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Abundance of mosquito species (diptera: culicidae) as vector of the Japanese encephalitis disease in the pig sties in north Sulawesi, Indonesia

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Abstract
The abundance of mosquito species in 4 locations of pig farms with different altitudes in North Sulawesi. This study has the objectives to measure the abundance of mosquito species suspected having a role as vector JE in the pig sties of the 4 different farm locations with different altitudes in North Sulawesi. The result of the research indicates that the differences in the altitudes of the pig farm locations do not affect the number and abundance of the mosquitoes caught, instead it increases availability of an ideal habitat for the breeding place of mosquitoes such as: wet rice paddies and ponds. This research also indicates that the *Cx. vishnui* and *Cx. gelidus* mosquitoes may also have roles as vector for the spread of the JE disease to the pigs. It was found that the highest abundance of *Cx. vishnui* was in Lemoh (24.32%), in Tara-tara (24.75%) and in Talikuran (25.90%), whereas in the Kalasey farms the highest abundance was *Cx. gelidus* (25%).

Keywords: Mosquito, abundance, pig farm, cage location, JE, North Sulawesi

1. Introduction
Vector is defined as arthropods that can spread, transfer and/or become a source of disease infection towards humans. Mosquitoes are one of the arthropod groups that become a vector of various diseases [1].

Disease groups originating from mosquitoes are still a crucial health problem in Indonesia. These diseases are: malaria, Dengue Hemorrhagic Fever (DHF) and Filariasis. The types of mosquitoes that become the main vector are *Aedes* sp., *Culex* sp., *Anopheles* sp. and *Mansonia* sp.

Mosquitoes are included in the Diptera order of the Culicidae family, with 3 subfamilies namely: Toxorhynchites (*Toxorhynchites*), Culicinae (*Aedes*, *Culex*, *Mansonia*, *Armigeres*) and Anophelinae (*Anopheles*). There are approximately 3200 mosquitoes from 39 genus known throughout the world. Mosquitoes of the genus *Anopheles, Culex, Aedes, Mansonia, Armigeres*, *Haemagogus, Sebethes, Culiseta* and *Psorophora* suck human blood and function as a disease vector [8]. A number of mosquito types can be found everywhere such as the *Culex quinquefasciatus* and *Aedes aegypti* hence are cosmopolitan. A number of mosquito types as vector or main transmitter of arbovirus disease such as the Dengue Hemorrhagic Fever (DHF), chikungunya, yellow fever, Japanese Encephalitis as well as other diseases caused by nematodes, for example filariasis and diseases caused by blood protozoa such as malaria [16].

The Japanese Encephalitis (JE) disease is a viral zoonotic transmitted by mosquitoes. This disease is caused by arbovirus (*Arthropod Borne Virus*) from the Flavivirus family that attacks the central nervous system (CNS). In nature, this virus is found to be sustainable living in wild poultry, such as cranes and other animals, especially in pigs. In humans, the *Case Fatality Rate* of this disease is about 20%-40% [3].

The JE disease agent can be transmitted through infected mosquito bites. The pigs infected by this disease will spread throughout the whole body through the blood circulation (viremia) at high levels and in a relatively long time [6]. Therefore pigs are considered an essential animal reservoir (Amplify Host) in the spread of this virus, whereas humans are dead-ends for the

infection of the JE virus. Several countries indicate that those functioning as vector for the transmission of the JE virus are types of mosquitoes such as: *Culex tritaeniorhynchus*, *Cx. fuscocephala*, *Cx. gelidus* and *Cx. quinquefasciatus*. These mosquitoes are mostly found spread throughout Asia, such as: Japan, Korea, China, India, Thailand, Philippines, Malaysia, Vietnam and Taiwan, including Indonesia [2, 4].

2. Method of Research

This research was initiated by determining 4 pig farm locations in different altitudes, namely: Farm I with an altitude of 0 – 200 m above sea level; farm II with an altitude of >200 – 400 above sea level; farm III with an altitude of >400 – 600 above sea level; and farm IV with an altitude of >600 – 800 above sea level. The mosquito catching is done in each pig farm location that has been determined. This research was carried in November 2015 and January– March 2016.

2.1 Mosquito Vector Observation

The observation has the objectives to measure the mosquito species abundance in 4 farm locations with different altitudes. Other supporting factors such as farm location, temperature, and moisture during the research, as well as the weather, are recorded in the observation.

The vector mosquito catching was conducted using 2 units of light traps with ultra violet lamps. The catching was executed for 12 hours, from 06:00pm-06.00am. Every two hours the mosquito trap shade is removed and replaced with empty shades. The mosquitoes that enter the trap were killed using chloroform and transferred to plastic bottles labeled with the time and location of the catch.

2.2 Mosquito Species Identification

The mosquitoes caught were taken to the Entomology and Plant Pest Laboratorium at the Faculty of Agriculture of Sam Ratulangi University for identification. Each species were counted based on the location of the farm and the time of capture and identification was done according to the keys given by Thielman and Hunter, 2007 [17]; J.I. Glick, 1992 [5]; Reuben *et al*., 1994 [14]; Rattanarithikul, 1982 [13]; Peyton *et al*., 1966 [12].

2.3 Data Analysis

After the identification, an analysis was performed on the abundance using the measurement of Abundance (%). The Abundance (%) is the number of individual of each species toward the total number of individuals caught during the research. There are five categories of Abundance (Abd), namely: (1) very low Abd= < 1%; (2) low Abd= 1%-10%; (3) medium Abd = > 10%-20%; (4) high Abd = > 20%-30% and (5) very high Abd = > 30%.

3. Results and Discussion

The study is done in 4 locations pigsty: (1) Farm I in Kalasey Village, Mandolang Sub-district, Minahasa Regency, with an altitude of 12 m above sea level, at the ordinates of 10,27'10.332" North Latitude and 1240,46'10.5132" East Longitude; (2) Farm II in Lemoh Village, Tombariri Sub-district, Minahasa Regency, with an altitude of 249 m above sea level, at the ordinates of 10,27'57.6984" North Latitude and 1240,41'41.8416" East Longitude; (3) Farm III in Tara-Tara Village, West Tomohon Sub-district, Tomohon City, with an altitude of 576 m above sea level, at the ordinates of 10,18'31.0716" North Latitude and 1240,47'16.7496" East Longitude; and (4) Farm IV in Talikuran Village, Sonder Sub-district, Minahasa Regency, with an altitude of 616 m above sea level, at the ordinates of 10,15'55.8648" North Latitue and 1240,46'20.316" East Longitude.

The locations of pig farms in North Sulawesi are spread throughout several regencies/cities, illustrates the geographical area conditions that are mountainous. In general, the farm areas are of moderate conditions with temperatures between 20 ^{0}C – 30 ^{0}C and moisture between 59% - 66.5%. These conditions are ideal for the development of mosquitoes as vector of disease infection. The pig farm maintenance system still dominated by the community farming kept close to the settlement areas highly support the transmission cycle of the vector mosquito, including the Japanese Encephalitis (JE) disease agent. The habitat or place of mosquito breeding or places for the mosquitoes to rest, such as: wet paddies, ponds, contained water, water ducts, moist cage corners and animal waste, are ideal conditions.

Table 1: The Percentage abundance of adult female mosquitoes caught in farm I, the Kalasey Village Farm

Type of Mosquito	Kalasey Farm												Total	Abd (%)
	18.00-20.00	Abd (%)	20.00-22.00	Abd (%)	22.00-24.00	Abd (%)	24.00-02.00	Abd (%)	02.00-04.00	Abd (%)	04.00-06.00	Abd (%)		
Cx. quinquefasciatus	2	12,5	1	9,1	3	15,79	3	18,75	0	0	1	25,00	10	13,89
Cx. pipiens	1	6,25	0	0	0	0	1	6,25	0	0	1	25,00	3	4,17
Cx. gelidus	5	31,25	4	36,36	3	15,79	3	18,75	2	33,33	1	25,00	18	25,00
Cx. vishnui	5	31,25	2	18,18	5	26,32	3	18,75	2	33,33	0	0	17	23,61
Cx. pseudovishnui	0	0	2	18,18	2	10,52	3	18,75	0	0	0	0	7	9,72
Cx. tritaeniorhynchus	1	6,25	2	18,18	6	31,57	1	6,25	0	0	0	0	10	13,89
Cx. fuscocephala	0	0	0	0	0	0	1	6,25	1	16,67	0	0	2	2,78
An. maculatus	0	0	0	0	0	0	0	0	1	16,67	0	0	1	1,39
An. crucians	2	12,5	0	0	0	0	0	0	0	0	0	0	2	2,78
Ae. stimulans	0	0	0	0	0	0	1	6,25	0	0	1	25,00	2	2,78
	16	100	11	100	19	100	16	100	6	100	4	100	72	100

In the result of the mosquito catch at the farm in Kalasey Village, as shown in Table 1, the total number of female mosquitoes caught between 06:00pm – 06:00am are 72. There were 11 types of mosquitoes identified, consisting of 3 genus, namely: Genus *Culex* 67; genus *Anopheles* 3; and genus *Aedes* 2. The dominating mosquito species is *Cx. gelidus* with

a real abundance of 25%. This mosquito has the highest activity between 08:00 pm - 12:00 pm with a total number of 4 mosquitoes (Abd= 36.36%). The highest mosquito activity was found in the total number of 19 mosquitoes caught between 10:00pm - 12.00pm, and *Cx. tritaeniorhynchus* mostly caught (6).

This environmental condition around the farm relatively supports the proliferation of the vector mosquitoes, where there are ponds with flowing water and still water around the pig sties. The semi-permanent pig sty conditions and the groups of surrounding animal farms are closely located. Feeding the animals with leftovers from the restaurants causes the surrounding sties to be very dirty. Aside from that, the location of the farms very close to the settlement increases the possibility of disease transmission through vector mosquitoes.

Table 2: The Percentage Abundance of the Adult Female Mosquitoes caught in Farm II, Lemoh Village

Type of Mosquito	Lemoh Farm												Total	Abd (%)
	18.00-20.00	Abd (%)	20.00-22.00	Abd (%)	22.00-24.00	Abd (%)	24.00-02.00	Abd (%)	02.00-04.00	Abd (%)	04.00-06.00	Abd (%)		
Cx. quinquefasciatus	1	16,67	2	25,0	1	12,5	1	14,29	1	20	1	33,33	7	18,92
Cx. pipiens	1	16,67	1	12,5	1	12,5	0	0	1	20	0	0	4	10,81
Cx. gelidus	0	0	1	12,5	1	12,5	2	28,57	1	20	1	33,33	6	16,22
Cx. vishnui	2	33,33	0	0	2	25,0	3	42,86	1	20	1	33,33	9	24,32
Cx. pseudovishnui	1	16,67	1	12,5	1	12,5	0	0	0	0	0	0	3	8,11
Cx. tritaeniorhynchus	1	16,67	2	25,0	1	12,5	0	0	1	20	0	0	5	13,51
An. maculatus	0	0	1	12,5	0	0	1	14,29	0	0	0	0	2	5,41
An. roberi	0	0	0	0	1	12,5	0	0	0	0	0	0	1	2,70
	6	100	8	100	8	100	7	100	5	100	3	100	37	100

The type of female mosquitoes caught in the Lemoh farm location, as shown in Table 2 are 2 genus consisting of 6 species from *Culex* and 2 species from genus *Anopheles*. The dominating mosquito species is the *Cx. vishnui* with an abundance of 24.32%. This mosquito has the highest activity between 12:00pm - 02:00am with a total number of 3 mosquitoes (Abd= 42.86%). The highest female mosquito activity found was between 08:00pm – 12:00pm with 16 caught. The dominant type of female mosquito caught between those hours were *Cx. quinquefasciatus*, *Cx. vishnui* and *Cx. tritaeniorhynchus* each 3 caught with an real abundance of 18.75%.

The total number of adult female mosquitoes caught in this farm is the lowest compared to other farms. This is assumed to be caused by the farm conditions that are relatively clean, the floors of the sties are slanted and the water ducts that are kept clean as well as little contained water within the sties. The farm located in the clove plantation areas and far from ponds, wet paddies and settlement areas caused the breeding place of the mosquitoes to be relatively few.

Table 3: The Percentage Abundance of the Adult Female Type caught in Farm III, Tara-Tara Village

Type of Mosquito	Tara-Tara Farm												Total	Abd (%)
	18.00-20.00	Abd (%)	20.00-22.00	Abd (%)	22.00-24.00	Abd(%)	24.00-02.00	Abd (%)	02.00-04.00	Abd (%)	04.00-06.00	Abd (%)		
Cx. quinquefasciatus	10	13,33	9	8,41	2	5,13	5	10,42	5	21,74	1	11,11	32	10,56
Cx. pipiens	9	12	9	8,41	2	5,13	0	0	2	8,7	2	22,22	24	7,92
Cx. gelidus	21	28	15	14,02	4	10,26	8	16,67	4	17,39	2	22,22	54	17,82
Cx. vishnui	17	22,67	32	29,91	8	20,51	10	20,83	7	30,43	1	11,11	75	24,75
Cx. pseudovishnui	7	9,33	17	15,89	7	17,95	9	18,75	4	17,39	3	33,33	49	16,17
Cx. tritaeniorhynchus	6	8,0	9	8,41	2	5,13	5	10,42	1	4,35	0	0	23	7,59
Cx. fuscocephala	0	0	1	0,93	0	0	0	0	0	0	0	0	1	0,33
An. minimus	0	0	0	0	1	2,56	1	2,08	0	0	0	0	2	0,66
An. maculatus	2	2,67	15	14,02	13	33,33	4	8,33	0	0	0	0	34	11,22
An. crucians	2	2,67	0	0	0	0	6	12,5	0	0	0	0	8	2,64
Ae. Stimulans	1	1,33	0	0	0	0	0	0	0	0	0	0	1	0,33
Total	75	100	107	100	39	100	48	100	23	100	9	100	303	100

The type of female mosquitoes caught in the Tara-Tara farm location, as shown in Table 3 are 3 genus consisting of 7 species from genus *Culex* and 3 species from genus *Anopheles* and 1 species from genus *Aedes*. The dominating mosquito species was *Cx. vishnui* (75 mosquitoes) with an abundance of 24.75%. This type of mosquito has the highest activity between 08:00pm-10:00pm with a total number of 32 mosquitoes (Abd= 29.91%). The highest female activity was mostly caught between 08:00pm – 10:00pm with 107 mosquitoes. The dominant type of female mosquito caught within those hours was *Cx. Vishnui* (32 mosquitoes) with an abundance of 18.75%.

The farm located around the wet paddies and a flowing river behind which becomes a place for the breeding of vector mosquitoes. The total number of mosquitoes caught in this farm was high, which were 303 mosquitoes of 11 different species.

Table 4: The Percentage Abundance Type of Adult Female Mosquitoes caught in Farm IV, Talikuran Village

Type of Mosquito	Talikuran Farm												Total	Abd (%)
	18.00-20.00	Abd (%)	20.00-22.00	Abd (%)	22.00-24.00	Abd (%)	24.00-02.00	Abd (%)	02.00-04.00	Abd (%)	04.00-06.00	Abd (%)		
Cx. quinquefasciatus	9	18,37	4	11,76	2	5,88	1	3,33	0	0	0	0	16	9,63
Cx. pipiens	8	16,33	2	5,88	4	11,76	3	10,0	2	18,18	1	16,67	20	12,05
Cx. gelidus	1	2,04	1	2,94	5	14,71	2	6,67	2	18,18	1	16,67	12	7,23
Cx. vishnui	16	32,65	7	20,59	8	23,53	5	16,67	2	18,18	3	50,0	43	25,90
Cx. pseudovishnui	3	6,12	3	8,82	3	8,82	5	16,67	1	9,09	0	0	15	9,04
Cx. tritaeniorhynchus	11	22,45	9	26,47	1	2,94	5	16,67	0	0	0	0	26	15,66
An. minimus	0	0	0	0	1	2,94	3	10,0	0	0	0	0	4	2,41
An. kochi	0	0	0	0	3	8,82	3	10,0	2	18,18	0	0	8	4,82
An. maculatus	0	0	3	8,82	4	11,76	1	3,33	1	9,09	0	0	9	5,42
An. crucians	0	0	4	11,76	2	5,88	1	3,33	1	9,09	0	0	8	4,82
An. roberi	0	0	1	2,94	1	2,94	1	3,33	0	0	1	16,67	4	2,41
Ae. stimulans	1	2,04	0	0	0	0	0	0	0	0	0	0	1	0,60
	49	100	34	100	34	100	30	100	11	100	6	100	166	100

The type of female mosquitoes caught in the Talikuran Village farm location, as shown in Table 4 were 3 genus consisting of 6 species from genus *Culex* and 5 species from genus *Anopheles* and 1 species from genus *Aedes*. The dominating mosquito species is *Cx. vishnui* (43 mosquitoes) with an abundance of 25.90%. This type of mosquito has the highest activity between 06:00pm-08:00pm with a total number of 16 mosquitoes caught (Abd= 32.65%). The highest female activity was caught between 06:00pm – 08:00pm with a total of 49 mosquitoes. The dominant type of female mosquito caught within those period is *Cx. vishnui* (16 mosquitoes) with an abundance of 32.65%.

The farm in Talikuran Village is surrounded by wet paddies and flowing river behind the farm. On the eastern side of the farm is a pond planted with the kangkung vegetable and the nilla fish cultivated in the pond. This condition is ideal for the breeding place of mosquitoes, which is proven by the total number of mosquitoes caught within 12 hours as many as 166 mosquitoes from 12 different species.

Table 5: The Type of Female Mosquitoes caught in 4 farm locations of different Altitudes

Type				Farm Location					Total	Abd (%)
	Kalasey	Abd (%)	Lemo	Abd (%)	Tara-Tara	Abd (%)	Talikuran	Abd (%)		
Culex quinquefasciatus	10	13,89	7	18,92	32	10,56	16	9,63	65	11,25
Culex pipiens	3	4,17	4	10,81	24	7,92	20	12,05	51	8,82
Culex gelidus	18	25,00	6	16,22	54	17,82	12	7,23	90	15,57
Culex vishnui	17	23,61	9	24,32	75	24,75	43	25,90	144	24,91
Culex pseudovishnui	7	9,72	3	8,11	49	16,17	15	9,04	74	12,80
Culex tritaeniorhynchus	10	13,89	5	13,51	23	7,59	26	15,66	66	11,42
Culex fuscocephala	2	2,78	0	0	1	0,33	0	0	3	0,52
Anopheles minimus	0	0	0	0	2	0,66	4	2,41	6	1,04
Anopheles kochi	0	0	0	0	0	0	8	4,82	8	1,38
Anopheles maculatus	1	1,39	2	5,41	34	11,22	9	5,42	46	7,96
Anopheles crucians	2	2,78	0	0	8	2,64	8	4,82	18	3,11
Anopheles roberi	0	0	1	2,70	0	0	4	2,41	5	0,87
Aedes stimulans	2	2,78	0	0	1	0,33	1	0,60	4	0.69
Total	72	100	37	100	303	100	166	100	578	100

The overall capture of mosquitoes shown in Table 5 from the four farm locations with different altitudes based on the number and species of dominant mosquitoes caught, shows the following results: (1) the farm in Tara-Tara Village with an altitude of 576 m above sea level, the total number of mosquitoes caught was 303, with the dominant species *Cx. vishnui* (75 mosquitoes, Abd 24.75%); (2) the farm in Talikuran Village with an altitude of 616 m above sea level, the total number of mosquitoes caught was 166, with the dominant species of *Cx. vishnui* (43 mosquitoes, Abd 25.90%); (3) the farm in Kalasey Village with an altitude of 12 m above sea level, the number of mosquitoes caught was 72, the dominant species is *Cx. gelidus* (18 mosquitoes, Abd 25%); and (4) the farm in Lemoh Village with an altitude of 249 m above sea level, the total number of mosquitoes caught was 37, the dominant species is *Cx. vishnui* (9 mosquitoes, Abd 24.32%).

In this regard, the difference of altitudes of the farm locations does not affect the number of mosquitoes caught. According to the conditions of the farm surrounding which was noted in the observation, the availability of habitat and a large area of breeding place affect the number of mosquitoes caught. For example, the most mosquitoes caught were in Tara-Tara Village and Talikuran Village that are surrounded by wet paddies area which becomes ideal habitats for the breeding of mosquitoes.

The data obtained shows that the species of mosquitoes mostly caught was *Culex* sp. This research found 6 species of Culex caught in all the farm locations, namely: *Cx. vishnui*, *Cx. gelidus*, *Cx. pseudovishnui*, *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus* and *Cx. pipiens*. Whereas the *Cx. fuscocephala* was only caught in the Kalasey Village and

Tara-Tara village farm areas. Aside from this, the *An. maculatus* mosquitoes can also be found in all the farm locations, whereas the *Ae. stimulans* was only found in 3 farm locations (Kalasey, Tara-Tara and Talikuran).

In relation to the role of mosquitoes as the main vector of Japanese Encephalitis disease infection, the *Culex* sp. has a large role in transmitting the JE virus to pigs because this type of mosquito is known to prefer biting animals (zoophilic) [6]. The *Culex* type of mosquitoes that has a role as vector for transmitting the JE virus are: *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus*, and *Cx. fuscocephala* [7, 15, 18]. Further, the JE virus has been isolated to see the genotype strain of JE virus on *Culex quinquefasciatus* mosquitoes in Thailand [11]. Therefore, the result of this research shows that *Cx. vishnui*, and *Cx. gelidus* with a high abundance and dominance, can be suspected as having a role as a vector of JE disease on pigs.

The *Culex* sp. mosquitoes usually rest outdoor (exophilic), active in biting and sucking blood at night (nocturnal) as well as in the early evening (crepuscular). The living habitat of the mosquito larva is in the wet paddies, such as the *Cx. tritaeniorhynchus* that they are often known as wet paddy mosquitoes. The *Culex* sp. mosquitoes has zoophilic and anthropophilic characteristics. Whereas the *Anopheles* sp. mosquitoes have the habit of being active at night. These mosquitoes usually choose to rest indoor (endophilic), however there are some types that are exophilic. The aquatic habitat of these mosquitoes is in gutters, ditches, and mud holes. The *Aedes* sp. type of mosquitoes are more active in biting and sucking blood inside the home (endophilic, endophagic), therefore these types of mosquitoes are known as the house mosquito group, such as the *Aedes aegypti*. The *Aede albopictus* are mostly found outdoor (exophilic) and often more known as the garden mosquitoes [9, 10].

4. Conclusion

The result of this study shows that the difference in location of the pig farms does not affect the abundance of mosquito species caught. The availability of the ideal habitat for the breeding of mosquito highly affects the number of mosquitoes and the abundance of mosquitoes caught. This can be seen in the total number of the *Culex* sp. mosquitoes caught in the pig farm locations in Tara-Tara Village and Talikuran Village, where in these locations the farms are surrounded by wet paddies that is the aquatic habitat of the *Culex* sp. larva.

As the vector for transmission of JE virus is a mosquito commonly found around the pigsty, among other *Culex* species. The result of the mosquitoes caught shows that 6 species of *Culex* are found in the four farm locations, namely *Cx. vishnui*, *Cx. gelidus*, *Cx. pseudovishnui*, *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus* and *Cx. pipiens* in large numbers and high abundance. The result of the mosquitoes caught shows that *Cx. vishnui* mosquitoes have the highest abundance in three location, that is in Lemoh, in Tara-tara and Talikuran farm. Whereas in the Kalasey farm, the highest abundance is the *Cx. gelidus*. These mosquitoes bite at dusk and night. With this, the *Cx. vishnui* and *Cx. gelidus* can be suspected as having roles in transmuting of the Japanese Encephalitis (JE) in pigs.

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