Universitas Ahmad Dahlan Public Health Conference (UPHEC)
https://seminar.uad.ac.id/index.php/uphec/index
Volume December 2024

Entomological Investigation of Japanese Encephalitis Vector in Bali, Indonesia

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ARTICLE INFO

Article history
Received Sept 26, 2024
Revised Oct 28, 2024
Accepted Oct 29, 2024

Keywords
Japanese encephalitis;
Mosquito density;
Pig population;
Vector control;

ABSTRACT

Background: Japanese encephalitis (JE), caused by the Japanese encephalitis virus (JEV), is a mosquito-borne disease prevalent in Southeast Asia. Pigs act as amplifying hosts, and their presence influences mosquito population dynamics. This study investigates the relationship between mosquito density and pig population in three regencies of Bali, Indonesia: Jembrana, Badung, and Karangasem.
Method: The research was a cross-sectional study using spot survey method in three regencies in Bali. The samples were all mosquitoes captured in the research locations. Mosquitoes were collected using techniques like human landing catches (HLCs) and animal-baited traps across different ecosystems—urban, forest, and coastal. Data on pig populations were obtained from the Bali Central Bureau of Statistics.
Results: *Culex tritaeniorhynchus, Cx. vishnui*, and *Anopheles vagus* were the dominant mosquito species across all regencies. Higher mosquito densities were observed in areas with significant pig populations, such as Negara and Mengwi, suggesting a link between pig farming and mosquito populations. However, some areas with high pig potential, like Rendang, showed lower mosquito densities, indicating the influence of other ecological factors.
Conclusion: The study highlights an observed association between mosquito density and the presence of pig populations, suggesting that livestock may play an important role in areas where Japanese Encephalitis Virus (JEV) transmission is prevalent. Recognizing this pattern could help inform future vector control strategies. Further observational research may be needed to investigate additional environmental conditions that coincide with higher mosquito populations in these regions.

1. Introduction

The pathogen that causes Japanese encephalitis (JE) is the Japanese encephalitis virus (JEV), which belongs to the *Flavivirus* genus and Flaviviridae. Mosquitoes spread the illness, targeting intermediate hosts such as pigs, cows, and poultry. JEVs are widely distributed, with the Western Pacific, South and South-east Asia, and northern Australia being the most popular locations (1), (2), (3). Currently, Indonesia has identified sixteen mosquito species as JE vectors in Indonesia: *Culex tritaeniorhynchus, Cx. gelidus, Cx. vishnui, Cx. quinquefasciatus, Cx. fuscocephala, Cx. bitaeniorhynchus, Cx. sitiens,* *Cx. infula, Coquilletidia crassipes, Anopheles vagus, An. annularis, An. kochi, An. peditaeniatus, Armigeres subalbatus, Mansonia dives*, and *Aedes lineatopennis* (4), (5).

Since 1960, the JE virus has been circulating in Indonesia; as of right now, 32 provinces have shown signs of infection. Reports indicate cases of JE in Bali, West Kalimantan, East Nusa Tenggara, West Java, and East Java. The absence of regular national surveillance for JE has made it difficult to precisely determine the burden of diseases. Since 2014, sentinels have carried out surveillance for JE in 11 provinces, involving 60 government and private hospitals. According to JE surveillance data, three provinces are endemic for JE: Bali, West Kalimantan, and East Nusa Tenggara (4), (6), (7).

The disease is particularly notable for its association with domestic pigs, which serve as amplifying hosts for the virus. In Bali, the interplay between mosquito populations and pig farming practices has become a focal point for understanding the epidemiology of JE. The objective of this article is to investigate the relationship between mosquito density and pig population in Bali, Indonesia, and to identify the mosquito species that act as vectors for JEV transmission. Research indicates that pigs are crucial in the transmission cycle of JEV due to their higher susceptibility and the significant levels of viraemia they exhibit when infected (1), (8). The high density of pigs in Bali, with a population ratio of humans to pigs estimated at 2:1, exacerbates the risk of JE outbreaks, as these animals attract mosquito vectors that can transmit the virus to humans (8).

The close proximity of both pigs and mosquitoes creates an environment conducive to the transmission of the virus. There is evidence from seroepidemiology that the number of mosquitoes and the presence of JEV antibodies in pigs correlates with the density of mosquito populations, indicating a direct relationship between these two factors (9), (10). Furthermore, understanding the diversity of mosquito species in areas surrounding pig farms is essential for assessing the potential risk of JE transmission (11), (12). In summary, the investigation of mosquito density in relation to pig populations in Bali is critical for developing effective control strategies against Japanese encephalitis. By elucidating the relationship’s dynamics, we can better understand the epidemiological patterns of JEV and implement measures to mitigate the risks associated with this zoonotic disease.

2. Method

2.1. Location determination

This study is a component of the nationwide vector-borne disease (Rikhus Vektora) research project, which ran from 2015 to 2018. We selected Badung, Karangasem, and Jembrana as the three regencies in Bali as research locations. The research was a cross-sectional study using spot survey method in three regencies in Bali. The samples were all mosquitoes captured in the research locations. We purposively sampled mosquitoes in three districts within each chosen province. There are three types of ecosystems in each district: forest, urban/rural, and coastal areas. We selected two sites for the study in each habitat, both near and far from human dwellings. We conducted mosquito surveys at each study site. We allocated thirty days for the sample process in each district, or six points, as each point takes five days to complete (13).

2.2. Mosquito collections

We collected mosquitoes using a variety of techniques, such as human landing captures (HLCs), animal-baited traps, and cattle-baited cowsheds. Three residences were used for each indoor and outdoor mosquito collection using HLCs. We selected the houses close to areas where mosquitoes were expected to breed. Mosquito collections was placed between 6 p.m. and 6 a.m. For twelve hours, the duration was fifty minutes every hour. We identify the mosquitoes using identifying keys (13), (14), (15), (16).

2.3. Pig Potentials

The pig potential data was obtained from the Bali Central Bureau of Statistics to collect the information about pig density (17).

3. Result

The research was carried out in 3 regencies: Jembrana Regency (Melaya, Negara, Jembrana, and Pekutatan Districts), Badung Regency (Petang, Mengwi, and Kuta Districts), and Karangasem Regency (Rendang, Selat, Manggis, and Karangasem Districts) **(Figure 1)**.

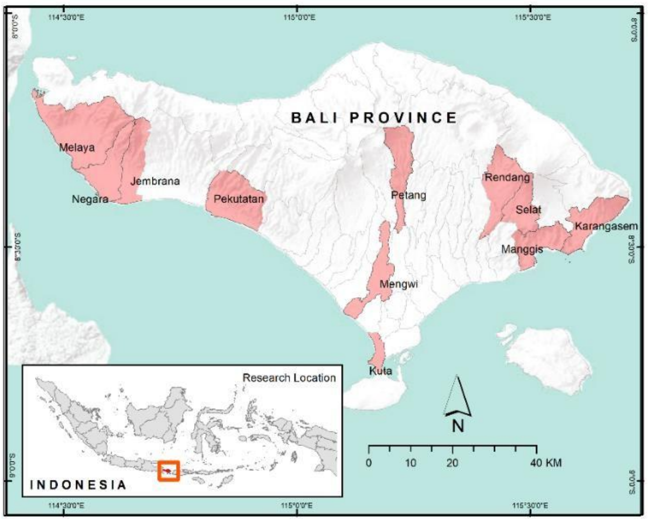

Figure 1. Reseach location

Tables 1-3 show the results of mosquito collections for each species in each ecosystem in the regencies of Jembrana, Badung, and Karangasem in sequential order. In Jembrana, *Culex tritaeniorhynchus* is dominan species, together with *Cx. vishnui*, *Cx. fuscocephala*, and *An. vagus. Culex vishnui* and *An. vagus* are found in most ecosystems (forest, urban, and coastal) (**Table 1**).

Table 1. Mosquito species composition based on ecosystem in Jembrana Regency

No	Vector Spesies	FNH* *Pekutatan***	FFH* *Jembrana***	UNH* *Negara***	UFH* *Melaya***	CNH* *Melaya***	CFH* *Melaya***
1	Culex tritaeniorhynchus	-	-	396	1	3	11
2	Culex gelidus	-	-	-	-	-	-
3	Culex vishnui	182	-	48	83	132	35
4	Culex quinquefasciatus	-	-	1	-	1	-
5	Culex fuscocephala	2	-	227	12	1	-
6	Culex bitaeniorhynchus	1	-	-	**1**	-	1
7	Culex sitiens	2	-	-	1	30	2
8	Culex infula	-	-	-	-	-	-
9	Coquilletidia crassipes	-	-	-	-	3	-
10	Anopheles vagus	8	-	326	9	91	1
11	Anopheles annularis	-	-	-	-	1	-
12	Anopheles kochi	3	-	7	6	20	-
13	Anopheles peditaeniatus	-	-	-	-	-	-
14	Armigeres subalbatus	5	-	16	3	12	-
15	Mansonia dives	-	-	-	-	-	-
16	Ae. lineatopennis	-	-	-	-	-	-

Notes:

*FNH	:	Forest area nearby human dwellings	*UFH	:	Urban area far from human dwellings
*FFH	:	Forest area far from human dwellings	*CNH	:	Coastal area nearby human dwellings
*UNH	:	Urban area nearby human dwellings	*CFH	:	Coastal area far from human dwellings

** district location in Jembrana Regency

The common species in Badung Regency according to **Table 2** are *Cx. tritaeniorhyncus, Cx. vishnui, Cx. quinquefasciatus,*and *An. vagus.* Urban and coastal areas have various mosquito species.

Table 2. Mosquito species composition based on ecosystem in Badung Regency

No	Vector Spesies	FNH* *Petang***	FFH* *Petang***	UNH* *Mengwi***	UFH* *Petang***	CNH* *Kuta***	CFH* *Mengwi***
1	*Culex tritaeniorhynchus*	-	-	198	2	2	260
2	*Culex gelidus*	-	-	10	-	1	4
3	*Culex vishnui*	-	-	331	5	20	573
4	*Culex quinquefasciatus*	59	-	7	-	199	57
5	*Culex fuscocephala*	2	1	81	3	-	88
6	*Culex bitaeniorhynchus*	-	-	6	1	-	-
7	*Culex sitiens*	-	-	-	-	3	18
8	*Culex infula*	-	-	-	-	-	-
9	*Coquilletidia crassipes*	-	-	-	-	-	-
10	*Anopheles vagus*	-	-	559	10	-	269
11	*Anopheles annularis*	-	-	-	-	-	-
12	*Anopheles kochi*	-	-	2	14	-	5
13	*Anopheles peditaeniatus*	-	-	-	-	-	-
14	*Armigeres subalbatus*	104	-	-	-	110	5
15	*Mansonia dives*	-	-	-	-	-	-
16	*Ae. lineatopennis*	-	-	-	-	-	-

Notes:

**FNH*	*:*	*Forest area nearby human dwellings*	**UFH*	*:*	*Urban area far from human dwellings*
**FFH*	*:*	*Forest area far from human dwellings*	**CNH*	*:*	*Coastal area nearby human dwellings*
**UNH*	*:*	*Urban area nearby human dwellings*	**CFH*	*:*	*Coastal area far from human dwellings*

** district location in Badung Regency

In Karangasem Regency, higher mosquito density was seen in *An. vagus, Cx. vishnui,* and *Cx. tritaeniorhyncus* in coastal and urban ecosystems, while *An. kochi* is found mostly in CNH (**Table 3**).

Table 3. Mosquito species composition based on ecosystem in Karangasem Regency

No	Vector Spesies	FNH* *Rendang***	FFH* *Rendang***	UNH* *Karangasem***	UFH* *Selat***	CNH* *Manggis***	CFH* *Karangasem***
1	Culex tritaeniorhynchus	-	-	32	11	171	22
2	Culex gelidus	-	-	1	-	5	-
3	Culex vishnui	1	-	149	37	329	119
4	Culex quinquefasciatus	13	1	-	-	20	-
5	Culex fuscocephala	2	-	-	3	5	1
6	Culex bitaeniorhynchus	-	-	-	-	4	-
7	Culex sitiens	-	-	-	-	-	-
8	Culex infula	-	-	-	-	-	-
9	Coquilletidia crassipes	-	-	-	-	-	-
10	Anopheles vagus	1	-	395	33	743	395
11	Anopheles annularis	-	-	-	-	-	-
12	Anopheles kochi	-	-	-	-	148	-
13	Anopheles peditaeniatus	-	-	-	-	-	-
14	Armigeres subalbatus	2	-	5	-	85	-
15	Mansonia dives	-	-	-	-	-	-
16	Ae. lineatopennis	-	-	-	-	-	-

Notes:

*FNH	:	Forest area nearby human dwellings	*UFH	:	Urban area far from human dwellings
*FFH	:	Forest area far from human dwellings	*CNH	:	Coastal area nearby human dwellings
*UNH	:	Urban area nearby human dwellings	*CFH	:	Coastal area far from human dwellings

** district location in Karangasem Regency

Although at different densities, *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. fuscocephala*, *An. vagus*, *An. kochi*, and *Ar. subalbatus* are present in all three regencies consistently.

Table 4-6 illustrates the relationship between the density of mosquitoes and the presence of pig farming in each district in the regencies of Jembrana, Badung, and Karangasem.

Table 4. The Relationship between mosquito density and pig population in Jembrana Regency

No	District	Ecosystem*	Pig Potential**	Mosquito Density
1	Melaya	UFH CNH PJP	26,566	116 294 50
2	Negara	UNH	4,998	1,021
3	Jembrana	FFH	3,166	-
4	Mendoyo	-	6,999	-
5	Pekutatan	FNH	1,581	203

Notes:

*FNH	:	Forest area nearby human dwellings	*UFH	:	Urban area far from human dwellings
*FFH	:	Forest area far from human dwellings	*CNH	:	Coastal area nearby human dwellings
*UNH	:	Urban area nearby human dwellings	*CFH	:	Coastal area far from human dwellings

** report from Bali Central Bureau of Statistics, 2013 (17)

The table 4 indicates that There is a notable mosquito density (1,021) in districts with higher pig potential (Negara). Even in places with reduced pig potential, such as Pekutatan, there is a noticeable mosquito density (203). While in Jembrana, although the pig density is higher (4,998), there are no JE-vector mosquito collected.

Table 5. The Relationship between mosquito density and pig population in Badung Regency

No	District	Ecosystem*	Pig Potential**	Mosquito Density
1	Kuta Selatan	-	1,975	-
2	Kuta	CNH	254	335
3	Kuta Utara	-	3,992	-
4	Mengwi	UNH CFH	18,267	1194 1279
5	Abiansemal	-	21,863	-
6	Petang	FNH FFH UFH	17,148	165 1 35

Notes:

*FNH	:	Forest area nearby human dwellings	*UFH	:	Urban area far from human dwellings
*FFH	:	Forest area far from human dwellings	*CNH	:	Coastal area nearby human dwellings
*UNH	:	Urban area nearby human dwellings	*CFH	:	Coastal area far from human dwellings

** report from Bali Central Bureau of Statistics, 2013 (17).

From three district, Kuta, Mengwi, and Petang where the mosquito collection conducted, there is a various density of pig. **(Table 5).**

Table 6. The Relationship between mosquito density and pig population in Karangasem Regency

No	District	Ecosystem*	Pig Potential**	Mosquito Density
1	Rendang	FNH FFH	8,180	19 1
2	Kubu	-	46,996	-
3	Abang	-	47,099	-
4	Karangasem	UNH CFH	20,375	582 537
5	Manggis	CNH	23,124	1,510
6	Bebandem	-	12,874	-
7	Selat	UFH	3,022	84
8	Sidemen	-	16,352	-

Notes:

*FNH	:	Forest area nearby human dwellings	*UFH	:	Urban area far from human dwellings

*FFH	:	Forest area far from human dwellings	*CNH	:	Coastal area nearby human dwellings
*UNH	:	Urban area nearby human dwellings	*CFH	:	Coastal area far from human dwellings

** report from Bali Central Bureau of Statistics, 2013 [17]

Higher of mosquito densities (1,510 and 1,119) are seen in Manggis and Karangasem, areas with great pig potential. Despite having a respectable pig potential (8,180), Rendang has low mosquito density, suggesting the presence of additional ecological concerns (**Table 6**).

4. Discussion

Species Distribution

The results presented in Tables 1-3 highlight the diversity and distribution of mosquito species across different ecosystems in the regencies of Jembrana, Badung, and Karangasem. The data indicate that *Culex tritaeniorhynchus*, *Cx. vishnui*, and *An. vagus* are consistently dominant across all three regencies, with notable variations in their densities depending on the specific ecosystem type. In Jembrana, *Cx. tritaeniorhynchus* was particularly abundant in urban areas, while *An. vagus* showed significant presence in urban and coastal ecosystems. This pattern suggests that certain mosquito species exhibit adaptability to various ecological niches, which is crucial for understanding their potential as vectors for diseases (18).

In Badung Regency, the presence of *Cx. tritaeniorhynchus*, *Cx. vishnui*, and *An. vagus* was again prominent, in urban and coastal ecosystems. Karangasem Regency presents a similar trend, with *An. vagus*, *Cx. vishnui*, and *Cx. tritaeniorhyncus* dominating the mosquito populations, especially in coastal and urban ecosystems. The significant presence of *An. kochi* in coastal areas suggests that specific species may have unique habitat preferences that could be tied to environmental factors such as salinity and water quality, which are known to affect mosquito breeding and survival (19). The consistent presence of these species across different regencies underscores the ecological resilience of these mosquitoes and their potential role in disease transmission across varied environments (18) .

The data reveal that urban areas support a diverse mosquito population, which aligns with findings from other studies indicating that urbanization can enhance mosquito abundance due to increased human activity and available breeding sites (20). The high densities of *An. vagus* in urban settings may also indicate a shift in habitat preferences, potentially influenced by anthropogenic factors that alter natural ecosystems (18) , while in another paper, it is stated that *An. vagus* has zoophilic habit (21).

Impact of Pig Cattle

The relationship between mosquito density and pig farming, as illustrated in Tables 4-6, reveals a complex interaction between livestock presence and mosquito populations. In Jembrana, high mosquito densities were observed in districts with substantial pig populations, particularly in Negara, where the mosquito density reached 1,021. This correlation suggests that livestock may provide additional breeding sites or food sources for mosquitoes, thereby influencing their population dynamics (22). Similarly, in Badung, the district of Mengwi exhibited a significant relationship between pig potential and mosquito density, reinforcing the idea that agricultural practices can impact vector populations (22).

In Karangasem, the districts with the highest mosquito densities, such as Manggis and Karangasem, also had considerable pig potential. However, the low density in Rendang, despite its respectable pig potential, indicates that other ecological factors may be at play, potentially including habitat degradation or competition with other species (22). This variability highlights the need for further investigation into the ecological interactions that govern mosquito populations in relation to livestock farming.

Additionally, the data shows that there is a relationship between mosquito density and pig cattle prevalence; higher densities are seen in districts with larger pig numbers, especially in Badung and Karangasem. In Badung and Karangasem regencies in particular, higher mosquito levels are frequently correlated with larger pig numbers. There are, however, certain oddities (such as low density despite high pig potential), which point to additional environmental impacts. Locations with high pig populations in all three regencies exhibit linear relationships with mosquito densities, particularly for the three major species, *An. vagus, Cx. vishnui,* and *Cx. tritaeniorhyncus.*

Certain mosquitoes that carry the JE virus prefer the presence of pigs as blood feed. A study conducted in Asia showed that *Cx. tritaeniorhyncus* and *Cx. vishnui* choose pigs or cows as the main blood feed (23). *Anopheles vagus* has a history of favouring animal blood over human food (21).

These results highlight how crucial it is to comprehend ecological relationships and species distribution in order to control mosquito populations and reduce the spread of diseases carried by vectors. It is advised that more investigation be done on the underlying environmental elements influencing mosquito dynamics in these regencies.

5. Conclusion

The data from these three regencies show how closely related ecosystem type, agricultural methods, and mosquito species diversity are. The association between pig farming and the consistent presence of critical species reveals that mosquitoes are well-adapted to exploit available resources. Additionally, the presence of livestock can have a substantial impact on mosquito population dynamics. To effectively control vectors and prevent diseases, future study should concentrate on comprehending the underlying ecological mechanisms underpinning these patterns.

Acknowledgment

The research was encouraged by the Ministry of Health, for which the authors are grateful. The technical and managerial team that assisted with the data collection is also appreciated. We also thank the local administration for allowing us to conduct the research and all of the field investigators and coordinators who worked on the project.

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