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# Vector bionomic of *Culex* species in possible Japanese Encephalitis Transmitted areas of Ein-me Township, Ayayawady Region in Myanmar

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## ABSTRACT

Japanese encephalitis is a public health problem in many parts of Myanmar. The diseases are transmitted to human by the biting of vector mosquitoes. The study was conducted in JE suspected cases occurred villages ThaPhan Pinseit and ThaPyu Pinseit villages in ThaPhan Pinseit RHC and PaukGone and SarPhyusu villages in PaukGone village in PaukGone RHC from Ayeyawady Region were selected for JE vector surveillance from December 2023 to March 2024. 50 houses from the pigs were randomly selected from each village. Mosquitoes were collected in all selected houses and pig farms using WHO sucking tubes. Larvae were collected in and around 1 Kilometer radius from study sites. Result found that JE main vector *Cx. tritaeniorhynchus* was abundantly collected in all study village and the highest number of vectors *Cx. tritaeniorhynchus* was collected from SarPhyusu village 2284 followed by ThaPyu Pinseit 1890 and lowest number of main vectors was collected in ThaPhan Pinseit village (1268). In overall collected mosquitoes, the highest density was found 74.18% of main vector *Cx. tritaeniorhynchus* followed by suspected vector *Cx. vishnui* 10.04%, and lowest density was observed *Mansonia* and *An. vagus* 0.33% each. Peak biting time of main vector *Cx. tritaeniorhynchus* was found 9-10 pm. Suspected vector *Cx. vishnui* was also collected in high number in all villages. Nine species of mosquitoes were collected from the study villages. Of this only four species of *An. hyrcanus, An. barbirostris, An. vagus* and *An. tesellatus* were collected. These *Anopheles* species were available in PaukGone and SarPhyusu villages. *Anopheles hyrcanus* was collected from all villages. *Culex tritaeniorhynchus* larvae were abundantly collected from rice fields, polluted water pools and stagnant water pools with plenty of hyacinth plants and also larvae were collected in concrete jars, Bago jars and metal drums water which water was stored for fire water. High density of main vector and suspected vector were collected in all JE suspected villages, pig farms, pigs, and breeding sources of vectors mosquitoes were available in the villages and also JE suspected cases are available in the all villages. This study provides information of high collection of main vector adult and larvae on risk of further transmission in the study areas. Environmental and ecological factors are responsible for the spread of JE virus. Therefore, awareness of community to vector borne diseases control activities and health education to all family members should be strengthened to prevent JE transmission in the villages.

**Keywords:** Japanese Encephalitis; JE; Main vector; *Cx. tritaeniorhynchus*; Larvae; Habitats; Rice fields; Water pools; Pig farm; Density; Positivity.

## 1. Introduction

Mosquitoes are responsible for the spread and transmission of several harmful diseases such as Japanese Encephalitis, Lymphatic filariasis, Malaria, Zika, Chikungunya and Dengue. It is known to infect over 700 million people causing 1 million deaths each year especially in developing regions of the world [1]. Japanese encephalitis (JE) is a zoonotic disease caused by JE virus (JEV) which is a mosquito-borne flavivirus spreading to human through the bite of *Culex* mosquitoes. *Culex tritaeniorhynchus* is the primary vector. The virus cycles among amplifier hosts such as pigs, wading birds and human [2]. Most of JE virus infection in human is asymptomatic and less than 1% of infected people develop symptoms and the incubation period is 5-15 days. Case fatality rate is approximately 20-30% and among survivors, 30-50% has serious neurologic or psychiatric sequalae [3]. To date, threats of JE outbreaks are mostly found in 27 countries in Asia and the Pacific Regions including Myanmar. In 1974, Myanmar reported its first outbreak of JE and in 2014, there was an outbreak in 46 villages of nine Townships of Rakhine State coupled with lack of awareness of JE transmission and prevention among the villagers [4]. As JE is endemic in many parts of Myanmar, the existence of source, vectors and seed virus outbreak can occur at any time. Cases reported were slightly higher in rural population than in urban population. Simultaneously, VBDC implements awareness raising activities to improve alertness but there are limitations. Department of Public

Health indicated a total of 491 JE cases occurred in the whole country especially in Yangon, Bago, Tanintharyi and Ayeyarwady Regions and Kayin, Rakhine and Shan States [5]. In Dawbon Township, Yangon Region JE virus infection was detected in 52.1% of pigs. The known JEV vector mosquito species, especially *Cx. tritaeniorhynchus*, were found in the study area but no concurrent human JEV infections were elicited [6]. In Myanmar, isolates of virus from pigs in Dike U pig farm were identified as genotype III by PCR [7] and isolates from pig blood sample from Thakeyta Township were found to be Genotype 1 by DNA sequencer [8].

The virus is now known to be endemic in a large area of Asia with cases being reported from Japan, China, India, the Philippines and Pakistan [9]. There have been no autochthonous cases of JEV in Africa, Europe or the Americas. JEV is the leading cause of human encephalitis in eastern and southern Asia, and currently numerous genotypic forms are recorded in Asia [10].

Japanese encephalitis (JE) is a vector borne zoonosis and one of the world's leading encephalitic diseases, particularly in the Asia-Pacific region [11]. The disease is endemic in 24 countries in South and Southeast Asia from Pakistan to Japan, northern Australia and Oceania and putting more than three billion people at risk of infection [12]. The annual incidence of JE is about 69,000 cases and annual loss of 709,000 disability-adjusted life years, JE has even a higher disease burden than dengue [13-15].

### 1.1. Background and Justification

The overall incidence of JE is 1.8 per 100 000 per year in endemic countries, it is 5.4 among 1 to 15 years old children. The infection can lead to severe complications with high case fatality [13]. There is no specific treatment till date. However, this disease can be easily prevented by protection from mosquito bite, mosquito control and safe and effective vaccination [2]. Vaccination is the most effective form of prevention. A 30-year retrospective study conducted in Taiwan reported that the vaccine had an estimated effectiveness of 97% against disease incidence, 98% against disease mortality in adult and 19.3% in decreasing the fatality of confirmed JE in 1 to15 year old children [4].

As JE is endemic in many parts of Myanmar, the existence of source, vectors and seed virus outbreak can occur at any time. Cases reported were slightly higher in rural population than in urban population. Simultaneously, VBDC implements awareness raising activities to improve alertness but limited. As of August 2017, data from Department of Public Health indicated a total of 491 JE cases occurred in the whole country especially from Yangon, Bago, Tanintharyi and Ayeyawady Regions and Kayin, Rakhine and Shan States [16]. According to Department of Public Health, Ayeyawady Region was found high prevalence of JE (171 cases) and also observed high risk in Pantanaw, Nyaungdon, Wakema and Hinthada respectively in their Townships [17]. And also, Japanese encephalitis (JE) suspected cases were reported in Ein-me Township Hospital [18]. Moreover, community has poor understanding about the interplay of human health, animal health, changing agricultural practices, environment and socioeconomic factors, and health care infrastructure. There is a need to focus diseases prevention by mitigating the transmission risk through vector surveillance and control programs incorporating one health concept in rural areas of vulnerable sites in Ayeyawady Region. Therefore, the study aims to access the vector bionomics of *Culex* species in possible Japanese Encephalitis suspected areas of Ein-me Township, Ayeyawady Region in Myanmar.

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### 1.2. Study Objectives

(i) To assess the vector bionomic of *Culex* species in possible Japanese Encephalitis Transmitted areas of Ein-me Township, Ayayawady Region. (ii) To determine the density of mosquito species and behavior of main vector *Cx. tritaniorhynchus*. (iii) To search main vector larval habitats. (iv) To determine the susceptible of insecticides. (v) To Map of the location of mosquito collection sites, vector breeding sites, pig farming sites and human settlement areas in the study villages.

## 2. Materials and Methods

### 2.1. Study area and Study period

The study area of this study was conducted in 4 villages of Ein-me Township, Ayeyawady Region. The selection is based on the 2023-24 Township Hospital Record of JE suspected cases reported (TDOPH 2023-24). Ein-me Township was found high JE suspected cases reported during 2022-2023. Therefore, the study was conducted from January 2024 to December 2024 in ThaPhan Pinseit and ThaPyu Pinseit, PaukGone and SarPhyusu villages in Ein-me Township, Ayeyawady in Myanmar.

### 2.2. Study design

A cross-sectional descriptive study design was done.

### 2.3. Study population

Study population consisted pig farmers, veterinarians, village authorities, health staff from rural health centers, village volunteers and insect collectors were involved in this study.

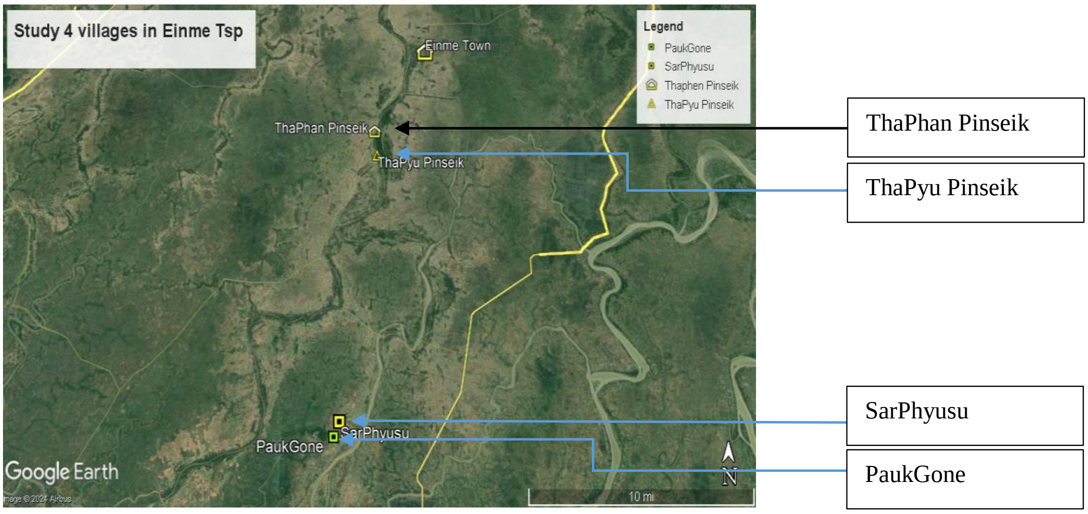


**Figure 1.** Map of the study areas (Ein-me Township, Ayeyawady Region, Myanmar)

### 2.4. Sample size determination and sampling procedure

The occurrence of JE vectors and it breeding sites and density were measured in four villages of Ein-me Township, Ayeyawady Region. From the Ayeyawady Region, Ein-me Township that reported high JE suspected cases in 2023 was selected purposively followed by random selection of ThaPhan Pinseit RHC and PaukGone RHC were selected. In the ThaPhan Pinseit RHC, ThaPhan Pinseit and ThaPyu Pinseit villages and in PaukGone RHC, PaukGone and SarPhyusu villages were selected. ThaPhan Pinseit and PaukGone villages have high density of pig farms and ThaPyu Pinseit and SarPhyusu villages had low density of pig farms. Therefore, this study covered two RHC and four villages.

### 2.5. Study Tools/Data Collection Methods

Mosquitoes were collected from fixed mosquito catching stations between pig farm and human households of selected ThaPhan Pinseit, ThaPyu Pinseit, PaukGone and SarPhyusu villages of both field areas of ThaPhan Pinseit and PaukGone RHCs using Kanda big mosquito nets (330 x 330 x 180 cm) (K-net) for animal bait and CDC light traps method was used for indoor and outdoor mosquito collection, Pig farms and cow shed collection in the selected areas of four villages with WHO sucking tubes from 18:00 to 06:00 hours of the next day. All collected mosquitoes were kept in individual labeled paper cups.

### 2.6. Larva collection

For identification of breeding sites, larval surveys were conducted in polluted water sources in and around one kilo-meters radius from the study site, such as ponds, rice fields, slowly running water, streams, creeks, foot prints, irrigation ditches, hyacinth vegetation pond, water pools, and all different types of water holding places were examined for larvae by 3 Dips/water holding place with WHO dipper [19]. The captured larvae and pupae were put in labeled plastic bags and brought back to the laboratory for species identification and colonization.

### 2.7. Susceptibility test

Susceptibility of *Culex* adult mosquitoes was done with WHO test kit as Deltamethrin 0.05%, Cyfluthrin 0.15% and Pyrethrin 0.75% [20].

### 2.8. Morning Resting collection

Morning resting mosquitoes were collected in indoor and outdoor of the household, pig farms and cow shed from 6:00-7:00 am.

### 2.9. Study location

Map of the location of mosquito collection sites, vector breeding sites, pig farming sites and human settlement areas in the study villages were drawn by using Global Positioning System GPS device (GPSMap16 Garman, 18x-5HZ) software interface application method by expert person.

### 2.10. Identification of mosquitoes

Collected adult mosquitoes and adult emerged from larva survey were identified by species according to different identification keys [21-24].

### 2.11. Data Analysis

Data from various sources were triangulated for meaningful interpretation. Larval density was calculated by larvae/dip and percentage was computed for adult mosquito density per village by Excel software. Map of the study areas were drowning by GPS device (GPSMap16 Garman, 18x-5HZ) software interface application method by expert person.

## 3. Results

**Table 1.** Distribution and density of collected mosquitoes in 4 different villages in Ein-me Township

| S.No. | Species | ThaPhan Pinseit | | ThaPyu Pinseit | | PaukGone | | SarPhyusu | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of collected | Density | No. of collected | Density | No. of collected | Density | No. of collected | Density | No. of collected | Density |
| 1 | *Cx. tritaeniorhynchus* | 1268 | 84.08 | 1890 | 83.12 | 1470 | 66.16 | 2284 | 68.93 | 6914 | 74.18 |
| 2 | *Cx. vishnui* | 184 | 12.20 | 252 | 11.21 | 244 | 11.21 | 248 | 7.48 | 936 | 10.04 |
| 3 | *Cx. gelidus* | 16 | 1.06 | 10 | 0.44 | 30 | 1.58 | 14 | 0.45 | 76 | 0.82 |
| 4 | *Cx. quinquefasciatus* | 18 | 1.26 | 68 | 2.99 | 84 | 3.78 | 124 | 3.83 | 298 | 3.20 |
| 5 | *Mansonia* | 0 | 0.00 | 10 | 0.57 | 18 | 0.81 | - | 0.00 | 31 | 0.33 |
| 6 | *An. hyrcanus* | 12 | 0.86 | 24 | 1.19 | 220 | 10.04 | 580 | 17.59 | 846 | 9.08 |
| 7 | *An. barbirostris* | 8 | 0.53 | 0 | 0.00 | 104 | 4.68 | 22 | 0.69 | 135 | 1.45 |
| 8 | *An. vagus* | 0 | 0.00 | 0 | 0.00 | 20 | 0.90 | 10 | 0.33 | 31 | 0.33 |
| 9 | *An. tesellatus* | 0 | 0.00 | 10 | 0.48 | 19 | 0.86 | 22 | 0.69 | 53 | 0.57 |
| | Total | 1508 | 100 | 2275 | 100 | 2222 | 100 | 3315 | 100 | 9320 | 100.00 |
| | Density | | 16.18% | | 24.41% | | 23.84% | | 35.57% | | 100% |

Table 1 shows that total collected mosquito species and densities in the villages. A total of 9320 mosquitoes were collected from pig farms, cow sheds and households. Three groups of mosquitoes as *Culex*, *Mansonia* and *Anopheles* were collected. Of these 4 species of *Culex* mosquitoes i.e. *Cx. tritaeniorhynchus, Cx. vishnui, Cx. gelidus, Cx. quinquefasciatus*, one species of *Mansonia* and 4 species of *Anopheles* as *An. hyrcanus, An. barbirostris, An. vagus* and *An. tesellatus* were collected. Highest density of mosquitoes was collected from SarPhyusu village (35.75%) followed by ThaPyu Pinseit (24.41%) and lowest density was observed in ThaPhan Pinseit village (16.18%). Main vector of JE *Cx. tritaeniorhynchus* was collected in high density (74.18%) followed by *Cx. vishnui* (10.04%) in all collected villages lowest density was found *Mansonia* and *An. vagus* (0.33%). Highest density of main filarial vector *Cx. quinquefasciatus* was collected from SarPhyusu village (3.83%) followed by PaukGone village (3.78%) and lowest density was observed ThaPhan Pinseit village (1.26%).

**Table 2.** The distribution of JE vector and suspected vectors in four villages in Ein-me villages

| S.No. | Species | ThaPhan Pinseit | | ThaPyu Pinseit | | PaukGone | | SarPhyusu | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of collected | Density% | No. of collected | Density% | No. of collected | Density% | No. of collected | Density% | No. of collected | Density |
| 1 | *Cx. Tritaeniorhynchus* | 1268 | 87.33 | 1890 | 88.24 | 1470 | 85.76 | 2284 | 90.21 | 6914 | 88.08 |
| | Density% | 18.34 | | 27.34 | | 21.26 | | 33.03 | | 100% | |
| 2 | *Cx. Vishnui* | 184 | 12.67 | 252 | 11.76 | 244 | 14.24 | 248 | 9.79 | 936 | 11.92 |
| | Density% | 19.66 | | 26.92 | | 26.07 | | 26.50 | | 100% | |
| Total | | 1452 | 100% | 2142 | 100% | 1714 | 100 | 2532 | 100 | 7850 | 100 |
| Total Density% | | 18.50 | | 27.29 | | 21.83 | | 32.25 | | 100% | |

Table 2 shows that the distribution of JE vector and suspected vector in four villages in Ein-me villages and found that the highest total density of both main vector *Cx. tritaeniorhynchus* and suspected vector *Cx. vishnui* was observed in SarPhyusu village 2532(32.25%) and followed by ThaPyu Pinseit village 2142(27.29%) and lowest density was found 1452(18.50%). When compared with *Cx. vishnui*, the density of main vector *Cx. tritaeniorhynchus* was higher over 85% in all villages than suspected vectors of *Cx. vishnui* species. Of this the highest density of main vector was collected from SarPhyusu village (33.03%) and followed by ThaPyu Pinseit village (27.34%) and lowest density was collected from ThaPhan Pinseit ThaPhan Pinseit village. In suspected vector, the high density of suspected vector *Cx. vishnui* was collected from ThaPyu Pinseit (26.92%), SarPhyusu (26.50%) and PaukGone villages (26. 07%) followed by ThaPhan Pinseit village (19.66%).

**Table 3.** Mosquito collected by different collection methods in Ein-me Township Ayeyawady Region

| S.No. | Species | Cattle sheds | Pig farms | Indoor LT. | Outdoor LT | House holds | Morning Resting | Total | Density |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *Cx. tritaeniorhynchus* | 1246 | 2789 | 782 | 894 | 766 | 437 | 6914 | 74.18 |
| 2 | *Cx. Vishnui* | 251 | 443 | 56 | 93 | 48 | 45 | 936 | 10.04 |
| 3 | *Cx. Gelidus* | 21 | 34 | 6 | 9 | 6 | 0 | 76 | 0.82 |
| 4 | *Cx. quinquefasciatus* | 98 | 123 | 26 | 19 | 21 | 11 | 298 | 3.20 |
| 5 | *Mansonia* | 11 | 17 | 0 | 3 | | | 31 | 0.33 |
| 6 | *An. Hyrcanus* | 493 | 310 | 13 | 25 | 5 | 0 | 846 | 9.08 |
| 7 | *An. Barbirostris* | 68 | 56 | 2 | 8 | 1 | 0 | 135 | 1.45 |
| 8 | *An. Vagus* | 15 | 9 | 2 | 4 | 1 | | 31 | 0.33 |
| 9 | *An. Tesellatus* | 26 | 14 | 3 | 8 | 2 | 0 | 53 | 0.57 |
| | Total | 2229 | 3795 | 890 | 1063 | 850 | 493 | 9320 | 100.00 |
| | Percent | 23.92 | 40.72 | 9.55 | 11.41 | 9.12 | 5.29 | 100.00 | 74.18 |

Table 3 shows that mosquito collection by different methods and found that highest number of mosquitoes were collected in pig farms 3795 (40.72%) followed by cow shed 2229 (23.92%) and lowest number of mosquitoes were collected by morning resting collection. Main vector of *Cx. tritaeniorhynchus* and suspected vector of *Cx. vishnui* were also highest in pig farms and second most was Cow shed collection. All the collected mosquitoes were found higher in Cow shed and Pig farms than other collection methods. CDC light traps collection found that higher mosquitoes collected by Outdoor light trap collection 1063 (11.41%) than indoor light traps collection 890(9.55%). Indoor Households collection was found 850 mosquitoes and morning resting collection was found 493 mosquitoes is a lowest collection of mosquitoes by different mosquito collection methods. Higher number of main vectors *Cx. tritaeniorhynchus* and suspected vector *Cx. vishnui* were collected in all method. It may be due to high density of both mosquitoes are available in these areas.

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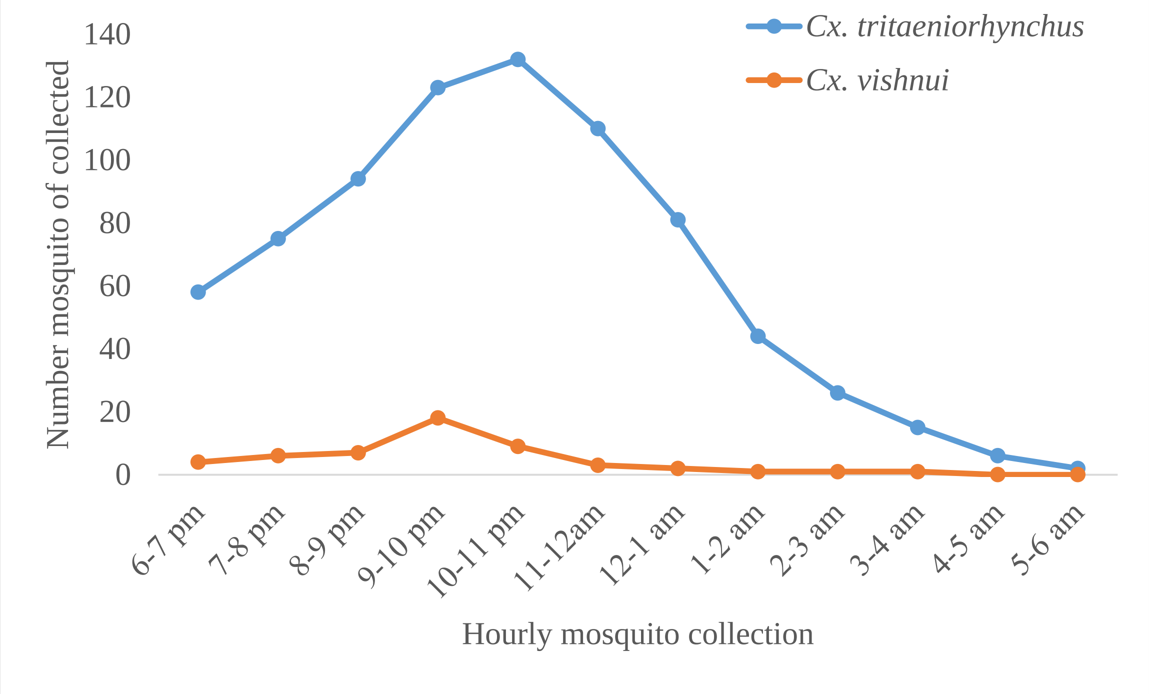


**Figure 2.** Hourly collection of main JE vector *Cx. tritaeniorhynchus* and suspected vector *Cx. vishnui* mosquitoes in house hold

Figure 2 shows that hourly collection of vectors *Cx. tritaeniorhynchus* and suspected vector *Cx. vishnui* mosquitoes in house hold and found that pick number of *Cx. tritaeniorhynchus* were collected in 10.11 pm then the number of collections was gradually decreased to 5-6 am, and *Cx. vishnui* was found pick collection at 9-10pm then it was decreased gradually to 4-5 am (0 catches).

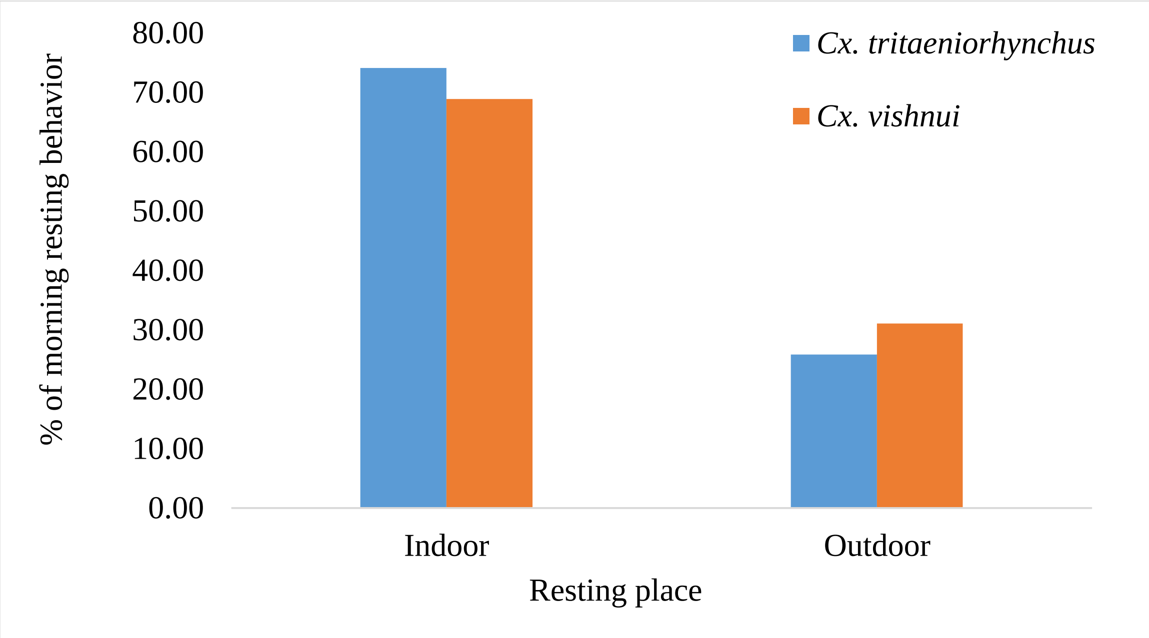


**Figure 3.** Morning resting behavior of main vector *Cx. tritaeniorhynchus* and suspected vector *Cx. vishnui*

Figure 3 shows that morning resting behavior of main vector and suspected vector in households, Cow shed and Pig farm and found that both *Cx. tritaeniorhynchus* and *Cx. vishnui* were higher resting behavior in Indoor (74.14% and 68.89%) than Outdoor (25.86% and 31.11%). It was 2.87 and 2.21 folds higher in Indoor than Outdoor.

**Table 4.** Breeding habitats and its larval density in selected villages in Ein-me Township

| S.No. | Species | ThaPhan Pinseit | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | WPs | RFs | Ps | Ss | PW Ps | SR Ws | Cs | FPs | HVs | CJs | BJs | MDs | Total |
| 1 | *Cx. tritaeniorhynchus* | 21 | 42 | 11 | 2 | 49 | 3 | 7 | 0 | 48 | 19 | 12 | 5 | 219 |
| 2 | *Cx. vishnui* | 4 | 21 | 3 | 2 | 10 | 3 | 2 | | 6 | 6 | | 2 | 59 |
| 3 | *Cx. gelidus* | | | | | 2 | | | | | | | | 2 |
| 4 | *Cx. quinquefasciatus* | 3 | 7 | | | 3 | | 1 | | 3 | | 2 | | 19 |
| 5 | *Mansonia* | | | | | | | | | | | | | 0 |
| 6 | *An. hyrcanus* | 3 | 13 | 4 | 4 | 0 | 6 | 5 | | 6 | 9 | | 2 | 52 |
| 7 | *An. barbirostris* | 3 | 11 | 2 | 0 | 1 | 4 | 4 | 2 | 5 | 7 | 3 | 2 | 44 |
| 8 | *An. vagus* | | | | | | | | 2 | | 3 | | | 5 |
| 9 | *An. tesellatus* | | | | | | | | | | | | | 0 |
| 10 | *Ae. aegypti* | | | | | | | | | | 4 | 4 | 6 | 14 |
| 11 | *Ae. albopictus* | | | | | | | | | | | 4 | | 4 |
| 12 | *Ae. albopictus* | 34 | 94 | 20 | 8 | 65 | 16 | 19 | 4 | 68 | 48 | 25 | 17 | 418 |
| | % Density | 8.13 | 22.49 | 4.78 | 1.91 | 15.55 | 3.83 | 4.55 | 0.96 | 16.27 | 11.48 | 5.98 | 4.07 | 100.00 |
| | Density | 15.60 | | | | | | | | | | | | |
| | | **ThaPyu Pinseit** | | | | | | | | | | | | |
| 1 | *Cx. tritaeniorhynchus* | 23 | 89 | 18 | 4 | 58 | 5 | 9 | 0 | 37 | 25 | 16 | 6 | 290 |
| 2 | *Cx. vishnui* | 6 | 28 | 4 | | 18 | 3 | 3 | | 5 | 9 | 3 | 4 | 83 |
| 3 | *Cx. gelidus* | | | | | 3 | | | | | | | | 3 |
| 4 | *Cx. quinquefasciatus* | 2 | 9 | | | 7 | | 2 | | 3 | | | | 23 |
| 5 | *Mansonis* | | 1 | 0 | 0 | 3 | | | | | | | | 4 |
| 6 | *An. hyrcanus* | 1 | 18 | 6 | 7 | | 10 | 7 | | 9 | 5 | | 4 | 67 |
| 7 | *An barbirostris* | | 9 | | | 0 | 0 | 1 | 3 | 1 | 3 | | | 17 |
| 8 | *An. vagus* | | | | | | | | 2 | | 0 | | | 2 |
| 9 | *An. tesellatus* | | 3 | | 1 | | 1 | 1 | | | 0 | | | 6 |
| 10 | *Ae. aegypti* | | | | | | | | | | 5 | 4 | 5 | 14 |
| 11 | *Ae. albopictus* | | | | | | | | | | | 3 | 2 | 5 |
| | Total | 32 | 157 | 28 | 12 | 89 | 19 | 23 | 5 | 55 | 47 | 26 | 21 | 514 |
| | % Density | 7.66 | 30.54 | 5.45 | 2.33 | 17.32 | 3.70 | 4.47 | 0.97 | 10.70 | 9.14 | 5.06 | 4.09 | 100.00 |
| | Density | 19.31% | | | | | | | | | | | | |
| | | **PaukGone** | | | | | | | | | | | | |
| 1 | *Cx. tritaeniorhynchus* | 28 | 92 | 22 | 3 | 65 | 6 | 12 | 0 | 32 | 58 | 38 | 18 | 374 |
| 2 | *Cx. vishnui* | 11 | 35 | 9 | | 19 | 3 | 3 | | 8 | 6 | 4 | 3 | 101 |
| 3 | *Cx. gelidus* | | | | | 6 | | | | | | | | 6 |
| 4 | *Cx. quinquefasciatus* | 4 | 8 | | | 7 | | 2 | | 3 | | | | 24 |
| 5 | *Mansonia* | | 0 | | | 5 | | | | | | | | 5 |
| 6 | *An. hyrcanus* | 11 | 26 | 7 | 11 | | 6 | 7 | | 11 | 6 | | | 85 |
| 7 | *An. barbirostris* | 4 | 23 | 5 | 11 | | 5 | 6 | 5 | 10 | 5 | 9 | 4 | 87 |
| 8 | *An. vagus* | | 3 | | | 1 | | | 21 | | 4 | | 4 | 33 |
| 9 | *An. tesellatus* | | 4 | | 2 | | 2 | 0 | | | 4 | | 1 | 13 |
| 10 | *Ae. aegypti* | | | | | | | | | | 4 | 4 | 3 | 11 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | *Ae. albopictus* | | | | | | | | | | | 2 | 3 | 5 |
| | Total | 58 | 191 | 43 | 27 | 103 | 22 | 30 | 26 | 64 | 87 | 57 | 36 | 744 |
| | % Density | 11.28 | 25.67 | 5.78 | 3.63 | 13.84 | 2.96 | 4.03 | 3.49 | 8.60 | 11.69 | 7.66 | 4.84 | 100.00 |
| | Density | 28.39 % | | | | | | | | | | | | |
| | | **SarPhyusu** | | | | | | | | | | | | |
| 1 | *Cx. tritaeniorhynchus* | 32 | 135 | 27 | 3 | 75 | 9 | 8 | 0 | 36 | 214 | 27 | 7 | 573 |
| 2 | *Cx. vishnui* | 14 | 37 | 7 | | 22 | 2 | 3 | | 7 | 8 | 5 | 4 | 109 |
| 3 | *Cx. gelidus* | | 1 | | | 9 | | | | | | | | 10 |
| 4 | *Cx. quinquefasciatus* | 7 | 5 | | | 3 | | 2 | | 4 | | | | 21 |
| 5 | *Mansonia* | | | | | 0 | | | | | | | | 0 |
| 6 | *An. hyrcanus* | 12 | 25 | 9 | 10 | | 5 | 8 | | 10 | 8 | | 4 | 91 |
| 7 | *An barbirostris* | 6 | 15 | 6 | 12 | 2 | 6 | 8 | 3 | 12 | 7 | 13 | 5 | 95 |
| 8 | *An. vagus* | | 4 | | | | | | 13 | | 4 | | 2 | 23 |
| 9 | *An. tesellatus* | | 7 | | 3 | | 2 | 2 | | | 5 | | | 19 |
| 10 | *Ae. aegypi* | | | | | | | | | | 3 | 4 | 4 | 11 |
| 11 | *Ae. albopictus* | | | | | | | | | | 2 | 3 | 4 | 9 |
| | Total | 71 | 229 | 49 | 28 | 111 | 24 | 31 | 16 | 69 | 251 | 52 | 30 | 941 |
| | % density | 9.54 | 23.83 | 5.10 | 2.91 | 11.55 | 2.50 | 3.23 | 1.66 | 7.18 | 26.12 | 5.41 | 3.12 | 100.00 |
| | | 36.70% | | | | | | | | | | | | |
| | Ground total | 195 | 671 | 140 | 75 | 368 | 81 | 103 | 51 | 256 | 433 | 160 | 104 | 2637 |
| | % Density | 20.29 | 25.45 | 5.31 | 2.84 | 13.96 | 3.07 | 3.91 | 1.93 | 9.71 | 16.42 | 6.07 | 3.94 | 100.00 |
| | | 100% | | | | | | | | | | | | |

Table 4 shows that breeding habitats and its larval density in selected villages in Ein-me Township and found that 12 types of larval habitats such as water pools, Rice fields, ponds, streams, polluted water pools, slowly running water, creek, foot prints, hyacinth vegetation, concrete Jars, Bago Jars and Metal drums were found in villages. Of this highest density of larvae were collected from Rice field 22.49% followed by water pools 20.29% lowest density was observed footprints 1.39%. High number of primary vectors *Cx. tritaeniorhynchus* and suspected vector *Cx. vishnui* larvae were collected than other mosquito species of larvae in all habitats. Highest density of larvae was collected from SarPhyusu village (36.44%) followed by PaukGone village (28.21%) and lowest density of larvae was collected from ThaPhan Pinseit village 15.85%.

In ThaPhan Pinseit village the highest density of *Cx. tritaeniorhynchus* larvae were collected from Polluted water pools (PWPs) and Hyacinth vegetation's (HVs) water (49 and 48) larvae followed by Rice fields (RFs) water (42). In ThaPyu Pinseit and PaukGone villages the highest number of *Cx. tritaeniorhynchus* larvae were collected in Rice fields water (RFs) (89 and 92) followed by Polluted water pools (PWPs) (58 and 65) and lowest number of larvae were collected *Cx. tritaeniorhynchus* from bed of Streams (2 and 4). Although in SarPhyusu village the highest density of *Cx. tritaeniorhynchus* larvae were collected from concrete Jars (214) followed by Rice fields water (135) and lowest number of *Cx. tritaeniorhynchus* larvae were collected from Streams (3).

In Rice field water sources, different species of *Culex* as *Cx. tritaeniorhynchus, Cx. vishnui, Cx. quinquefasciatus* and *Anopheles* as *An. barbirostris, An. hyrcanus, An. vagus* and *An. tesellatus* larvae were collected and in polluted water sources *Cx. tritaeniorhynchus, Cx. vishnui, Cx. quinquefasciatus* and *Cx. gelidus* were collected and only 1 and 2 larvae of *An. barbirostris* from ThaPhan Pinseit and SarPhyusu and only 1 *An. vagus* larvae was collected from PaukGone. Concrete Jars, Bago Jars and Metal drums were mostly used to store the water for fire water and *Aedes* larvae were also collected together with *Culex* larvae in these containers.

**Table 5.** Susceptibility status of collected mosquitoes against WHO recommended insecticides

| **S.No.** | **Species** | **No of tested mosquitoes** | **Insecticides** | **Susceptibility** | **Remarks** |
|---|---|---|---|---|---|
| 1 | *Cx. tritaeniorhynchus* | 30 | Deltamethrin 0.05% | “” | 20-30 minutes tolerance |
| 2 | *Cx. vishnui* | 30 | | “” | 20-30 minutes tolerance |
| 3 | *Cx. gelidus* | 20 | | “” | 20-30 minutes tolerance |
| 4 | *Cx. quinquefasciatus* | 30 | Permethrin 0.75% | “” | 20-30 minutes tolerance |
| 5 | *Mansonia* | 20 | | “” | Susceptible |
| 6 | *An. hyrcanus* | 30 | | “” | 20-30 minutes tolerance |
| 7 | *An. barbirostris* | 30 | Cyfluthrin 0.15% | “” | 20-30 minutes tolerance |
| 8 | *An. Vagus* | 20 | | “” | Susceptible |
| 9 | *An. tesellatus* | 20 | | “” | Susceptible |

Table 5 shows that the susceptibility status of collected mosquitoes against WHO recommended insecticides as Deltamethrin 0.05%, Permethrin 0.75% and Cyfluthrin 0.15% and found that all tested mosquitoes were susceptible to WHO recommended insecticides. Although Culex group as *Cx. Tritaeniorhynchus, Cx. Vishnui, Cx. Gelidus* and *Cx. quinquefasciatus,* and Anopheles group as *An. hyrcanus* and *An barbirostris* were found 20-30 minutes tolerated the all insecticides impregnated papers in WHO test Kits.

## 4. Discussion

Japanese encephalitis (JE), a vector-borne viral disease, is endemic to large parts of Asia and the Pacific regions [25]. An estimated 3 billion people are at risk, and the disease has recently spread to new territories globally [11]. The main epidemiological pattern is an enzootic cycle where the virus is transmitted between birds and/or pigs by mosquitoes [26]. Mosquitoes are still considered the key players in terms of virus transmission and therefore investigations of their density and susceptibility to efficiently manage the mosquito population to reduce JE transmission.

The present study mosquitoes were collected in pig farms, cow sheds, households and resting places and breeding sources were searched in the villages. A total of 9320 adult mosquitoes consist of 9 species as 4 species of *Culex* mosquitoes i.e. *Cx. tritaeniorhynchus, Cx. vishnui, Cx. gelidus, Cx. quinquefasciatus*, one species of *Mansonia* and 4 species of *Anopheles* as *An. hyrcanus, An. barbirostris, An. vagus* and *An. tesellatus* were collected. Highest density of mosquitoes was collected from SarPhyusu villages followed by ThaPyu Pinseit and lowest density was observed in ThaPhan Pinseit village. The main JE vector *Cx. tritaeniorhynchus* was found highest density in all villages, and suspected vector *Cx. vishnui* was also found second most in all villages. Same high density of main and secondary vector adults was collected in Chaung Sone Township Mon State and Wakema township in Ayeyawady Region, in Myanmar [27,28]. Auerswald and his associate revealed that based on the investigated publications, author classified 14 species as *Ae. albopictus, Ae. vexans, Ae. vigilax, Armigeres (Ar.) subalbatus, Cx. annulirostris, Cx. bitaeniorhynchus, Cx. fuscocephala, Cx. gelidus, Cx. pipiens, Cx. pseudovishnui, Cx. quinquefasciatus, Cx. sitiens, Cx. tritaeniorhyncus and Cx. vishnui* confirmed vectors for JEV due to their documented experimental vector competence and evidence of JEV found in wild mosquitoes. Additionally, author identified 11 mosquito species as *Ae. detritus, Ae. dorsalis, Ae. japonicus, Ae. kochi, Ae. nigromaculis, Ae. notosciptus, An. tessellatus, Cx. tarsalis, Cs. annulata, Cs. inornata* and *Ve. Funerea*, belonging to five genera, with an experimentally confirmed vector competence for JEV but lacking evidence on their JEV transmission capacity from field-caught mosquitoes [15].

Other researchers report that the most important vector is *Cx. tritaeniorhynchus*, which feeds on cattle in preference to humans. It has been proposed that moving swine away from human habitation can divert the mosquito away from humans and swine. The natural hosts of the Japanese encephalitis virus are birds, not humans, and many believe the virus will therefore never be completely eliminated [29]. A South Korea researcher observed that in November 2011, the Japanese encephalitis virus was presented in *Cx. bitaeniorhynch*us mosquitoes [30]. The common domestic animals include cows, buffaloes, goats, pigs, dogs and horses are available in Pantanaw. The pig is known to be the amplifier host of the JE virus (JEV). Despite this knowledge, unorganized piggeries and Pig farms are common in the study areas of the Ayeyawady Region. Many species of *Culex* mosquitoes can transmit JE. For Southern Asia, Eastern Asia, and Southeastern Asia, the main vector of JE *is Cx. tritaeniorhynchus*. For Northern Australia, the main vector is *Cx. annulirostris*. However, various other secondary vectors may be important [31]. Vectors observation study in India by Indian researchers they also revealed that Japanese encephalitis virus isolation has been made from a variety of mosquito species. *Culicine* mosquitoes mainly *Cx. vishnui* group (*Cx. tritaeniorhynchus, Cx. vishnui* and *Cx. seudovishnui*) are the major vectors of JE in different parts of India. In *Anopheles* group as An. *barbirostris An. paeditaeniatus* and *An. subpictus* isolates and Mansonia group as *Ma. Annulifera, Ma. indiana* and *Ma. uniformis* isolates were found JE virus positive [32]. *Cx. vishnui* groups are present in many countries of south-east Asia [33,34]. These mosquitoes are usually found in rural rice growing and pig-farming regions of Asia, but can also be found at the outskirts of cities in close proximity to human populations. They prefer to breed in rice fields, and outbreaks of JE are commonly associated with intensive rice cultivation [35,36]. Filarial vector *Cx. quinquefasciatus* was also collected from all villages in high number but not a vector of JE. Although JEV was detected in *Cx. quinquefasciatus* mosquitoes from India [37] and Vietnam [38]. Isolations were successful from mosquitoes in India [39], Vietnam [40], Thailand [41] and Taiwan [34]. Early vector competence studies with *Cx. quinquefasciatus* in laboratory colonies from Japan [42] and India [43,44] were able to observe concentration-dependent infection rates, and confirm transmission to young chickens, respectively. Weng and his party demonstrated transmission of a human JEV isolate from Okinawa to infant mice when the mosquitoes were infected by feeding on infectious blood presented on cotton [34]. Reeves and Hammon also used this technique for successfully infecting *Cx. quinquefasciatus* and demonstrating further transmission to mice, whereas they were not able to infect mosquitoes successfully by feeding them on viremic chicken [45].

In the present study, highest number of mosquitoes were collected in pig farms followed by cow shed and lowest number of mosquitoes were collected by morning resting collection. Main vector of *Cx. tritaeniorhynchus* and suspected vector of *Cx. vishnui* were collected, and also highest in pig farms and followed by Cow shed collection. All the collected mosquitoes were found higher in Cow shed and Pig farms than other collection methods. Mosquito collection by CDC light traps found higher by Outdoor light trap collection than indoor light traps collection. Indoor Households collection and morning resting collection was found lower than other mosquito collection methods. Higher number of main vectors *Cx. tritaeniorhynchus* and suspected vector *Cx. vishnui* were collected in all method. It may be due to the fact that plenty of breeding sources and high density of both mosquitoes are available in these areas. Same result has been found in Pantanaw Township Ayayawady Region and Letpadan Township Bago Region [28,46]. A South Korea researcher observed that the Japanese encephalitis virus was presented in *Cx. bitaeniorhynchus* mosquitoes [30]. The pig is known to be the amplifier host of the JE virus (JEV). Despite this knowledge, unorganized piggeries and Pig farms are common in the previous study areas of the Ayeyawady Region [28]. Vectors observation study in India by Indian researchers they also revealed that Japanese encephalitis virus isolation has been made from a variety of mosquito species. Culicine mosquitoes mainly *Cx. vishnui* group (*Cx. tritaeniorhynchus, Cx. vishnui* and *Cx. seudovishnui*) are the major vectors of JE in different parts of India [47].

In Ein-me Township, hourly collection of vectors *Cx. tritaeniorhynchus* and suspected vector *Cx. vishnui* mosquitoes in house hold and found that pick number of *Cx. tritaeniorhynchus* were collected in 10-11 pm then the number of collections was gradually decreased to 5-6 am, and *Cx. vishnui* was found pick collection at 9-10pm then it was decreased gradually to 4-5 am. Same pick biting pattern of *Cx. tritaeniorhynchus* and *Cx. vishnui* mosquitoes were found in Wakema Township Ayeyawady Region [28]. Other researcher observed that in Lapatan Township, the biting rates of *Cx. tritaeniorhynchus* and *Cx. vishnui* were found 3.81 and 4.52-fold higher on cattle and 3.5 fold and 2.1 fold higher on pig than on human. Biting rates of both *Cx. tritaeniorhynchus* and *Cx. vishnui* were observed 2.67 bites/hour and 3.5 bite/hour on human, 10.17bite/hour and 15.83 bite/hour on cattle and 9.33 bite/hour and 7.5 bite/hour on pig, respectively. *Cx. tritaeniorhynchus* and *Cx. vishnui* were observed as anthropophilic mosquitoes [46].

In the present study, morning resting behavior of main vector and suspected vector in households, Cow shed and Pig farm were found both *Cx. tritaeniorhynchus* and *Cx. vishnui* were higher resting behavior in Indoor (74.14% and 68.89%) than Outdoor (25.86% and 31.11%). It was 2.87 and 2.21 folds higher in Indoor resting than Outdoor resting. Same resting behavior of *Cx. tritaeniorhynchus* and *Cx. vishnui* has been found higher in indoor in Wakema Township [28]. In Pantanaw Township, Ayayawady Region JE vector *Cx tritaeniorhynchus* was collected in highest density in Pathew 30.22%, Kyonthet 32.08% and Aque 24.16 expect Kyontonekalay 20.04% and suspected vector *Culex vishnui* was highest density in Kyontonekalay 24.59 and followed in remaining villages. Both species are very common, widespread and new JE cases were reported in 2017 in Pantanaw and Wakema [28,48].

In Ein-me Township, 12 types of larval habitats were observed in 4 studied villages, such as water pools, Rice fields, ponds, streams, polluted water pools, slowly running water, Creek, foot prints, hyacinth vegetation, Concrete Jars, Bago Jars and Metal drums were found in villages. Of this highest density of larvae were collected from Rice field followed by water pools and lowest density was observed footprints. High number of primary vectors *Cx. tritaeniorhynchus* and suspected vector *Cx. vishnui* larvae were collected than other larvae of mosquito species in all habitats. Highest density of larvae was collected from SarPhyusu village followed by PaukGone village and lowest density of larvae was collected from ThaPhan Pinseit village. Other researcher also revealed that JE vector *Culex tritaeniorhynchus* and secondary vector *Cx vishnui* adult were collected by Cattle and pig farm collection and their larvae were abundantly collected from Rice field water and polluted water pools in Letpadan Township, Bago Region [46] and high density of *Cx. tritaeniorhynchus* and *Cx. vishnui* larvae were collected in rice field water and polluted water pools and water pools in Wakema Township in Ayeyawady Region, Letpadan Township Bago Region [28,46]. These mosquitoes are usually found in rural rice growing and pig-farming regions of Asia, but can also be found at the outskirts of cities in close proximity to human populations. They prefer to breed in rice fields, and outbreaks of JE are commonly associated with intensive rice cultivation [49,50]. Other researcher revealed that these vectors are primarily outdoor resting in vegetation and other shaded places but in summer may also rest in indoors. And breed in water with luxuriant vegetation mainly in paddy fields and the abundance is related to rice cultivation, shallow ditches and pools [51].

In ThaPhan Pinseit village the highest density of *Cx. tritaeniorhynchus* larvae were collected from Polluted water pools (PWPs) and hyacinth vegetation's (HVs) water followed by Rice fields water. In ThaPyu Pinseit and PaukGone villages, the highest number of *Cx. tritaeniorhynchus* larvae were collected in Rice fields water (RFs) followed by Polluted water pools (PWPs) and lowest number of *Cx. tritaeniorhynchus* larvae were collected from bed of Streams. Although in SarPhyusu village the highest density of *Cx. tritaeniorhynchus* larvae were collected from Concrete Jars (214) followed by Rice fields water (135) and lowest number of larvae were collected from Streams (3). In PaukGone and SarPhyusu villages Concrete Jars, Bago Jars and Metal drums were used to store of water for fire water. These containers were found high density of *Culex* and *Aedes* larvae. In *Anopheles* group as *An. barbirostris An. paeditaeniatus* and *An. subpictus* isolates and Mansonia group as *Ma. Annulifera, Ma. indiana* and *Ma. uniformis* isolates were found JE virus positive [47]. *Cx. vishnui* groups are present in many countries of south-east Asia [52,53]. These mosquitoes are usually found in rural rice growing and pig-farming regions of Asia, but can also be found at the outskirts of cities in close proximity to human populations. They prefer to breed in rice fields, and outbreaks of JE are commonly associated with intensive rice cultivation [27,28,49,50,54].

Other researchers observed that *Culex* mosquito's larvae were abundantly present in stagnant water pools and in rice field water and also knowledge of respondents about breeding site of *Culex* mosquito was high (61.6%) [28]. Yadav and Ahmad [55] revealed that only 36.3% of households agreed that stagnant water can be a potential breeding site for mosquitoes. A similar study from Pondicherry reported that 70% of house answered *Culex* mosquito's larvae in stagnant water and this type of community perception favors mosquito breeding in their areas [56,57].

In the present study, the susceptibility status of collected mosquitoes were found susceptible to Deltamethrin 0.05%, Permethrin 0.75% and Cyfluthrin 0.15% within 0ne hour. Although *Cx. tritaeniorhynchus, Cx. vishnui, Cx. quinquefasciatus, An. barbirostris* and *An. hyrcanus* were found 20-30 resistant to Deltamethrin 0.05%, Permethrin 0.75%, Cyfluthrin 0.15%. It means that JE vectors can be controlled by LLINs nets. In China and India, a population-based case-control study found that use of insecticide treated nets was associated with significant reduction in JE cases (Yadav and Ahmad) [56]. Same result has been found in Letpadan Township Bago Region, Chaung Zone and Ye Townships in Mon State and Wakema Township in Ayeyawady Region [27,28,48]. Although *An. barbirostris* was found Knockdown resistance to Deltamethrin 0.05% in Bunmout Township Sagaing Region [58].

In the villages over 80% of JE vaccination covered under15 age group, although JE transmission risk is very high in remaining children in Ein-me Township, and need to control mosquito population to reduce JE transmission risk. Study reveals the abundance and prevalence of the major vector *Cx. tritaeniorhynchus* and secondary vector *Cx. vishnui* in studied villages in Ein-me Township. The maximum number of main vectors *Cx. tritaeniorhynchus* and secondary vector *Cx. vishnui* mosquitoes and their larvae were prevalent in these areas and this will enable village's people and authority concerns to develop appropriate vector control strategies in villages.

An alternative to vaccination of livestock is to control the vector. This can target the adult through application of insecticide, and using insecticide treated nets, LLINs nets and fogging with chemicals such as pyrethrins, malathion, and spray the wall of houses and pig farms with insecticides to prevent mosquito bite or by introducing mosquito-proof screens to animal housing to prevent vector access. Alternatively, the juvenile stages can be targeted with larvicides such as Bacillus thuringiensis toxin and Abate (Temephos 1% sand granule). However, both are costly and in the case of insecticide use, can have negative environmental consequences on non-target species. Using Larvivorous fishes in water sources are more effective to control mosquito larvae and eco-friendly and it should be used in JE endemic areas.

Risk factor: Pig farms, mosquito breeding sources as rice fields and water pools are situated very close to human dwelling (5-15 meters). In each village, 20 to 50% of the households have 1-15 pigs in one to two pig farms and 90% of the pig farms are open type. Main JE vector breeding sites as polluted water creeks, pools, gutters, hyacinth vegetation's, long term water storage containers for fire and rice fields are situated in and beside the villages and also *Culex* larvae were abundantly presented. Most of the villagers and their family members have not used mosquitoes net when they go to sleep. All the human dwelling are situated within the flight range of JE vector (1.5 Kilo meter).

## 5. Conclusion

Japanese Encephalitis is rising throughout Asia, because epidemics are typically noticed only after outbreaks. This study helps not only to improve the awareness of JE in the community but also to reduce the transmission risk and to fill the knowledge gaps of stakeholders and community members to implement an integrated one health approach at village level in high-risk endemic regions. Pigs are main host for JE virus, *Cx. tritaeniorhynchus* adult and larvae were abundantly collected in polluted water pools and rice fields in all selected villages. Suspected JE cases were available in villages. Implementation of a vaccination program for young children, as well as modified agricultural practices, pig vaccination, rigorous monitoring, prevent man vectors contact, destroying breeding habitats, vector control and improved living standards can reduce the number of JE cases. This study provides further information on risk of JE transmission in study areas. Environmental and ecological factors are responsible for the spread of JE virus. All the collected mosquitoes were susceptible to WHO recommended insecticides. Therefore, the study recommended that distribute long lasting insecticides treated nets (LLINs nets) to all villages and health education to all family members to prevent JE transmission in villages. Further study of man, vector, pigs relationship should be determined. Risk factors of man, Households, pig and pig farms association should be searched. JE vector competence should be studied in local context under local climate condition to achieve reliable data.

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**Competing Interests Statement**

The authors declare no competing financial, professional, or personal interests.

**Consent for publication**

The authors declare that they consented to the publication of this study.

**Authors' contributions**

All the authors took part in literature review, analysis and manuscript writing equally.

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