



INCRIMINATION AND BLOOD FEEDING ON MOLECULAR MALARIA VECTORS IN PESAWARAN DISTRICT, LAMPUNG PROVINCE

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Abstract

There are 10 species of Anopheles spp. suspected as vectors of malaria in Lampung Province, namely An. vagus, An. sondaicus, An. barbirotris, An. acconitus, An. indefinite, An. kochi, An. subpictus, An. tessellatus, An. minimus, An. maculatus. Vector confirmation by PCR method is more effective as a tool to determine malaria vectors. Another thing that can support Anopheles spp mosquitoes to become malaria vectors is the preference for blood feed from the Anopheles. The female Anopheles mosquito feeds on human blood (anthropophilic) or livestock blood (zoophilic) at night. This study aims to confirm the presence of plasmodium in Anopheles spp species, determine Anopheles spp blood feed preferences and determine the distribution map of Anopheles spp. in Pesawaran Regency, Lampung Province. A sampling of Anopheles spp. carried out in 5 Coastal Villages in the working area of the Hanura Health Center, Tanjung Pandan District, Pesawaran Regency, Lampung Province. The Nested PCR test was carried out at the Laboratory of the Jakarta Environmental Health Engineering Center on female Anopheles spp. mosquitoes caught during night fishing. The results of this study confirmed that no Plasmodium was found in Anopheles sondaicus in the head and thorax, the blood in the stomach found 6 mosquitoes containing human blood, Anopheles sondaicus was most dominantly caught in 5 villages in Teluk Pandan District, Pesawaran Regency, Lampung Provinces. Density level An. sondaicus in Batu Meyan Village with MLR 4, Hanura Village MLR 3.75, Sidodadi Village MLR 3, Lempasing Village MLR 0.25 and in Tanjung Jaya Village with MLR 0. The activity of sucking blood from An. sondaicus began to be caught at 21.00 until 03.00 with the highest peak at 11.00-02.00 WIB (Western Indonesia Time).

Keywords: *Incrimination, blood feeding, map distribution, anopheles spp*

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INTRODUCTION

In accordance with the strategic plan of the malaria program until 2020, Lampung Province will achieve malaria-free. The achievements of the malaria program in 2018 are from 15 districts/cities in Lampung Province that have received malaria-free certificates as many as 10 districts (6.667%) namely Metro City, Pringsewu Regency, Tulang Bawang Regency, West Tulang Bawang Regency, Way Kanan Regency, East Lampung Regency, Central Lampung Regency, West Lampung Regency, North Lampung Regency, and Tanggamus Regency. When compared to 2018 there was a decrease in cases 2018. The Annual Parasite Incidence (API) figure was 0.37/1000 population, while in 2017 the API rate was 0.54/1,000 population. As many as 75% of malaria cases in Lampung Province were found in Pesawaran District(Lampung Health Office, 2018).

Anopheles spp. as a vector of malaria in Indonesia, there are about 80 species, while there are 20 species confirmed as malaria vectors (Elyazar et al., 2013). Data from Lampung Provincial Health Office(2016)There are 10 species of Anopheles suspected of being vectors, namely An.vagus, An.sundaicus, An.barbirotris, An.acconitus, An.indefinitus, An.kochi, An.subpictus, An.tesselatus, An. minimus, An. maculatus. Anopheles sunaicus was confirmed as a vector on the coast of Lampung using the ELISA technique, while the Nested PCR technique has never been done.

Vector confirmation by salivary gland surgery using a microscope is still the gold standard for the determination of Anopheles spp. as a malaria vector although the sensitivity of the ELISA and PCR tests is higher. Salivary gland surgery requires special skills, so the risk of errors during surgery is very high.ResearchBass et al., (2008) said the PCR method is more effective as a tool to determine malaria vectors, because it can detect 10 sporozoites in the salivary glands, compared to

using the CS antigen method which requires 200-400 sporozoites. According to researchIndriyati et al., (2017)found 3 species of Anopheles spp. positive for Plasmodium vivax using the Nested PCR method in Kota Baru, South Kalimantan Province.

Some things that can support the Anopheles spp can be a vector of malaria, namely the presence/abundance, preference for blood feed, and the age of the mosquito from the Anopheles. Anopheles females only mate once during their life, for egg growth requires blood, generally female Anopheles mosquitoes suck human blood (anthropophilic) or livestock blood (zoophilic) at night(Ministry of Health RI, 2013). Sugiarto et al., 2016 in his research stated that Anopheles vagus mosquitoes were caught in insider bait, outsider bait, and resting in the house with an abundant population.

Based on this description, this research aims to confirm Anopheles spp. as a malaria vector using molecular techniques in Pesawaran Regency, Lampung Province, knowing the blood feed preferences of Anopheles spp. in Pesawaran Regency, Lampung Province, and know the distribution map of Anopheles spp. in Pesawaran Regency, Lampung Province.

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METHOD

Research Design

This research is a descriptive study to determine the Anopheles spp. as a malaria vector in Pesawaran Regency, Lampung Province in May – November 2019. Mosquito capture, species identification, ovarian surgery, thorax and abdomen cutting, blood-feeding test, and vector determination using the Nested PCR method. A sampling of Anopheles spp. carried out in 5 (five) Coastal Villages in the working area of the Hanura Health Center, Tanjung Pandan District, Pesawaran Regency, Lampung Province, namely Sukaraja Lempasing, Hanura, Sidodadi, Tanjung Jaya and Batu Menyan Villages (Figure 1).





Figure 1. Map of malaria villages in Pesawaran District

Implementation of Nested PCR test to identify female *Anopheles* spp. caught at night and in a parous condition was carried out in the laboratory of the Jakarta Environmental Health Engineering Center. The independent variable in this study was the *Anopheles* spp. caught during nocturnal capture, the thorax, and abdomen of mosquitoes. While the dependent variable in this study was the mosquito species *Anopheles* spp. and blood feed preferences.

Plasmodium Identification

Examination of *Plasmodium* in *Anopheles* spp. mosquitoes using the Nested PCR technique with targets on the genus *Plasmodium* (rPLU5 and rPLU6), for each *Plasmodium* species, namely *P. viva* (rVIV1 and rVIV2), *P. falciparum* (rFAL1 and rFAL2), *P. malariae* (rMAL1 and rMAL2), *P. ovale* (rOVA1 and rOVA4) (Indriyati et al., 2017), while for *P. knowlesi* (Kn1f and Kn3r). Preference for sucking blood is measured by the number of mosquitoes that suck human blood divided by

the number of mosquitoes that suck blood, a ratio measuring scale.

Research Tools and Materials

Catching mosquitoes at night. Materials: Chloroform, 10% sugar water, NaCl liquid. Tools: aspirator, paper cup, gauze, rubber band, compound microscope, stereo microscope, cable roll, sexy needle, collection box, collection needle, petri dish, flashlight, cotton, rag, alarm, cardboard, collection tube, arrest form.

Blood feed test. Material: *Anopheles* spp. mosquito, circular filter paper, chlorophore. Tools: object glass, hot needle, silica gel, paper glass, gauze, rubber band, cotton, label.



Mosquito testing by PCR

Plasmodium DNA isolation from the head-thorax of the mosquito. Ingredients: Purelink Genomic DNA Kit (invitrogen), RNA/DNase Free Water, PBS, Absolute Alcohol (96%), Alcohol 70%. Equipment: Micro pipette (1000 µl, 200 µl, 100 µl, 20 µl, 10 µl), Vortex, Refrigerated Centrifuge, BSC-Class II, Spin column, Collection tube, Aerosol resistant bio clean tips (1000 µl, 200 µl, 100 µl, 20 µl, 10 µl).

Nested Plasmodium sp. PCR. Tools: Micro pipette (1000 µl, 200 µl, 100 µl, 20 µl, 10 µl), Vortex, microcentrifuge, PCR cabinet, thermal Cycler, microcentrifuge tube 1.5 ml, 20µ L, Cooler rack. Ingredients: Qiagen Top Taq Master Mix, Nuclease free water and Primer. The primers used in the study are shown in table 1.

Table 1 Plasmodium Primary Product

Species	Primary	Sequence (5-3)	PCR Product Size (BP)
<i>Plasmodium sp</i>	rPLU5	CCTGTTGTTGCCTTAAACTTC	1131bp
	rPLU6	TTAAAATTGTTGCAGTAAAAACG	1131bp
<i>P. vivax</i>	rVIV6	TAACGCCGTTAGCTAGATCCACAAGG	205bp
	rVIV7	CTGTAGTATTCAAAACGCGCAATGCTG	205bp
<i>P. falciparum</i>	rFAL1	TTAAACTGGTTTTGGAAAACCAATATATT	206bp
	rFAL2	ACACAATGAACTCAATCATGACTACCCGTC	206bp
<i>P. ovale</i>	rOVA1	ATCTCTTTTGCTATTTTTAGTATTGGAGA	419bp
	rOVA4	ACTGAAGGAAGCAATCTAAGAAAATTT	419bp
<i>P. malariae</i>	rMAL1	ATAACATAGTTGTACGTTAAGAATAACCGC	145bp
	rMAL2	AAAATTCCTATGCATAAAAAATTATACAAA	145bp
<i>P. knowlesi</i>	Kn1F	CTCAACACGGGAAAACACTACTAGTTTA	279bp
	Kn3r	GTATTATTAGGTACAAGGTAGCAGTATGC	279bp

Electrophoresis. Ingredients: 100 bp DNA ladder pilot gel (Qiagen, Cat: 239045), 5x DNA loading dye pilot gel (Qiagen, paint: 239901), Small Fragment agarose pilot gel (Qiagen, Cat: 129832), 10x TE buffer, Sybr Safe, and aqua dest. Tools: power supply, electrophoresis tank + accessories, microwave, analytical balance, beaker glass 100 ml, 1.5 ml microcentrifuge tube, micropipette 100 µl, 10 µl, aerosol resistant bio clean tips 100 µl, 10 µl.

Blood feed test. Ingredients: Using QiaAmp DNA mini kit, Absolute alcohol (Ethanol 96%), ATL, AW1, AW2, Al. Tools: Micropipette (1000 µl, 200 µl, 100 µl, 20 µl, 10 µl), Vortex, Refrigerated Centrifuge, BSC-Class II, water bath, Spin column, Collection tube, Aerosol resistant bio clean tips (1000 µl,200 µl, 100 µl, 20 µl, 10 µl).

RESULT

1. Molecular examination results/vector incrimination PCR

Mosquito Anopheles spp. which was tested by PCR as many as 99 mosquitoes out of 229 caught and only Anopheles sundaicus was tested. The results of PCR examination using primers rVIV1, rVIV2, and P. vivax Positive Control to detect P. vivax in Anopheles spp. not found positive for Plasmodium vivax in An mosquitoes. sundaicus. Samples were pooled at no. 1-12, 13-19 from a total of 99 An. sundaicus which was tested (Figure 2).





Figure 2. PCR results on *An. sundaicus* samples in pooling 1-13, 13-19, K-: negative control, K+: *P. vivax*, and leader 100 DNA markers

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The results of PCR examination using primers rFAL1, rFAL2, and *P. falciparum* Positive Control to detect *P. falciparum* in *Anopheles* spp. not found positive for *Plasmodium falciparum* in *An. sundaicus*. Samples were pooled at no. 1-12, 13-19 from a total of 99 *An. sundaicus* which was tested (Figure 3).

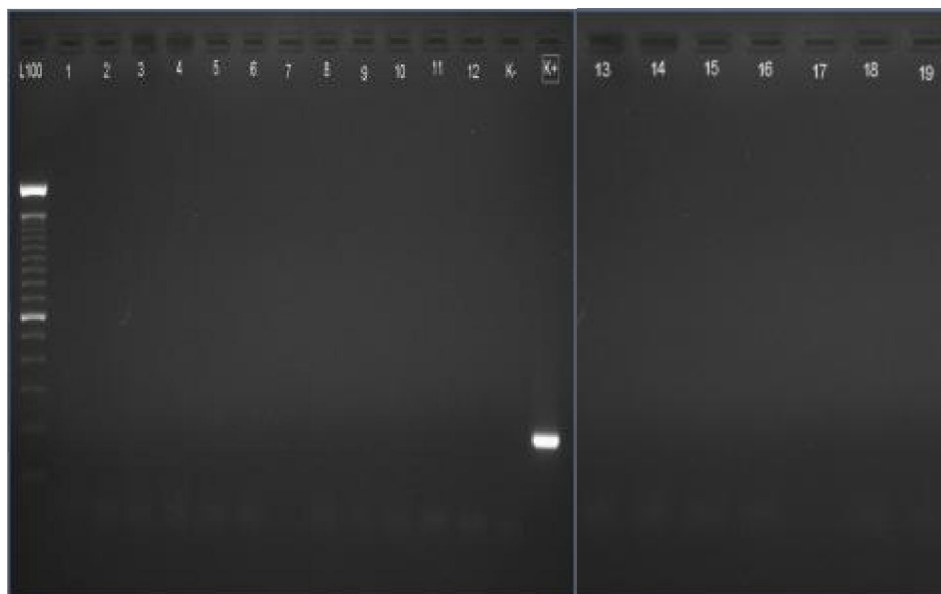


Figure 3. PCR results on *An. sundaicus* samples in pooling 1-13, 13-19, K-: negative control, K+: *P. falciparum*, and leader 100 DNA markers

The results of PCR examination using rOVA1 primer, rOVA4 to detect *P. ovale* in *Anopheles* spp. not found positive for *Plasmodium ovale* in *An. sundaicus*. Samples are pooled at numbers 1-12 from a total of 99 *An. sundaicus* which was tested (Figure 4).

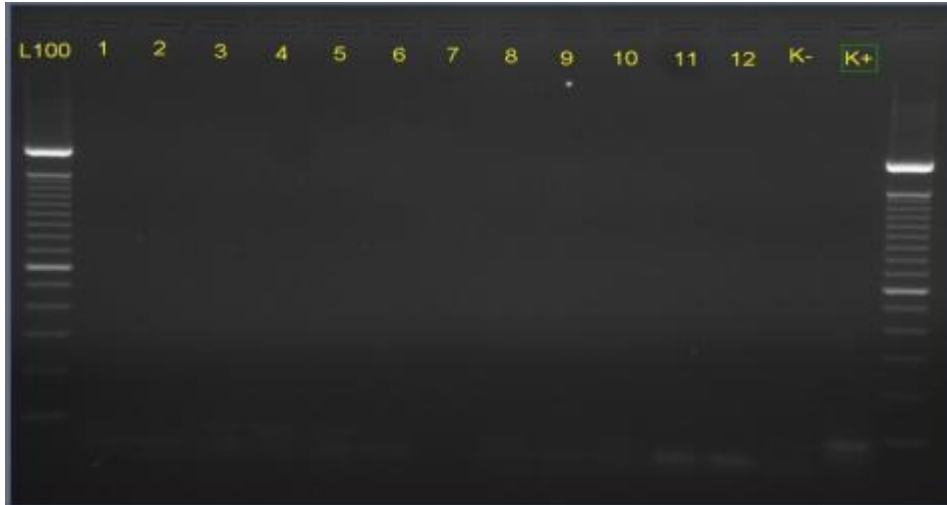


Figure 4 PCR results on *An. sundaicus* samples in pooling 1-12, K-: negative control, and leader 100 DNA markers

3801

The results of PCR examination using primers rMAL1, rMAL2 and *P. malariae* Positive Control to detect *P. malariae* in *Anopheles* spp. not found positive for *Plasmodium malariae* in *An. sundaicus* (Figure 5).



Figure 5 PCR results on *An. sundaicus* samples in pooling 1-13, K-: negative control, and leader 100 DNA markers

The results of PCR examination using primers Kn1F, Kn3r, and Positive Control *P. knowlesi* to detect *P. knowlesi* in *Anopheles* spp. not found positive for *Plasmodium knowlesi* in *An. sundaicus*.

sundaicus. The sample is pooled at numbers 1-19 from a total of 99 *An. sundaicus* which was tested (Figure 6).



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Figure 6 PCR results on *An. sundaicus* samples in pooling 1-13, 13-19, K-: negative control, and leader 100 DNA markers

2. The results of molecular examination/PCR blood feeding *Anopheles* spp.

Examination of blood feed on *Anopheles* spp. in Teluk Pandan District, Pesawaran Regency, Lampung Province by using primers Pig573F, Human741F, Dog368F, Cow121F, UNFOR403, UNREV1025.

Table 1. Human Blood Index (HBI)

No	Species	Number of Species	Positive Results		HBI %
			Animal	Man	
1	<i>an. sundaicus</i>	99	1	6	6.061

The results of the PCR test showed that *An. sundaicus* sucked the blood of 6 humans (334bp) and 1 animal (534bp), with a percentage of 6.061%. The picture below is a PCR result on a positive mosquito containing animal and human blood.

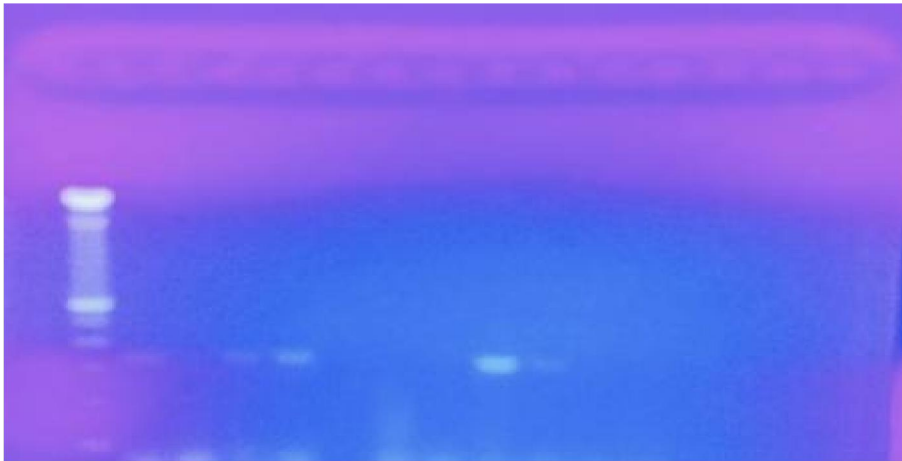
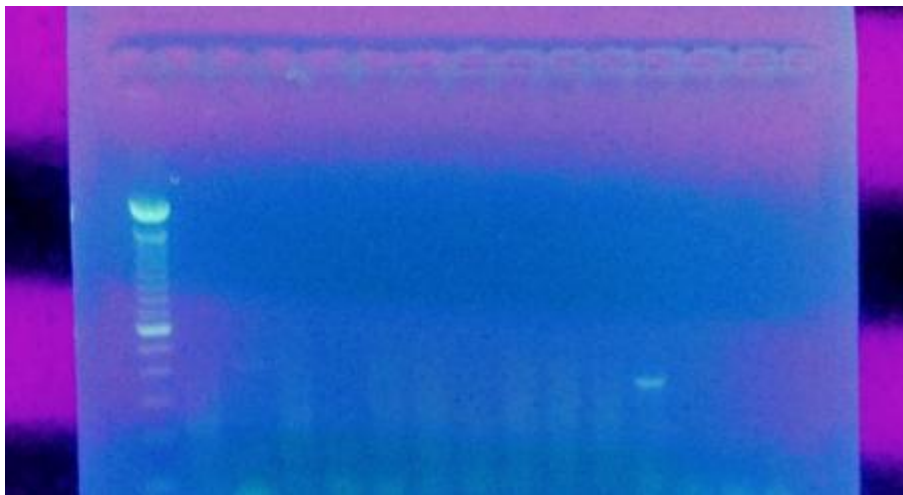


Figure 7. Results of Blood Feeding *An. sondaicus* in Lempasing Village, Pesawaran Regency, samples no. 1, 3, 4, 8, and 9 were positive using the primer above.



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Figure 8 Results of Blood Feeding *An. sondaicus* in Hanura and Batumeyan Villages, Pesawaran Regency, samples no. 14 and 23 were positive using the primer above.

3. Distribution of *Anopheles* spp. in Pesawaran Regency, Lampung Province

a. The catch of *Anopheles* spp. in Teluk Pandan District, Pesawaran Regency, Lampung Province

Catching *Anopheles* spp. in Teluk Pandan District, it was carried out in 5 villages namely Lempasing Village, Hanura Village, Sidodadi Village, Tanjung Jaya Village, and Batu Meyan Village with the results shown in the table below.

Table 2. Catching results of Anopheles mosquitoes per species

Species	Catching Method				Total	
	Insider Feed	Outsider Bait	Wall	Cage	N	%
<i>an. sundaicus</i>	44	60	31	92	227	99.12
<i>an. subpictus</i>	0	0	1	0	1	0.44
<i>an. vagus</i>	0	0	0	1	1	0.44
Total	44	60	32	93	229	100.00

Table 3. Catching results of Anopheles mosquitoes per species

Species	Catching Method				Total	
	Insider Feed	Outsider Bait	Wall	Cage	N	%
Lempasing						
<i>an. Sundaicus</i>	1	15	7	3	26	96.30
<i>an. Subpictus</i>	0	0	1	0	1	3.70
Total	1	15	8	3	27	100.00
Hanura						
<i>an. Sundaicus</i>	15	19	15	58	107	99.07
<i>an. Vagus</i>	0	0	0	1	1	0.93
Total	15	19	15	59	108	100.00
Sidodadi						
<i>an. Sundaicus</i>	12	10	2	5	29	100.00
Total	12	10	2	5	29	100.00
Tanjung Jaya						
<i>an. sundaicus</i>	0	1	1	2	4	100.00
Total	0	1	1	2	4	100.00
Batu Meyan						
<i>an. sundaicus</i>	16	15	6	24	61	100.00
Total	16	15	6	24	61	100.00



Table 4. Distribution of Anopheles mosquitoes per species

Species	VILLAGE					TOTAL	
	Lempasing	Hanura	Sidodadi	Tanjung Jaya	Batu Meyan	N	%
<i>an. sundaicus</i>	26	107	29	4	61	227	99.12
<i>an. subpictus</i>	1	0	0	0	0	1	0.44
<i>an. vagus</i>	0	1	0	0	0	1	0.44
TOTAL	27	108	29	4	61	229	100

b. Density of Anopheles spp. in Teluk Pandan District, Pesawaran Regency, Lampung Province

The density of Anopheles spp. was calculated based on the MBR formula from WHO but modified to MLR so that the only mosquitoes that were caught were those that landed, not the ones that bitten. The results of the density of Anopheles spp. in each village can be seen in the table below.

Table 5. Density of Anopheles spp. caught

Species	UOD		UOL	
	Quantity	MLR	Quantity	MLR
Lempasing				
<i>an. sundaicus</i>	1	0.25	15	3.75
Hanura				
<i>an. sundaicus</i>	15	3.75	19	4.75
Sidodadi				
<i>an. sundaicus</i>	12	3	10	2.5
Tanjung Jaya				
<i>an. sundaicus</i>	0	0	1	0.25
Batu Meyan				
<i>an. sundaicus</i>	16	4	15	3.75

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DISCUSSION

1. Molecular assay/vector incrimination PCR

Anopheles sundaicus based on several studies in Lampung Province has been declared as a vector of malaria using the ELISA method but in this study 99 An. sundaicus did not find Plasmodium in the head and thorax using Nested PCR examination. In contrast to this study, An. sundaicus confirmed as malaria vector in the study Sudomo, Susanti, (2000) with a sporozoite index of 0.41%. (Sugiarto et al, 2016) An. sundaicus in Sungai Mosquito

Village, Sebatik Archipelago, North Kalimantan Province which was caught in foreign capture was confirmed to contain Plasmodium falciparum using the ELISA method.

Anopheles sundaicus in Teluk Pandan District in this study caught in various methods so that this can be a risk of transmission in the area, although in this study no Plasmodium was found in these mosquitoes.

2. Molecular examination/PCR blood feeding Anopheles spp.



Anopheles sundaicus In Teluk Pandan Subdistrict, 99 mosquitoes were caught on bait outsiders and baited insiders, all the blood in their stomachs was tested by PCR to determine blood feed. The results showed that 6 mosquitoes were detected to contain human blood (334 bp) in samples numbered 1,3,4,8,9, 14 and 23, while one mosquito was detected to contain pig blood (453bp), in line with this study Sukowati, (2009) *An. sundaicus* in Lampung Province is anthropophilic. Other studies mention *An. sundaicus* was also caught on the UOL and UOD method(Sugiarto et al., 2016).

3. Distribution of *Anopheles* spp. in Pesawaran Regency, Lampung Province

a. Arrest of *Anopheles* spp. in Teluk Pandan District, Pesawaran Regency, Lampung Province

Catching *Anopheles* spp. in Teluk Pandan District, Pesawaran Regency, Lampung Province in 2019 carried out in 5 villages namely Lempasing Village, Hanura Village, Sidodadi Village, Batu Meyan Village, Tanjung Jaya Village. The results of the capture showed that 3 species were caught, namely *An. sundaicus*, *An. subpictus* and *An. vagus* with a total of 229 mosquitoes. *An. sundaicus* was the most dominant caught with 227 mosquitoes.

Teluk Pandan District is a coastal area in Lampung Province, in line with the results of catching *Anopheles* mosquitoes in that area, the species caught, namely *An. sundaicus*, *An. subpictus* and *An.vagus*.According to research(Nalim et al., 2000) *An. sundaicus* was confirmed to contain sporozoites from malaria with a sporozoite rate of 0.41% in Padang Cermin District. The location of Teluk Pandan District is adjacent to Padang Cermin District so that the presence of *Anopheles* species in the two sub-districts is not much different. Other studies that show *An. sundaicus* as a vector found in research in the coastal area of Sungai Nyamuk Village, Sebatik Islands, North Kalimantan, was positive for *Plasmodium falciparum*(Sugiarto et al., 2016). Another study in Bayah District *An. sundaicus* was caught

dominantly in coastal areas as many as 213 mosquitoes. In contrast to other studies in the coastal area of Sukarame Village, Carita District, Pandeglang Regency, *An. vagus* caught the most dominant as many as 704 mosquitoes with various catching methods (Astuti, 2016).

The female *Anopheles* mosquito bites humans and other animals for egg development and is active foraging at night from 18.00 to 06.00. *An. sundaicus* is an *Anopheles* mosquito that breeds in coastal areas such as fish ponds, shrimp ponds, lagoons, and others that are abandoned. Abandoned shrimp and fish ponds can be used as breeding grounds for *An. sundaicus* and *An. Subpictus* (Munif, 2009).

According to research Rosa, (2009) *An. sundaicus* caught mostly in the coastal area of Puri Gading, Sukamaju Village, Teluk Betung Barat District, Lampung Province, while *An. subpictus* is not dominant.

Different from other research Kazwaini (2015)in the coastal area of *An. vagus* and *An. subpictus* caught was most dominant in malaria endemic areas, Central Lombok Regency, NTB Province.



b. Density of Anopheles spp. in Teluk Pandan District, Pesawaran Regency, Lampung Province

The density of Anopheles spp. in each area is different, this is because it is influenced by environmental factors such as breeding sites, the presence of predators, and others.

Density level An. sondaicus as the most common species found in 5 villages where the catch was carried out in Teluk Pandan District was different per night, the highest was in Batu Meyan Village with an MLR 4 value, Hanaura Village MLR 3.75, Sidodadi Village MLR 3, Lempasing Village MLR 0.25 and the lowest is Tanjung Jaya Village with MLR 0.

The activity of sucking blood from An. sondaicus in 5 villages in Teluk Pandan District began to be caught at 21.00 until 03.00 with the highest peak at 11.00-02.00. inline with research (Maekawa, 2009). Other studies mention An. sondaicus began to be caught at 24.00-04.00 in the Padang Cermin area, Lampung Province with an MBR value of 6.3 to 101.1(Sukowati, 2009).

CONCLUSION

The results of this study confirmed that Anopheles sondaicus did not find plasmodium in the head and thorax, the blood in the stomach found 6 mosquitoes containing human blood, Anopheles sondaicus was most dominantly caught in 5 villages in Teluk Pandan District, Pesawaran Regency, Lampung Province. Density level An. sondaicus in Batu Meyan Village with MLR 4, Hanura Village MLR 3.75, Sidodadi Village MLR 3, Lempasing Village MLR 0.25 and in Tanjung Jaya Village with MLR 0. The activity of sucking blood from An. sondaicus began to be caught at 21.00 until 03.00 with the highest peak at 11.00-02.00 WIB (Western Indonesia Time).

RECOMMENDATION

Anopheles sondaicus was confirmed as a vector of malaria in Lampung Province. Proof through various methods so that early vigilance

is needed against the transmission of malaria. Malaria vector control in Teluk Pandan District, Pesawaran Regency should involve other sectors so that integrated vector control can be carried out.

THANK-YOU NOTE

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