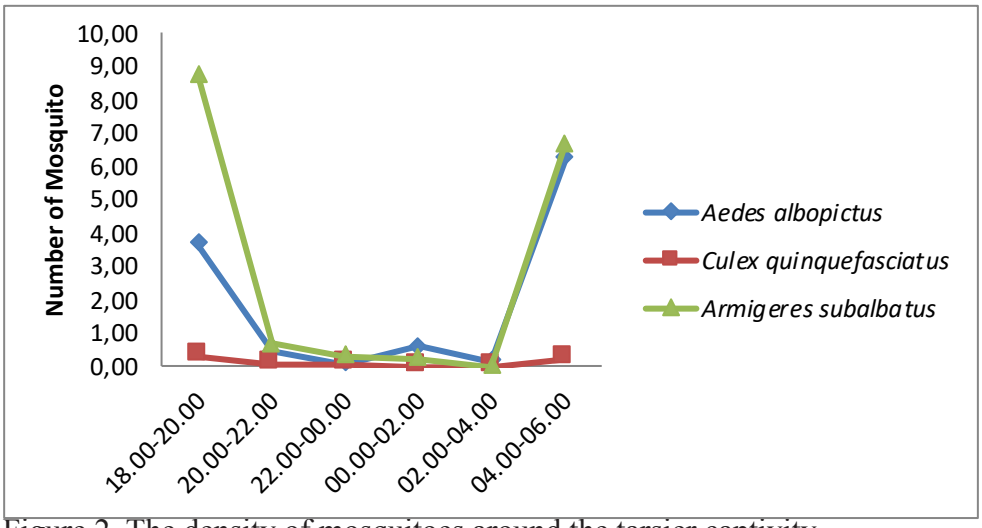

Figure 2. The density of mosquitoes around the tarsier captivity

Armigeres does the blood-sucking activity in the morning and late afternoon (crepuscular) (Astuti and Marina, 2009).This is also supported by the research of Pandian and Chandrashekaran in 1980 which showed that the peak of the fluctuation in the density of the Armigeres species occurred at 6 pm to 7 pm (GMT+7) due to Armigeres activity occurring before sunrise with exposure to the light above 17 lux and before sunset with exposure to light below 4 lux. Aedes albopictus was found mostly during the collection period from 4 am to 6 am and 6 pm to 8 pm (GMT+7). The Aedes albopictus sucks blood in the morning to evening, however, from observations done by Syahribulan in 2010 in Somba Opu sub-district, Makassar, South Sulawesi found Aedes aegypti and Aedes albopictus species to suck blood at night from 7 pm to 10 pm (GMT+8) (Syharibulan *et al.* 2012). Information about mosquito activity can be used in developing control strategies due to the risk of transmitting zoonotic diseases through mosquito vectors.

The results of the Dengue virus examination on samples consisting of *Aedes albopictus* and *Armigeres subalbatus* using the Reverse Transcriptase Real-time PCR method gave a negative result. Dengue virus serotypes 1, 2, 3, 4 were not found in isolated mosquito samples. The data showed the absence of Dengue virus serotypes 1, 2, 3, and 4 in both mosquito samples.

Until now, the *Aedes aegypti* and *Aedes albopictus* mosquitoes are known to be the main vectors of the Dengue virus, different than the mosquitoes from the genus Armigeres, especially *Armigeres subalbatus*, which are the main vectors of filarial worms including Brugia malayi, Brugia timori, and Wuchereria bancrofti which cause lymphatic filariasis or elephantiasis in humans (Mathers *et al.* 2007). A negative result can be caused by several factors. Tunissea *et al.* (2012) reported in their research that serotype 3 of Dengue virus RNA with an incubation period of 1-4 days in infectious mosquitoes could not be detected by RT-qPCR, positive results for Dengue-3 antigen could be seen at five days incubation. Yasmon *et al.* (2010) also stated that a negative result could be due to the number of virus particles being too low in the sample, so the negative result that was shown might be a false negative.

Based on the authors observation, the transmission of the dengue virus from primitive primates like tarsier has never been reported in scientific media. Until now, it is not known which specific primate species support the reservoir for sylvatic dengue virus (Vasikalis *et al.* 2011);

however, dengue virus was isolated directly from long-tailed monkeys (*Macaca fascicularis*) and leaf monkeys (*Presbytis obscura*) which were placed as sentinels in forest canopies (Rudnick, 1986). These primates did not show any clinical symptoms of DENV as experienced by humans. This supports a variety of Old-World Monkeys from Asia and Africa that are suspected of being the reservoir hosts to this virus (Vasilakis *et al.* 2011).

Transmission of the sylvatic dengue virus involves primates and Aedes mosquitoes that reside from the forest. Several primates, such as pigtailed monkeys (*Macaca nemestrina*), the leaf monkeys (*Presbytis cristata*), African green monkeys (*Chlorocebus sabaeus*), and one species of ape, the Bornean Orangutan (*Pongo pygmaeus*) (Wolfe *et al.* 2001) have also shown immunity to this virus although this immunity has not been definitively confirmed due to the cross-reaction of antibodies against several other viruses from the Flaviviridae family (Mansfield *et al.* 2011). This makes primates suspected of being involved in the global distribution of the dengue virus, both sylvatic and urban, which is transmitted through mosquitoes (Stabell *et al.* 2018). Although it can be isolated from primates, most of the sylvatic dengue viruses that have been isolated come directly from mosquitoes that come from the forest (Diallo *et al.* 2003). Dengue virus requires an incubation period of at least eight days in the mosquito's body before being transmitted to humans in a virulent form (Foote and Cooke 1959, Tunissea *et al.* 2012).

4. Discussion

The various types of mosquitoes found around the Tarsier captivity in IPB Primate Research Center were *Aedes albopictus*, *Culex quinquefasciatus*, *Armigeres subalbatus*, and *Armigeres foliatus*. The species most frequently found in each collection were *Armigeres subalbatus* and *Aedes albopictus*. The peak activity of the *Armigeres subalbatus* occurs from 6 pm to 8 pm (GMT+7), while *Aedes albopictus* occurs from 4 am to 6 am (GMT+7). The *Aedes albopictus* and *Armigeres subalbatus* mosquitoes that were tested for the presence of Dengue virus serotypes 1, 2, 3, and 4 using the Reverse Transcriptase Real-time Polymerase Chain Reaction (RT-qPCR) method gave a negative result. Although in the molecular analysis, there was no dengue virus in the sample detected, the presence of several types of mosquitoes that potentially act as Dengue virus vectors around the tarsier captivity in IPB PRC, which is also located near the housing area indicated the potential of mosquito-borne disease transmission in both tarsier and humans.

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References

Andini, W. R. 2008. Ektoparasit Pengganggu pada Orangutan (*Pongo pygmaeus*) di Habitat Ex-situ [Undergraduate Thesis]. Bogor (ID): Institut Pertanian Bogor.

Astuti, E. P., dan Marina, R. 2009. Ovoposisi dan perkembangan nyamuk Armigeres pada berbagai kontainer. Aspirator. 1(2):87-93.

Bhattacharya, S., Basu, P. 2016. The southern house mosquito, *Culex quinquefasciatus*: profile of a smart vector. Journal of Entomology and Zoology Studies. 4(2): 72-81.

Departemen Kesehatan RI. 2008a. Kunci Identifikasi Nyamuk Aedes. Jakarta (ID): Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan.

Departemen Kesehatan RI. 2008b. Kunci Identifikasi

Nyamuk Culex. Jakarta (ID): Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan.

Diallo, M., Ba, Y., Sall, A. A., Diop, O. M., Ndione, J. A., Mondo, M., Girault, L., Mathiot, C. 2003. Amplification of the sylvatic cycle of dengue virus type entomologic findings and epidemiologic considerations. Emerging Infectious Diseases. 9:362–367.

Foote, R. H., Cooke, D. R. 1959. Mosquito Transmitted Disease. Washington D.C (US): Government Printing Office.

Hadi, U. K., Koesharto, F. X. 2006. Hama Permukiman Indonesia: Pengenalan, Biologi&Pengendalian. Bogor (ID): IPB Press.

Harbach, R. 2008. Genus *Armigeres Theobald* 1901 [Internet]. [Downloaded 15 May 2020]. Available in: http://mosquito-taxonomic-inventory.info/genus-armigeres-theobald-1901.

Mansfield, K. L., Horton, D. L, Johnson, N., Li, L., Barrett, A. D., Smith, D. J., Galbraith, S. E., Solomon, T., Fooks, A. R.. 2011. Flavivirus-induced antibody cross-reactivity. Journal of General Virology. 92:2821–2829.

Mathers, C. D., Ezzati, M., Lopez, A.D. 2007. Measuring the burden of neglected tropical diseases: the global burden of disease framework. PLOS Neglected Tropical Disease. 1(2): 114.

Niebylski, M. L., Savage, H. M., Nasci, R. S., Craig, G. B. 1994. Blood of Ae. albopictus in the United States. Journal of The American Mosquito Control Association. 10 (3): 447-450.

Pandian, R. S., Candrashekaran, M. K. 1980. Rhythms in biting behaviour of a mosquito *Armigeres subalbatus*. Oecologia Journal. 47:89-95.

Ponlawat, A., Harrington, L. C. 2005. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. Journal of Medical Entomology. 42 (5): 844-9.

Rudnick, A.. 1986. Dengue Virus Ecology in Malaysia. Institute of Medical Research Malaysia. 23:51–152.

Sawyer, A. L. 2018. Dengue viruses cleave STING in humans but not in nonhuman primates, their presumed natural reservoir. eLife Research Article. DOI: https://doi.org/10.7554/eLife.31919.

Seema, Jain, S. K. 2005. Molecular mechanism of pathogenesis of dengue virus: entry and fusion with target cell. Indian Journal of Clinical Biochemistry. 20(2) 92-103.

Soegijanto, S. 2006. Demam Berdarah Dengue Edisi ke-2. Surabaya (ID): Universitas Airlangga Press.

Stabell, A. C., Meyerson, N. R., Gullberg, R. C., Gilchrist, A. R., Webb, K. R., William, M. O., Perera, R., Sawyer, A. L. . 2018. Dengue viruses cleave STING in humans but not in nonhuman primates, their presumed natural reservoir. eLife Research Article. DOI: https://doi.org/10.7554/eLife.31919

Sukendra D. M., Shidqon, M. A. 2016. Gambaran perilaku menggigit nyamuk *Culex* sp. sebagai vektor penyakit filariasis Wuchereria bancrofti. Jurnal Pena Media. 6 (1):19-33.

Supriatna J., Manansang J., Tumbelaka L., Andayani N., *et al.* 2001. Conservation Assessment and Management Plan for Primates of Indonesia: Final Report.Conservation Breeding Specialist Group (SSC/IUCN). California (USA): Apple Valley.

Syahribulan, Biu, F. M., Hassan, M. S. 2012. Waktu aktivitas menghisap darah *Aedes aegypti* dan *Aedes albopictus* di Desa Pa'lanassang Kelurahan Barombong Makassar Sulawesi Selatan. Jurnal Ekologi Kesehatan. 11(4): 306-314.

Tunissea, A., Widiastuti, D., Wijayanti, N. 2012. Uji Teknik RT-PCR untuk pemeriksaan virus Dengue-3 pada nyamuk *Aedes aegypti* yang diinfeksi secara intrathorakal. Widyariset. 15 (2).

Vasilakis, N., Cardosa, J., Hanley, K. A., Holmes, E. C., Weaver, S. C. 2011. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. Nature Reviews Microbiology 9: 532–541.

Wolfe, N. D., Kilbourn, A. M., Karesh, W. B., Rahman, H. A., Bosi, E. J., Cropp, B. C., Andau, M., Spielman, A., Gubler, D. J. 2001. Sylvatic transmission of arboviruses among Bornean orangutans. The American Journal of Tropical Medicine and Hygiene 64:310–316.

World Health Organization. 2005. Dengue: Guidelines for Laboratory and Field Testing of Mosquito Larvicides. Geneva (SZ).

Yasmon, A., Fatmawati, Ibrahim, F., Bela, B. 2010. A second generation of RT-PCR assay for detection of human immunodeficiency virus type 1 (HIV-1) infection. Medical Journal of Indonesia. (19): 154-7.

Zompi S., Santich B.H., Beatty P. R., Harris E. 2012. Protection from secondary dengue virus infection in a mouse model reveals the role of serotype cross-reactive B and T cells. The Journal of Immunology. 188 (1): 404-416.