UNIVERSITI PUTRA MALAYSIA

# A REVIEW ON THE POSSIBLE ROLE OF RODENT IN THE TRANSMISSION OF MOSQUITO-BORNE FLAVIVIRUS

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# A REVIEW ON THE POSSIBLE ROLE OF RODENT IN THE TRANSMISSION OF MOSQUITO-BORNE FLAVIVIRUS

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**DEDICATIONS**

To those who encouraged me until the very end.

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| | |
|---|---|
| BAGV | Bagaza virus |
| BKWV | Batu Kawa virus |
| CPE | Cytopathic effect |
| DENV | Dengue virus |
| ELISA | Enzyme-linked immunosorbent assay |
| ENSO | El Nino and La Nina-Southern Oscillation |
| ENTV | Entebbe bat virus |
| JEV | Japanese encephalitis virus |
| KFDV | Kyasanur forest disease virus (KFDV) |
| LGTV | Langat virus |
| LIV | Louping ill virus |
| MVEV | Murray Valley encephalitis virus |
| NA | Not available |
| NS | Non-structural |
| PRNT | Plaque reduction neutralization test |
| RNA | Ribonucleic acid |
| SOKV | Sokuluk virus |
| SLEV | St Louis encephalitis virus |

| | |
|---|---|
| TABV | Tamana bat virus |
| TBEV | Tick-borne encephalitis virus |
| WHO | World Health Organization |
| WN-KOTUV | Koutango-lineage of West Nile virus |
| WNV | West Nile virus |
| YFV | Yellow Fever virus |
| YOKV | Yokose virus |
| ZIKV | Zika virus |

## ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir.

**TINJAUAN TERHADAP PERANAN RODENT DI DALAM PENULARAN FLAVIVIRUS BAWAAN NYAMUK**

**Oleh**

**Shariffah Salmiah Binti Syed Mohd**

**2021**

**Penyelia: Dr Nor Yasmin Abd Rahaman**

*Flaviviridae* adalah famili virus envelop, RNA single-stranded positif yang menjangkiti mamalia dan burung, dan mamalia dikenali sebagai perumah buntu. Flavivirus adalah salah satu genera yang dikelaskan di bawah keluarga *Flaviviridae* dan disebarkan oleh vektor arthropoda seperti nyamuk dan sengkenit. Flavivirus bawaan nyamuk adalah genus yang diketahui dan melibatkan pelbagai perumah vertebrata. Flavivirus bawaan nyamuk juga dikenalpasti tersebar di kalangan roden seperti virus Denggi, virus West Nile, virus Japanese Encephalitis, virus Usutu dan virus Wesselsbron. Kebanyakan spesis roden yang hidup secara bersama dengan populasi manusia membawa kepada pelbagai penjalaran penyakit, sama ada secara langsung atau tidak langsung. Oleh itu, kemungkinan penglibatan roden dalam mengedarkan Flavivirus bawaan nyamuk boleh meningkatkan penjalaran virus kepada manusia. Walaupun masih kekurangan bukti tentang peranan roden, terdapat bukti kukuh perkaitan roden dan flavivirus bawaan nyamuk. Pengesanan virus membantu dalam memahami beberapa kemungkinan peranan roden, seperti perumah penguat di mana roden membina tahap viremia yang mencukupi dan dapat menjalarkan virus kepada nyamuk. Roden juga boleh menjadi perumah reservoir di mana roden membawa flavivirus secara asimtomatik. Jika roden tidak membina tahap viremia yang mencukupi untuk menjangkiti nyamuk, mereka dikenali sebagai perumah buntu dalam penjalaran virus. Kawalan populasi roden dan kawalan pembiakan nyamuk serta vaksinasi boleh dilakukan untuk membendung penjalaran virus itu. Lebih banyak penyelidikan dan pengamatan diperlukan terhadap interaksi antara roden, nyamuk dan virus untuk mengenalpasti peranan roden dalam penjalaran flavivirus bawaan nyamuk.

**Kata kunci:** Flavivirus; nyamuk; roden; peranan; penjalaran

**ABSTRACT**

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD4999 – Final Year Project.

**A REVIEW ON THE POSSIBLE ROLE OF RODENT IN THE TRANSMISSION OF MOSQUITO-BORNE FLAVIVIRUS**

By

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**2021**

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*Flaviviridae* is an enveloped, positive-stranded RNA virus family that mainly infects mammals and birds, with mammals known to be the dead-end host. Flavivirus is one of the genera classified under the *Flaviviridae* family and is transmitted by arthropod vectors such as mosquitoes and ticks. Mosquito-borne Flavivirus are the most common in the genus and involve a wide range of vertebrate hosts. Mosquito-borne Flavivirus is also known to be circulating among rodents such as Dengue virus, West Nile virus, Japanese Encephalitis virus, Usutu virus and Wesselsbron virus. The numerous species of rodents living with the human population leads to various disease transmission, either directly or indirectly. Hence, the possible involvement of rodents in circulating the mosquito-borne Flavivirus may enhance the transmission of the virus to humans. Even though there is still a lack of evidence on the definite role of rodents, there is strong evidence on the association of rodents and the mosquito-borne Flavivirus. Detection of the virus helps in understanding several possible roles of rodents, such as an amplifying host whereby the rodents develop sufficient viremia levels and are able to pass the virus to the mosquitoes. Rodents may also become a reservoir host such that the rodents are asymptomatically carrying the flavivirus. If the rodents do not develop sufficient viremia levels to infect the mosquitoes, they are known to be a dead-end host in the transmission of the virus. Rodent population and mosquito breeding control can be done to control the disease transmission. Vaccination can also be done to curb the spread of the virus. More research and surveillance are warranted on the interaction between rodents, mosquitoes and the virus to establish the definite role of rodents in the transmission of mosquito-borne flavivirus.

**Keywords**: Flavivirus; mosquito; rodents; role; transmission

# 1.0 INTRODUCTION

Flaviviridae is an enveloped, positive-strand RNA virus that mainly infects mammals and birds, with mammals known to be the dead-end host (Beth *et al.,* 2009). There are four genera categorised under the Flaviviridae family known as Hepacivirus, Pegivirus Pestivirus and Flavivirus (Payne, 2017). Briefly, the Hepacivirus consist of the Hepatitis C virus strain which is known to be a blood-borne hepatitis virus that causes chronic infection and persists in infected hosts for decades (Payne, 2017). In Pestivirus, three viruses are known to be significant in the veterinary field including bovine viral diarrhoea virus (BVDV), border disease virus (BDV) of sheep and goats, and classical swine fever virus (CSFV) (Payne, 2017). These viruses are known to be transmitted through contact and also to the fetus via vertical transmission (Payne, 2017). Pegivirus is known to cause persistent infection in humans and also animals, however, there is still a lack of information on its role in disease development (Payne, 2017).

Meanwhile, viruses' species under flavivirus genus is one of the common arboviruses (arthropod-borne virus), are believed to be existing and circulate between the hematophagous arthropod and varieties of the vertebrate host (Blasdell *et al.*, 2021). Mosquito-borne flavivirus largely contributes to the emerging and re-emerging of flavivirus infection worldwide, infecting various host species involving vertebrate hosts (Sotomayor *et al.*, 2018). Millions of human populations along with domestic animals and wildlife are affected with the mosquito-borne Flavivirus (Sotomayor *et al.*, 2018). The major concern in the infection of the mosquito-borne Flavivirus is that, this virus can cause infection in the nervous system that can result in high morbidity and mortality in host (Chesnut *et al.,* 2019).

Several reports have shown the detection and isolation of mosquito-borne flavivirus in rodents. In a report mentioned by (Sotomayor *et al.,* 2018), WNV has been isolated in rodents (*Sciuris Carolinensis*) and DENV are isolated in the urban rodents (*Mus musculus and Rattus rattus*) in the United States of America. Fall *et al.,* (2021) had also reported the first isolation of Koutango lineage of West Nile virus (WN-KOUTV) in Tatera Kempi, the wild rodent in Senegal in 1968. Other than WN-KOUTV, (Diagne *et al.*, 2019) had reported the first evidence of isolation of Usutu virus in rodents in Senegal with all the rodents showing no clinical signs or asymptomatic. Other than that, (Diagne *et al.,* 2017) described the emergence of Wesselsbron virus in rodents in the Eastern Senegal. In Malaysia, the first mosquito-borne flavivirus, Batu Kawa virus was isolated in a peridomestic rodent species (*Sundamays Muelleri*) (Blasdell *et al.,* 2021). Even though the virus was isolated in only one rat, the virus was detected in multiple organs and also in the serum indicating the true infection of the virus in the rat (Blasdell *et al.,* 2021). In China, large numbers of Rattus norvegicus and Rattus losea Swinhoe have been reported to have reactive antibodies against Japanese encephalitis virus (Chen et al., 2016). However, there was no detection of the viral RNA in the rodents detected with the antibodies against JEV (Chen et al., 2016).

Rodents are known to be the largest mammal group and have a very close association to human populations (Musser, 2020). Rodents are known to be the reservoir host for at least 60 zoonotic diseases, who play vital role in the transmission of these zoonotic diseases (Dahmana *et al.*, 2020). Even though mosquito-borne flavivirus are known to be one of the zoonotic diseases, there is still absence of critical review on the role or rodent in the transmission of mosquito-borne flavivirus globally.

Therefore, the objectives of this review are;

1. To review on the virological aspects related to the knowledge of mosquito-borne flavivirus infection.
2. To discuss on the possible role of rodents in transmission of the mosquito-borne flavivirus.

# 2.0 LITERATURE REVIEW

## 2.1 VIROLOGICAL ASPECTS OF FLAVIVIRUS

### 2.1.1 Properties of flavivirus genus

Flavivirus is categorized under the family Flaviviridae. Flavivirus is an enveloped, non-segmented single stranded positive sense RNA virus with the genome of approximately 11 kb. The diameter of the flavivirus virions are between 40 and 60 nm (1 nm = $10^{-9}$ metre) with spherical shapes virus particle (Britannica, 2018, November 30). Generally, flavivirus consist of three structural proteins, which are capsid (C protein), membrane (prM), and envelope (E protein), and seven non-structural proteins, which are NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (Mukhopadhyay *et al.*, 2005).

### 2.1.2 Vector-borne Flavivirus and its common host

The Flavivirus genus consists of arboviruses that are mainly transmitted by mosquitoes and ticks (Laureti et al., 2018). It consists of more than 70 small, positive-sense, single-stranded RNA viruses including the viruses that infect humans globally such as West Nile virus (WNV), Japanese encephalitis virus (JEV), dengue virus (DENV), Murray Valley encephalitis virus (MVE), tick-borne encephalitis virus (TBEV), Yellow Fever virus (YFV), and Zika virus (ZIKV) (Laureti *et al.*, 2018). The general flavivirus species with its respective vectors can be observed in Table 1 (Kuno *et al.,* 2017). Overall, mosquito-borne flaviviruses are the most common with 40 flaviviruses found known to be transmitted by mosquitoes, followed by 18 flaviviruses found with unknown host and 14 flaviviruses are known to be transmitted by ticks (Laureti *et al.*, 2018). Most flaviviruses are known to be detected in a wide range of vertebrate hosts. However, the dengue virus and zika viruses are known to be specifically infecting primates and humans (Laureti *et al.*, 2018). However, recent studies have shown that dengue viruses are able to be isolated from dogs, making dogs one of the potential hosts for the dengue virus (Laureti *et al.*, 2018)

Other than that, flaviviruses are also detected in the avian species, especially poultry (Liu *et al.*, 2013). The Flaviviruses isolated in the avian species are Usutu virus, Bagaza virus, Israel Turkey meningoencephalitis virus, West Nile virus and Tembusu virus. Usutu virus cause death in the migratory birds and poultry, Bagaza virus and Israel Turkey meningoencephalitis virus resulted in neurological diseases and deaths in domestic turkeys (*Meleagris gallopavo*), red-legged partridges (*Alectoris rufa*), ring-necked pheasants (*Phasianus colchicus*) and common wood pigeons (Sudeep *et al.,* 2013). Chickens, sparrows and ducks infected with Tembusu virus will develop encephalitis (Liu *et al.*, 2013).

**Table 1. Flavivirus and its respective vectors and major host**

| Vector | | Virus | Major host |
|---|---|---|---|
| **Vector** | Mosquito | Bagaza Virus (BAGV) | birds |
| | | Dengue virus | primates (monkeys and humans |
| | | Japanese encephalitis virus (JEV) | birds; pig; horse |
| | | Murray Valley encephalitis virus (MVEV) | ardeid water birds |
| | | St. Louis encephalitis virus (SLEV) | birds |
| | | West Nile virus (WNV) | birds; horse |
| | | Yellow fever virus (YFV) | primates |
| | | Zika virus (ZIKV) | primates |
| | Ticks | Kyasanur forest disease virus (KFDV) | rodents; monkey |
| | | Langat virus (LGTV) | Rodents |
| | | Louping ill virus (LIV) | grouse; sheep |
| | | Tick-borne encephalitis virus (TBEV) | Rodents |
| **Non-vector** | | Entebbe bat virus (ENTV) | Bats |
| | | Sokuluk virus (SOKV) | Bats |
| | | Tamana bat virus (TABV) | Bats |
| | | Yokose virus (YOKV) | Bats |

Note: This table shows the vector and non-vector borne flavivirus species with its respective hosts. Vector for the transmission of flavivirus mainly involve the mosquitoes and ticks with most of the virus are transmitted by the mosquitoes. The major hosts involve in the vector borne flavivirus varied while most of the non-vector borne involve bats as the major host.

### 2.1.3 Mosquito species and type of Flavivirus

Mosquito-borne Flavivirus largely contributes to the emerging and re-emerging of flavivirus infection worldwide, infecting various host species involving vertebrate hosts (Sotomayor *et al.*, 2018). The diseases are transmitted by many mosquito species that feed on varieties of vertebrate hosts. The two mosquito species that are involved in flavivirus transmission are the *Stegomyia* mosquito species such as *Aedes*, and *Culex* mosquito species (Huhtamo *et al.*, 2014). Yellow fever virus and dengue virus are known to be transmitted by Aedes, while West Nile virus, Japanese encephalitis virus and St Louis encephalitis virus are known to be transmitted by Culex species which are characteristically maintained in life cycles involving birds (Huhtamo *et al.*, 2014). The mosquito-borne Flavivirus are transmitted through two ways which are horizontal transmission and vertical transmission (Huang *et al.*, 2014). The horizontal transmission is responsible for causing infection to the vertebrate host by transferring the viral particles when the mosquitoes feed on the host (Huang *et al.*, 2014). The vertical transmission also termed transgenerationally, is mainly involved in maintaining the viral particle solely in the mosquitoes and in the environment (Huang *et al.*, 2014). The abundance of mosquitoes in the environment aids in the Flavivirus infection among humans, domestic animals and wildlife populations (Sotomayor et al., 2018). Some of the worldwide threat mosquito-borne flavivirus includes the dengue virus, zika virus, and West Nile virus (Sotomayor *et al.*, 2018).

### 2.1.4 Mosquito-borne Flavivirus in Rodents

From the previous studies, mosquito-borne flavivirus is also known to be circulating in the rodent population. Serological studies have shown evidence of several virus detected in rat populations such as DENV and WNV. In the USA for instance, WNV has been isolated in rodents (*Sciurus carolinensis*) and DENV have been isolated from neotropical rodents that inhabit the sylvatic, rural and urban areas (Sotomayor *et al.,* 2018). DENV is also isolated from the urban rodents (*Mus musculus* and *Rattus rattus*) (Sotomayor *et al*., 2018). Koutango lineage of West Nile virus (WN-KOUTV) was first isolated in Tatera Kempi, the wild rodent in Senegal in 1968 also shows evidence of isolation of mosquito-borne flavivirus in rodents (Fall *et al.,*2021). In South-East Asia, Malaysia, the first mosquito-borne flavivirus, Batu Kawa virus (BKWV) was detected in a peridomestic rodent species, *Sundamys Muelleri* in 2016, Sarawak (Blasdell *et al*., 2021). Even though the BKWV was isolated only in one rat, indicating that this species might not be the natural host of the virus, the BKWV was detected in multiple organs together with its serum, providing evidence of genuine infection in the rat (Blasdell *et al*., 2021).

In China, even though there was neither detection of JEV RNA in the rodents species nor any evidence on the role of rodents in the transmission of the JEV in China, large numbers of Rattus norvegicus and Rattus losea Swinhoe were found to have reactive antibodies against JEV (Chen *et al*., 2016), thus further research on the involvement of rodents in the transmission of JEV are warranted. Other than that, the Usutu virus, which is a Culex-associated mosquito-borne Flavivirus, was also isolated from rodent species with its first evidence of isolation in Senegal (Diagne *et al.*, 2019). All rodents detected with the Usutu virus were asymptomatic. Generally, humans and birds are the common hosts infected by the Usutu virus that will elicit neurological signs. Lastly, other than the WNV and Usutu Virus, (Diagne *et al.*, 2017) also described the emergence of the Wesselsbron virus in Eastern Senegal. The detection of mosquito-borne Flavivirus and its antibodies in rodents based on the worldwide geographical region is demonstrated in Figure 1 and the species of rodent detected in each flavivirus are summarised in Table 2.

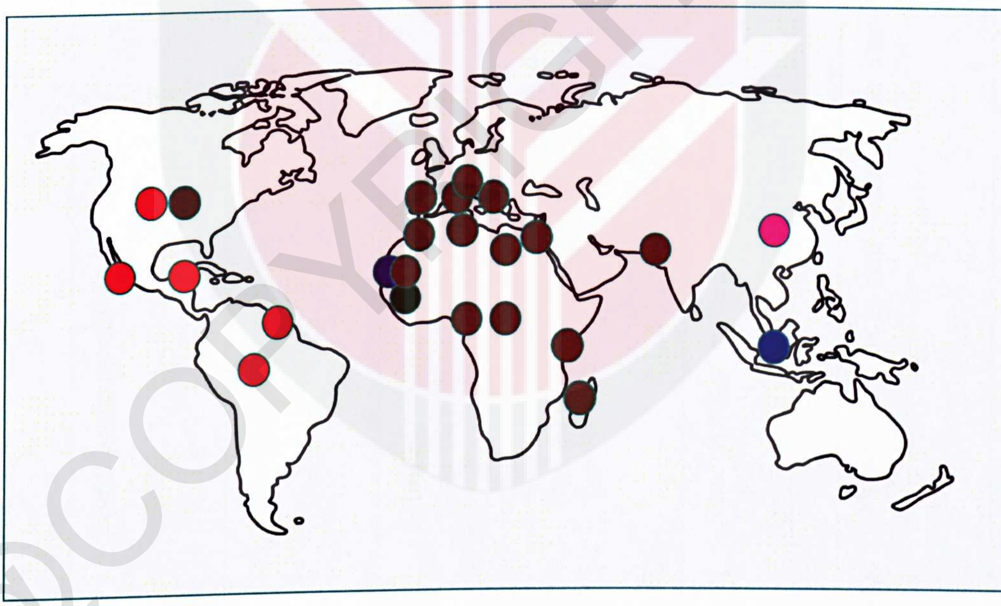

**Figure 1. The worldwide distribution of mosquito-borne flavivirus detected in rodents. Brown (West Nile virus), red (dengue virus), blue (Batu Kawa virus), light purple (Japanese encephalitis virus), green (Usutu virus), darker purple (Wesselsbron virus)**

**Table 2. Species of rodents detected with mosquito-borne flavivirus**

| Flavivirus species | Species of rodent | References |
| --- | --- | --- |
| Batu kawa virus | *Sundamys muelleri* | (Blasdell *et al.*, 2021) |
| Usutu virus | *Mastomys natalensis* & *Rattus rattus* | (Diagne *et al.*, 2019) |
| Wesselsbron virus | *Rattus rattus* | (Diagne *et al.*, 2017) |
| Dengue virus | *Mus musclus* & *Rattus rattus* | (Cigarroa, 2016) |
| West Nile virus | *Sciurus niger*, *Rattus rattus* | (Jeffrey, 2012) |
| Japanese encephalitis virus | *Rattus norvegicus and Rattus losea Swinhoe* | (Chen *et al.*, 2016) |

## 2.2 CLASSIFICATION OF RODENTS

### 2.2.1 Species of rodents

Rodents under the order of Rodentia, are mammals that are characterised by the lower and upper pairs of incisor teeth. The incisor teeth are rootless and ever-growing. Rodents contribute to the largest group of mammals and consist of half of the mammalia's class with approximately 4660 species (Musser, 2020). There are a total of five suborders that are classified under the order of Rodentia. The suborder, families and species that are categorized under the order of Rodentia (Musser, 2020) are summarized in Table 3.

**Table 3. Latest classification of Rodentia**

| Suborder | Family | Species |
|---|---|---|
| **Myomorpha (mouse-like rodents)** | 1) Muridae | True rats and mice |
| | 2) Dipodidae | Birch mice, jumping mice and jerboas |
| | 3) Geomyidae | Pocket gophers |
| | 4) Heteromyidae | Forest spiny mice, pocket mice, kangaroo rats and kangaroo mice |
| | 5) Myoxidae | Dormice |
| **Sciuromorpha (squirrel-like rodents)** | 1) Sciuridae | Squirrels |
| | 2) Castoridae | Beavers |
| | 3) Aplodontidae | Mountain beaver or sewellel |

| | | |
|---|---|---|
| **Hystricognatha (porcupine-like rodents** | 1) Echimyidae | American spiny rat |
| | 2) Octodontidae | Rock rats, degus, viscachas, viscacha rats, coruro, and tuco-tucos |
| | 3) Capromyidae | Hutias |
| | 4) Erethizontidae | North American, prehensile-tailed, stump-tailed, hairy dwarf, and thin-spined porcupines |
| | 5) Caviidae | Guinea pigs, cavies & maras |
| | 6) Dasyproctidae | Agoutis & acouchis |
| | 7) Bathyergidae | Blesmols |
| | 8) Hystricidae | African & Asian porcupines |
| | 9) Abrocomidae | Chinchilla rats |
| | 10) Chinchillidae | Plains viscacha & Chinchillas |
| | 11) Agoutidae | Pacas |
| | 12) Thryonomyidae | Cane rats |
| | 13) Dinomyidae | Pacarana |
| | 14) Hydrochoeridae | Capybara |
| | 15) Petromuridae | Dassie rat |
| | 16) Myocastoridae | Nutria |
| **Anomaluromorpha** | 1) Anomaluridae | Anomalures |
| | 2) Pedetidae | Spring hare |

| | | |
|---|---|---|
| **Sciuravida** | 1) Ctenodactylidae | Gundis |

The numerous numbers of families and species classified under the order of Rodentia allow the rodents to become reservoir hosts for many zoonotic pathogens that are transmissible to humans (Bordes *et al.*, 2015). The current trend of declining in the numbers of individuals in a population, also known as anthropocene defaunation due to urbanization creates a huge window of opportunities for the rodents and other small mammals to become abundant and dominate the urban environment (Bordes *et al.*, 2015). This will lead to closer interaction between the human population and the innumerable species of rodents that increase the potential of disease transmission between small mammals and human beings. Hence, understanding the method of disease transmission is vital to further comprehend this unfavorable interaction.

### 2.2.2 Transmission of disease from rodents to human

Rodents, as discussed previously, are able to harbor numerous pathogens that can be transmitted to humans. In total, 85 unique zoonotic pathogens have been documented to be carried by the rodent population making them one of the major taxa to be carrying zoonotic diseases. In addition to that, rats and mice alone have been reported to play an important role in spreading 35 zoonotic diseases rat bite fever, tularemia, hantavirus, lymphocytic choriomeningitis virus, other arenavirus infections, leptospirosis, salmonellosis, yersiniosis, pathogenic E. coli infections, campylobacterosis, giardiasis, and Lyme disease (Musser, 2020). Therefore, understanding the method of disease transmission is important to prevent the rise of cases circulating between rodents and humans. Generally, rodents can transmit diseases to humans directly and indirectly. Direct transmission primarily involves the contact of an infectious animal (Wesley *et al.*, 2010), by handling live or dead animals, animal's bite, and saliva containing the pathogen.

In an indirect transmission, rodents' excreta such as urine and faeces will contaminate the environment such as soil, and when in contact, humans will be infected (Wesley *et al.*, 2010). Other than environmental contamination, the disease can also be transmitted through aerosol. For instance, Hantavirus that is carried by rodents usually causes spillover to humans by inhaling the viral particles that are aerosolized (Wesley *et al.*, 2010). Indirect transmission can also occur through ticks, fleas, mites and mosquitoes that have previously fed on the infected rodents also known as vector-borne. Flavivirus specifically is arboviruses that are mainly transmitted through vectors such as ticks and mosquitoes. Previously, there is evidence of mosquito-borne flavivirus that was potentially transmitted from and infected rodents. This will further be discussed throughout the review.

## 2.3 RISK FACTORS OF MOSQUITO-BORNE FLAVIVIRUS

### 2.3.1 Vector

Mosquitoes is a competent vector that is responsible in the transmission of the mosquito-borne flavivirus (Ruckert & Ebel, 2018). A competent vector is a vector that acquired viruses through feeding that will then cause the multiplication of the virus in the vector (Ruckert & Ebel, 2018). The virus will then be inoculated to the host cutaneous vasculature when the vector feed on the host that will cause the establishment of an infection (Ruckert & Ebel, 2018). By looking at the feeding pattern of the mosquitoes, it can be classified as anthropophilic mosquitoes that feed on human blood (Scott & Takken, 2012) and also ornithophilic mosquitoes that feed on the bird blood (Chathuranga et al., 2017). In an anthropophilic mosquitoes, it is known that the bloodmeal is ingested by adult female during each ovarian cycle (Scott & Takken, 2012). Mosquito *Aedes aegypti* that carries the yellow fever virus are reported to ingests multiple human bloodmeal each time when laying egg (Scott, 1993). The multiple blood feeding by the mosquito *Aedes Aegypti* causes the increase in feeding frequency of the mosquitoes that contributes to the increase in the reproductive rate of mosquito-borne pathogens, hence elevating the transmission of the virus such as the yellow fever virus and the dengue virus (Scott & Takken, 2012). For the ornithophilic mosquitoes, the bloodmeal preference are the bird's blood and have higher chance of transmitting various pathogenic agents (Cerny & Svobodova, 2011). In the context of mosquito-borne flavivirus, (Cerny *et al.,* 2011) also reported that the transmission of the West Nile virus from birds to mammals are facilitated by the ornithophilic mosquitoes. Lastly, in discussing the competency of the mosquitoes as a vector in the transmission of mosquito-borne flavivirus, a species of mosquito is also able to carries multiple pathogens such as mosquito *Aedes aegypti* (Ruckert & Ebel, 2018). The *Aedes aegypti* carries multiple mosquito-borne flavivirus such as dengue virus, yellow fever virus and zika virus (Ruckert & Ebel, 2018). So, when a host is bitten by an Aedes aegypti, they are also at risk of infection of these three viruses (Ruckert & Ebel, 2018).

### 2.3.2 Agent

The Flavivirus is also able to cause persistent infection in a host and favors in the transmission of the virus to other vertebrate hosts (Goic & Saleh, 2012). In a persistent infection, the flavivirus will not be cleared up by the immune system of the host and are able to stay in the host cell for a long period of time. In addition to that, the persisting flavivirus is able to infect other viable host cells (Salas-Benito & De Nova-Ocampo, 2015). Based on a previous study, the evidence of persistent infection of the flavivirus has been demonstrated experimentally by inoculating the DENV in a mosquito cell line (Chen *et al.,* 1994; Kanthong *et al.,* 2008; Juarez-Martinez *et al.,* 2012). Cytopathic effect (CPE) can be observed four to seven weeks post-infection, but, as the infection progresses, the CPE can no longer be observed (Kanthong *et al.*, 2008; Juarez-Martinez *et al.*, 2013). However, the viral protein and genome can still be detected in the infected cell (Chen *et al.*, 1994; Tsai *et al.*, 2007). This shows the evidence of the non-pathogenic, persistent, flaviviral infection. Not just that, the detection of the viral protein without showing any pathogenic changes or abnormalities also suggests the balance between the viral replication and also the antiviral immune response elicited by the host cell (Oldstone, 2006; Asgari, 2014).

Rodents are also known to be the potential reservoir for flavivirus that is responsible for transmitting the disease to other vertebrate hosts. In 2013, Wesselsbron virus, which is a mosquito-borne Flavivirus, was isolated in *Rattus rattus* (black rat) and also in two humans with acute febrile illness (AFI) in Eastern Senegal (Diagne *et al.*, 2017). Interestingly, based on the result of the analysis, the strain of the Wesselsbron virus isolated in the black rat is the same as in the infected human (Diagne *et al.*, 2017). This result indicates that the rodents may contribute to the transmission of the mosquito-borne Flavivirus to humans (Diagne *et al.*, 2017).

### 2.3.3 Host

Mosquito-borne Flavivirus can be detected in a wide host range involving vertebrate hosts such as human and non-human primates, birds, pigs, and even rodents (Sotomayor *et al.*, 2018). The transmission of flavivirus between the vector and the hosts involve three cycles which are enzootic cycle (sylvatic cycle), epizootic cycle (rural cycle) and epidemic cycle (urban cycle) that can be observed in Figure 2 (Health Jade Team, 2020). In the enzootic cycle or in the jungle cycle, wild animals, also known as the reservoir vertebrate host transmit the flavivirus to the mosquitoes (Health Jade Team,2020). The virus will multiply and amplified in the mosquitoes to be transmitted to other hosts (Health Jade Team, 2020). As a reservoir host, the wild vertebrate hosts do not elicit any clinical signs or illness in transmitting the virus (Health Jade Team, 2020). Next, in the epizootic cycle or in the rural cycle, domestic animals, also known as the amplifying host transmit the flavivirus to the mosquitoes (Health Jade Team, 2020). This may result in an epidemic outbreak whereby the virus is amplified in the domestic animals and to some extent human can also be affected (Health Jade Team, 2020). Lastly, in the epidemic cycle or the urban cycle, mosquitoes are infected with the flavivirus from human due to the high viremia level develop in the human (Health Jade Team, 2020). If human does not develop sufficient viremia level, then the transmission of the virus does not occur between the human and mosquitoes (Health Jade Team, 2020). In this scenario, humans are known to be the dead-end host or the incidental host in the transmission of the flavivirus (Health Jade Team, 2020).

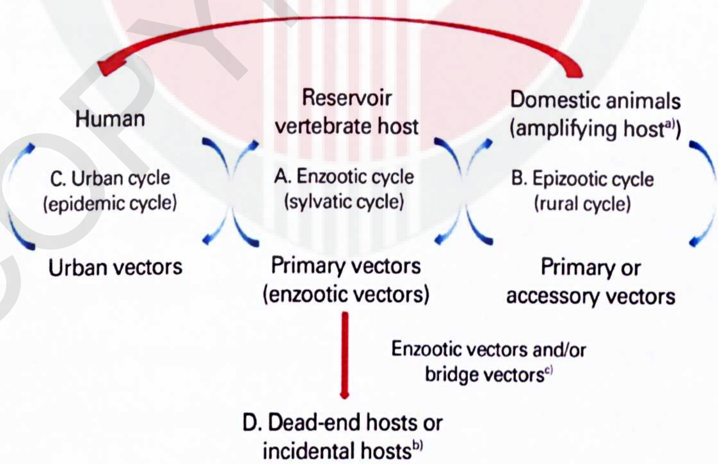


**Figure 2. Arbovirus vertebrate host and vector transmission cycles. (Health Jade Team, 2020)**

In the transmission of the Japanese encephalitis virus (JEV) where *Culex* spp are known to be the vector, pigs and aquatic birds are known to be the amplifying host for the virus (Huang *et al.*, 2014). Humans infected with the Japanese encephalitis virus are not able to have sufficient viremia to reinfect the mosquitoes, thus, humans are known to be the dead-end host for JEV (Halstead & Thomas, 2010; Rosen L, 1986; Go *et al.,* 2014). In an enzootic cycle of the West Nile virus (WNV), humans and horses are known to be the dead-end host (Go *et al.,* 2014; Blitvich, 2008), while mammalian and avian are known to be the reservoir host for the virus, and the WNV is maintained in the environment via the reservoir host (Huang *et al,* 2014). In some recent studies, some species develop sufficient viremic thresholds that are able to cause transmission of the virus to the vectors, including certain species of tree squirrels (Sciurus spp.), eastern chipmunks (Tamias striatus), and eastern cottontail rabbits (Sylvilagus floridanus) (Jeffrey Root, 2012).

In a Yellow fever virus (YEFV), that is transmitted by *Aedes* spp, the virus is found circulating in both human and non-human primates. According to the World Health Organisation (WHO), there are three types of transmission involving the human and non-human primates, which are the Sylvatic cycle (Jungle), the intermediate cycle (semi-domestic) and lastly is the urban cycle. In a sylvatic cycle, monkeys play an important role as the primary reservoir host and cause the virus to be transmitted within the monkeys and occasionally humans. Intermediate cycles commonly involve both the human and also the monkeys and lastly, the urban cycle mainly involves the human-to-human transmission with a highly dense mosquito environment. Next, the dengue virus (DENV) is transmitted between non-human primates and humans. The hosts are infected with the dengue virus mainly through the urban cycle and also the sylvatic cycle. Apart from primates and humans, the detection of DENV RNA in the rodents, marsupials and bats provide sheer evidence that non-primates can also be the reservoir hosts for DENV (de Thoisy *et al.* 2009).

The severity and characteristics of the mosquito-borne flavivirus in a host depend on several factors. The immunity of a host is one of the factors that determine the infection of the virus. For instance, according to WHO, low immunity to the yellow fever virus is one of the factors affecting the transmission of the virus in the human population. Other than that, the age of the host also determines the severity of the Flavivirus infection. It was reported that, in a dengue virus infection, infants are more prone to have a severe infection in comparison to the other age groups (Pierson & Diamond, 2020). In addition to that, experimentally, younger mice are more susceptible to the lethal effect of the Japanese encephalitis virus than the older mice when injected peripherally, and to some extent, even when the virus is injected intracerebrally (Kimura *et al.*, 2010). The higher resistance to Japanese encephalitis virus in older animals when inoculated intracerebrally, or also known as age-dependent resistance are more prominent in the rats than mice (Kimura *et al.*, 2010). Lastly, the antibody-dependant enhancement (ADE) by the host antibody is also one of the factors that contribute to the severity of flavivirus infection. In a dengue virus infection, a host that was primarily infected with one of the four serotypes of DENV will develop more severe clinical signs when reinfected with a different serotype of DENV (Halstead *et al*., 1970; Kliks *et al*., 1989; Katzelnick *et al.,* 2017). It is believed that the available antibodies from the first infection, when bound to the new serotype of DENV, will enhance the secondary infection through the engagement of the Fc receptor of the antibody (OhAinle *et al.,* 2011).

### 2.3.4 Environment

Environmental factors significantly affect the transmission and maintenance of the mosquito-borne Flavivirus. Mosquitoes play a very important role in maintaining the replication of the virus in the mosquito as the vector to transmit the virus to the host. Favourable conditions of the environment aids in the rapid transmission of the mosquito-borne Flavivirus. Climate change influences the transmission of the Flavivirus from mosquitoes to the hosts throughout the mosquito life cycle (Impoinvil *et al*., 2013). Temperature is known to have a significant impact on the infectivity periods, viral replication and also adult vector survival (Wilder-Smith *et al*., 2008). According to (Kumar *et al*., 2018), the percentage of arboviruses that are transmitted by mosquitoes increases, and the incubation time of the infection decreases as the temperature increases. (Philander, 1990) suggest that the transmission of arboviruses is also largely influenced by the El Nino and La Nina-Southern Oscillation (ENSO), which is an irregular periodic warming phase of the sea surface of the tropical Eastern Pacific Ocean that affects most of the tropics and subtropics. This climate change has been reported to be one of the driving factors for Japanese encephalitis virus, West Nile virus and dengue virus infection (Tipayamongkholgul *et al.*, 2009; Paz, 2015).

Agricultural sites are also known as one of the mediums for mosquito breeding areas. Rubber plantations, for instance, provide a conducive environment for the mosquito to breed (Kumar *et al.*, 2018). Latex-collection cups, tree holes, and water storage tanks are the potential mosquito breeding sites in the rubber plantation (Thammapalo *et al.*, 2009; Paily *et al.*, 2013). In Thailand, an abundance of *Culex* spp is found in its rubber plantation together with its forest areas (Kumar *et al.*, 2018). Not just that, it was reported that dengue is an epidemic in rubber plantations (Tangena *et al.*, 2016). Other than rubber plantations, paddy fields are common grounds for the breeding of mosquitoes, mainly *Culex* spp. and *Anopheles* spp. (Kumar et al., 2018). This can also aid in the transmission of mosquito-borne Flavivirus. As an example, some studies reported that JEV cases in Korea are known to be related to the mosquito population in the rice fields (Richards *et al.*, 2010; Masuoka *et al.*, 2010). Rice farming is also known to be involved in the JEV transmission in several countries including Bangladesh, North Korea, Cambodia, Indonesia, Laos, Myanmar and Pakistan (Erlanger *et al.*, 2009). Next, Aedes spp can also be found in the oil palm plantation with Aegypti spp. found in the monoculture and the highest abundance of *Aedes* spp. 60.9% are known to be found in the polyculture in the rainforest of Southern Cote d'Ivoire (Zahouli *et al.*, 2017).

Apart from agricultural sites, the urbanisation process is also one of the major factors in causing the increase of mosquito breeding. An urbanisation process is known as the changing of the breeding site of the mosquitoes from the natural habitat to the artificial habitat. Artificial habitat in the urban area that permits the growth of mosquitoes are empty leftover tanks, used tires, and disposable containers. (Bartlett-Harley *et al.*, 2012) reported that in the United States, pupal and larval can be found higher in an urban area. Urban areas are also known to have fewer predators that leads to a higher capacity in supporting the larval evolution in urban habitats (Li *et al.*, 2014). In focusing on the transmission of flavivirus involving rodents, urbanisation is also known as one of the possible transmission methods. In one of the cases involving the transmission of Wesselsbron Virus (WSLV) between black rats, which is believed to be the reservoir host of the virus, and humans in the Kedougou region in Eastern Senegal, gold mining area and also environmental changes due to urbanisation is known to become one of the concerns that cause the transmission of the virus (Diagne *et al.*, 2017). Black rats are recognised as one of the rat species that are found abundantly in urban areas that contributes to the transmission of mosquito-borne flavivirus (Diagne *et al.*, 2017). The abundance of black rats in the urban setting leads to closer interaction between humans and also rodent species, increasing the threat of mosquito-borne Flavivirus transmission.

Furthermore, the accessibility to food and shelter plays an important role in increasing the rodent population in an area. Improper waste disposal, dirty and dishevelled gardens, together with the rising occupancy of domestic animals in an urban setting and human residences such as dogs, cats, livestock and other animal pets generally related to the rodent invasion (Feng & Himsworth, 2014; Bonnefoy *et al.,* 2008). The availability of shelters such as cracks in roofs, access points to sewer, high building density, abandoned buildings and soils contribute to the presence of rodents (Feng & Himsworth, 2014; Bonnefoy *et al.,* 2008). With the capability of colonizing areas within short distances, rodents are known to be abundant in high housing density (Bonnefoy *et al.*, 2008). Climate and season are also known to affect the rat population. When temperature increases, specifically in the winter season, rodents tend to look around for shelter, invading the housing area, this will cause closer interaction between humans and the rodents, lower the competition for food with higher access to food, subsequently leading to a higher growth rate of the rodents (Feng & Himsworth, 2014). However, there is still a lack of research and studies related to environmental factors that contribute to the transmission of mosquito-borne Flavivirus in rodents.

## 2.4 CLINICAL MANIFESTATIONS OF MOSQUITO-BORNE FLAVIVIRUS IN RODENTS

Generally, mosquito-borne flavivirus leads to various clinical manifestations depending on the type of virus involved. It was reported that nearly 50-80% of flavivirus infections resulted in asymptomatic to little or absence of illnesses (Burger-Calderon *et al.,* 2018; Endy *et al.,* 2002; Mostashari *et al.,* 2001). Self-limiting flu-like illness, headache, rash and myalgia are the most common symptomatic signs of flavivirus infection without having long-term consequences (Pierson & Diamond, 2020). However, flavivirus infection may also cause the hosts to manifest neurotropic disease, visceral disease and can even be congenital (Pierson & Diamond, 2020). Some flavivirus, such as the Zika virus, a mosquito-borne flavivirus, can cause both neurological and visceral disease (Pierson & Diamond, 2020). The differences in clinical signs shown are the results of cellular and tissue tropism of each virus, and the ability of the virus to evade the host immune system (Pierson & Diamond, 2020). The clinical manifestation of common mosquito-borne flaviviruses in humans, wild rodents and rodents used as models are summarised in Table 4.

### 2.4.1 Neurotropic disease

Several mosquito-borne flaviviruses that manifest neurotropic disease in the infected hosts are WNV, JEV and ZIKV. The neurotrophic characteristic of the virus leads to encephalitis, cognitive impairment, seizure disorders and paralysis (Maximova & Pletnev, 2018). In the hosts infected with these viruses, particularly humans, the infections may persist for a long period of time even after recovering from acute illness (Pierson & Diamond, 2020). It is known that these viruses cause damage to the neuron and lesions can be identified in the brainstem, cerebral cortex, hippocampus, thalamus, cerebellum or spinal cord (Ludlow *et al.*, 2016). In rodents, squirrels have been known to be susceptible to WNV neurological disease in a natural setting and the infected squirrels show signs of uncoordinated movement, paralysis, circling, lethargy and death (Padgett *et al.*, 2007). Mice that were inoculated with WNV causes encephalitis disease, whereby the virus crosses the blood-brain barrier and replicates in the nervous tissue (Byas & Ebel, 2020). Next, even though wild rodents are regarded as insignificant maintenance hosts for JEV (Anna, 2016), mice models inoculated with JEV through the conjunctival route exhibit viral encephalitis after four days post-inoculation (Sethi *et al.*, 2019). Lastly, wild rodents are known to be resistant to ZIKV due to innate genetic resistance (Kuno & Chang, 2005). However, (Dick, 1952) reported that after intracerebral inoculation of the ZIKV, the Swiss mice model became ill and died after one day due to the infection.



### 2.4.2 Visceral disease

Visceral disease related to mosquito-borne flavivirus are hemorrhagic fever, shock syndrome, hepatitis and hepatic failure. This manifestation can be elicited after being infected with DENV, YFV and ZIKV which are also known as the principal flaviviruses leading to visceral diseases in humans (Pierson & Diamond, 2020). DENV predominantly infect the myeloid cells in blood and tissues that causes vascular leakage, thrombocytopenia, abnormal bleeding, hemoconcentration and low blood pressure via immunopathogenesis cascade (Nhan *et al.*, 2001; Rothman, 2011). The NS1 protein of the virus is capable of binding to the endothelial cells that will result in the alteration of the vascular permeability due to the interference of the underlying glycocalyx, leading to low blood pressure (Beatty *et al.*, 2015; Puerta *et al.*, 2016). Ideally, (Yauch & Shresta, 2008) states that wild mice are resistant to DENV-induced disease. However, when used as animal models, certain immunodeficient mice manifest severe signs that are similar to the vascular-leak syndrome seen in humans having severe dengue after being inoculated with mouse-adapted DENV (Zompi & Harris, 2012). Next, the manifestation of visceral disease in a YFV infection such as severe hepatitis, renal failure, hemorrhage, shock and death are observed in humans due to high replication of YFV in the liver cells (Vieira *et al.*, 1983; Monath & Vasconcelos, 2015). According to WHO, YFV only causes infection in human and non-human primates, and to date, there is still a lack of information on YFV in wild rodents. Commonly, small animal models will show encephalitic signs when inoculated with YFV (Meier *et al.*, 2009). However, small animal models such as mice that lack type 1 interferon exhibit viscerotropic disease that is similar to humans with viral replication are observed in visceral organs such as the spleen and liver (Meier *et al.*, 2009). Type 1 interferon is responsible for protecting rodents from viscerotropic YFV infection. Lastly, ZIKV is known to infect progenitor cells, epithelium and myeloid cells, together with the peripheral tissues will cause damage to the reproductive tracts and eyes (Miner & Diamond, 2017). In a model mouse, apart from replication in the central nervous system causing neurological signs, ZIKV also replicates in multiple organs such as the liver and testis (Li *et al.*, 2018). However, wild type rodents are known to be resistant to ZIKV (Li *et al.*, 2018).

### 2.4.3 Congenital

The congenital disease can be seen in a host infected with a mosquito-borne Flavivirus such as ZIKV. Apart from being viscerotropic and neurotropic, ZIKV is also known as teratogenic, whereby it infects and causes injury to a developing placenta (Minor Diamond, 2017). Even though there is still lacking evidence on the ZIKV infection in rodents that can cause congenital disease, in vivo studies of mice models shows that ZIKV in pregnant mice are able to cross the transplacental barrier to infect the fetus that may cause death and if alive, the neonates develop microcephaly or other malformations of the brain (Li *et al.*, 2018).

**Table 4. Clinical manifestation of mosquito-borne flavivirus**

| Mosquito-borne flavivirus | Human | Rodents | | References |
|---|---|---|---|---|
| | | Wild | Models | |
| DEV | Vascular leakage, thrombocytopenia, abnormal bleeding, hemoconcentration and low blood pressure | Asymptomatic | Vascular leak syndrome | (Pierson & Diamond, 2020), (Yauch & Shresta, 2008), (Simon & Eva, 2012) |
| ZIKV | 1. Damage to the reproductive tracts and eyes (VD),<br>2. Encephalitis, cognitive impairment, seizure disorders and paralysis (ND)<br>3. Congenital | Asymptomatic | 1. Replicates in multiple organs such as the liver and testis (VD)<br>2. Encephalitis (ND)<br>3. Microcephaly or other brain malformations in neonates if not die (congenital) | (Pierson & Diamond, 2020), (Kuno & Chang, 2005), (Dick, 1952), (Li *et al.*, 2018) |
| YFV | Severe hepatitis, renal failure, haemorrhage, shock and death | NA | 1. Encephalitis (ND)<br>2. Viscerotropic disease that is similar to humans (VD) | (Pierson & Diamond, 2020), (Kathryn et al., 2009) |
| WNV | Encephalitis, cognitive impairment, seizure disorders and paralysis (ND) | Uncoordinated movement, paralysis, circling, lethargy and death | Encephalitis | (Pierson & Diamond, 2020), (Padgett et al., 2007), (Alex & Gregory, 2020) |
| JEV | Encephalitis, cognitive impairment, seizure disorders and paralysis (ND) | Asymptomatic | Encephalitis | (Anna, 2018), (Menaka et al., 2019) |

## 2.5 METHODS OF DETECTION AND DIAGNOSIS OF MOSQUITO-BORNE FLAVIVIRUS IN RODENTS

In facing the increasing threat and danger of flavivirus in various host species involving both human and animal health such as dengue, yellow fever, West Nile, Japanese encephalitis and many more, detecting and diagnosing the virus is very crucial to combat the disease. In diagnosing viral infection, two approaches can be used that involve the detection of specific antibody response, also known as serological methods, and detection of the presence of the viral antigen itself (Vainionpaa & Leinikki, 2008). In detecting the viral antigen, virus isolation is done from the patient's specimens, whereas polymerase chain reaction (PCR) is extensively used to detect viral nucleic acid (Vainionpaa & Leinikki, 2008) also known as the molecular method. Didier & Philippe (2020), suggested that in human-associated flavivirus infection, the diagnosis is initially based on the detection of the pathogen, its nucleic acid and specific viral antigen during the acute phase of infection followed by detecting specific antibodies at least a week after the infection. In the surveillance of mosquito-borne flavivirus in rodents, similar methods are used in detecting the presence of the antigen with its nucleic acid and evaluating the presence of circulating antibodies as an indicator of exposure.

Molecular diagnostic methods involve the demonstration of viral nucleic acid and viral antigen detection. Polymerase chain reactions (PCR) with specific primers are widely used for viral nucleic acid detection. In detecting mosquito-borne flavivirus, which is an RNA virus, reverse-transcriptase polymerase chain reaction (RT-PCR) are used, whereby the viral RNA is first transcribed by reverse transcriptase enzyme (RT) to a complementary DNA. Viral nucleic acid detection using the RT-PCR method is known to be rapid and more sensitive than virus isolation, thus faster diagnosis can be made (Vainionpaa & Leinikki, 2008). In rodents, samples such as blood, serum, swab sample from the nasopharyngeal and rectal, spleen, liver and cerebrospinal fluid can be taken from a rodent in detecting the presence of the mosquito-borne flavivirus viral RNA (Ayers *et al*., 2006).

In serological detection of mosquito-borne flavivirus, the two most common methods are the plaque reduction neutralization test (PRNT) and enzyme-linked immunosorbent assay (ELISA). PRNT is a test that measures neutralizing antibodies that neutralizes virions and prevent the virions from infecting the cells that are cultured (Sirivichayakul *et al.,* 2014). Currently, PRNT is known to be the gold standard in the detection of mosquito-borne flavivirus and flavivirus in general (Maeda & Maeda, 2013). In detecting the mosquito-borne flavivirus in rodents, serum sample can be taken to run the test. Even though PRNT is known to be serotype-specific in the flavivirus, it is known to be time consuming and take days to receive the results. Apart from PRNT, ELISA is also one of the rapid and common methods used to detect the mosquito-borne flavivirus in rodents. The detection of the antibody's presence is commonly used to indicate the surveillance to detect the previous infection or exposure to the virus (Chen *et al.,* 2006). Other than molecular and serological detection of mosquito-borne flavivirus, cell culture can also be used to detect the virus. The cell lines that are commonly use to culture the mosquito-borne flavivirus are mosquito cell lines, Aedes Albopictus C6/36, and mammalian cell line, African green monkey Vero cells (Huhtamo *et al.,* 2014). The cytopathic effect of the cell culture can be seen 4-7 weeks post-infection (Hoshino *et al.,* 2009). Cell lines are also known as an applicable model in studying the virus-host interaction (Fredericks *et al.,* 2019). However, the cell culture method is not commonly used in the detection of mosquito-borne flavivirus in rodents.

## 2.6 POSSIBLE ROLE OF RODENTS

The close association between rodents and humans is one of the major factors that concern the zoonotic potential of the disease carried by the rodent, including mosquito-borne Flavivirus. Detection and isolation of viral nucleic acid of the mosquito-borne Flavivirus and also antibodies against the virus in rodents provide evidence in the involvement of the rodent in the transmission of mosquito-borne flavivirus. As mentioned by (Sotomayor *et al.,* 2018), several roles can be played by the rodent, either as an amplifying host, reservoir host or the dead-end host.

### 2.6.1 Reservoir host

Most evidence suggests that rodents may play an important role as the reservoir host in the transmission of mosquito-borne Flavivirus. For instance, the Dengue viral RNA was detected in wild rodents in the USA suggesting that they might be a reservoir host for the Dengue virus (de Thoisy *et al.* 2009). Other than the Dengue virus, (Diagne *et al.*, 2019) suggest that rodents (*R. Rattus* and *M. natalensis*) are the possible reservoir for the Usutu virus. This is because the rodents do not show any symptoms or changes pathogenetically at the time of detection. Apart from the viruses mentioned, rodents are also known to possibly be a reservoir host for the Wesselsbron virus from the detection of the virus in the asymptomatic black rats (Diagne *et al.,* 2017). However, the writer emphasized that the reservoir status of the rodents in the transmission of the Wesselsbron virus is only based on the detection and isolation of the virus in a Cape short-eared Gerbil in southern Africa. West Nile virus detection in squirrels and other rodent species in several countries also suggest that rodents might be a competent reservoir host for the mosquito-borne Flavivirus (Root, 2012).

### 2.6.2 Amplifying host

(Root,2012) states that recent studies show several squirrel species develop a high level of viremia that can transfer the virus to a biting mosquito and that the West Nile virus is also detected in a moribund, sick and dead squirrel. This evidence might also indicate rodents such as squirrels could be the amplifying host for the West Nile virus. Other than the West Nile virus, (Diagne *et al.*, 2019) also suggest that rodents are possible as an amplifying host for the Usutu virus.

### 2.6.3 Dead-end host.

Lastly, there is still no research evidence indicating rodents as a dead-end host for any mosquito-borne Flavivirus. However, the status of rodents as a dead-end host cannot be ruled out as there are still possibilities for the rodents to develop a very low level of viremia that is not possible to transmit the virus to mosquitoes. For example, experimentally, small mammals shows no or low level of viremia when inoculated with mosquito-borne flavivirus (Root, 2012). Hence, more research and studies are needed to emphasize on the role of rodent as the dead-end host for mosquito-borne flavivirus.

## 2.7 CONTROL AND PREVENTION

In evaluating the threat posed by the well-established, recently emerged, and those that may emerge mosquito-borne Flavivirus, it is an ultimatum to hasten the actions in controlling and preventing the disease. According to (Pierson & Diamond, 2020), the Dengue virus, West Nile virus and Japanese encephalitis virus are among those that are well established and pose a great threat worldwide. Mosquito control, vaccination and rodent control in a population will be discussed in this section.

### 2.7.1 Mosquito Control

Since the mosquito is the main vector in transmitting the virus, mosquito control is very crucial in order to mitigate the elimination of the disease. Mosquito surveillance is one of the methods that have been implemented to control the emerging pathogens from the vector. For instance, surveillance for West Nile virus (WNV) and other flaviviruses in adult mosquitoes is frequently done by a vector surveillance national program in Portugal, REVIVE, that was established in 2008 (Osorio *et al.*, 2014). The purpose of the surveillance is vital as an advanced effort to detect the invasive species carrying the pathogens (Osorio *et al.,* 2014). Usage of pyrethroid-based insecticides together with the destruction of mosquito habitats in the affected area can be done to control the mosquito population (Garcia-Bocanegra., 2010). In reducing the West Nile virus infection in humans, the application of ultra-low-volume insecticides is also known to be effective (Carney *et al.,* 2008; Reisen & Wheeler, 2016). Lastly, Wolbachia method are also known to be effective in reducing the spread of the viruses carried by the mosquitoes (World mosquito program, 2011). Wolbachia is a bacterium that is harmless in human and can be naturally occurring in insects including the mosquitoes (World mosquito program, 2011). It is known that mosquitoes carrying the Wolbachia bacteria will reduce the replication of the virus in the mosquitoes and reduce the spread of the virus (World mosquito program, 2011). In the context of mosquito-borne flavivirus, (World mosquito program, 2011) also mentioned that the transmission of dengue virus, zika virus and yellow fever virus are known to be reduced when the Aedes aegypti transmitting the virus are naturally carrying the Wolbachia bacteria. So, breeding the Wolbachia-carrying mosquitoes and releasing them into the mosquito-borne diseases affected area is also one of the measures to curb the spread of the virus by the mosquitoes.

### 2.7.2 Rodent control

Other than mosquito control, rodent control in a population is also important to control and prevent the spread of mosquito-borne flavivirus. The first isolation of the Usutu Virus in rodents in Senegal with the possibilities of rodents as an amplifying host or reservoir host of the virus emphasizes the urge for active surveillance and public health effort to control the rodent population (Diagne *et al,* 2019). Controlling the rodent population ideally may contribute to the reduction of flavivirus infection. (Mara *et al.,* 2020) Suggest that eradication or minimization of rat and mouse populations is known to be one of the effective control measures. By minimizing the rodent population help to reduce the risk of exposure of human to the rodents that are carrying diseases (Barnes, 1978). Cleanliness education is also one of the methods that can be implemented to control the rodent population (Mara *et al.,* 2020). For instance, integrating the environmental health education on environmental sanitation in school helps the students to understand the importance of cleanliness in reducing the population of the disease carrying rodents (Hanchke & Suarez, 2014). Limiting the access of rodents to residences, water and food sources should also be implemented as an extensive control and preventive action (Mara *et al.*, 2020). This will reduce the contact between the human populations and also the rodent's population and helps to reduce the transmission of the disease to human (Mara et al., 2020). A total of 1529 dwellings in low-income regions of Sao Paulo, Brazil had been involved in a survey on synanthropic rodent infestation that shows a drop in the rate of rodent infestation from 40% to 14% after the implementation of cleanliness education and pest control (de Masi *et al.*, 2009).

### 2.7.3 Vaccines

Some Flavivirus infections including the mosquito-borne Flavivirus may lead to a high mortality rate in the infected host especially humans (Ishikawa *et al.*, 2014). Vaccines are known to be a practical method in preventing the spread of mosquito-borne Flavivirus since it is very challenging to eradicate the virus (Ishikawa *et al.*, 2014). In the last several decades, research has been intensively done in developing prophylactic vaccination as a medium to combat the Flavivirus infection since most of the Flaviviruses cause explosive outbreaks in nature (Geerling *et al.,* 2020). To date, licensed vaccination for humans is available for several mosquito-borne Flavivirus such as Yellow Fever virus, Dengue virus and Japanese Encephalitis virus (Pierson & Diamond, 2020). Yellow Fever virus vaccines are highly effective and known to be among the most successful of all vaccines to prevent viral infection (Pierson & Diamond, 2020). (Stephenson, 1988) also mentioned that due to the high safety and efficient live-attenuated Yellow Fever vaccine, several countries that adequately practise the vaccination program are able to eliminate the threat of this disease. Together with the Yellow Fever virus vaccine, vaccines against Japanese Encephalitis are also known to express long-standing protective efficacy (Ishikawa *et al.,* 2014). Currently, chemically-inactivated viruses of cell culture-derived viruses are used as

Japanese Encephalitis vaccines (Fischer *et al.,* 2010). Even though they exert protective immunity, frequent boosters are needed to maintain the protective immunity (Pierson & Diamond, 2020).

Next, vaccination for the dengue virus faces challenges due to its characteristic of having four different serotypes (1-4) that poses a risk of incomplete immunity stimulation that may worsen the pathogenesis with the subsequent natural infection (Wong *et al.,* 2019). Thus, the main goal for dengue virus vaccination is protective immunity against all four serotypes of dengue virus or tetravalent (Castano-Osorio & Giraldo, 2018; Murphy & Whitehead, 2011). In 2016, the first live-attenuated tetravalent Dengvaxia vaccine, which was developed by Sanofi Pasteur for Dengue virus was licensed for individuals aged 9 years old and above (Hadinegoro *et al.*, 2015). Dengvaxia vaccine was approved by the United States Food and Drug Administration (FDA) only for individuals aged between 9-16 years of age and was confirmed in the laboratory of previous dengue infection and living in an endemic area. This is because Dengvaxia is known to cause severe dengue disease in an individual that was not previously infected with the Dengue virus and also was found to induce poor protective immunity against Dengue virus serotype 2 (Castano-Osorio & Giraldo, 2018; Wilder-Smith, 2018). Dengvaxia is also applicable to only individuals from the endemic region and is contraindicated in the non-endemic regions due to the high possibility of a seronegative individual from the non-endemic region (Wilder-Smith, 2018). However, the vaccine is known to be safe and have high efficacy for seropositive individuals (Castano-Osorio & Giraldo, 2018). To date, vaccination against the West Nile virus is still not available. However, West Nile virus vaccination in horses is being implemented with the recent vaccine available is a recombinant DNA vaccine.

All in all, several strategies need to be employed in order to fight against the uprising threat of the mosquito-borne Flavivirus. With the possible involvement in the transmission of the virus, the rodent population should be taken into account as one of the threats in mitigating the viral transmission to the human population especially with the advantage of having closer interaction within the living environment. Not just that, mosquito control and vaccination against the Flavivirus as discussed previously should also be practiced to further combat this mosquito-borne Flavivirus.

## 3.0 CONCLUSION

In conclusion, being the largest group of mammals, rodents are known to be involved in the transmission of mosquito-borne Flavivirus which is one of the important viruses that affect various vertebrate hosts globally including humans. Rodents can either be an amplifying host, reservoir host or dead-end host in the transmission of the mosquito-borne flavivirus.

## 4.0 RECOMMENDATIONS

Even though there is strong evidence that suggests the association between rodents and mosquito-borne Flavivirus, there is still a lack of definite proof based on the lack of detection of the mosquito-borne Flavivirus in rodents. In order to truly understand the involvement of rodents in the transmission of the virus, more active surveillance is needed in the region that is possible for the virus to be circulating among rodents. Serological and molecular survey should be conducted to assess the prevalence of mosquito-borne flavivirus in the rodents in an endemic area of mosquito-borne flavivirus. Previously positive and currently positive rodents for the mosquito-borne flavivirus can be detected with the serological survey. Active surveillance on the detection of the virus in the rats and also the human population allows further understanding of the extensiveness of the virus, especially on its zoonotic potential. Apart from that, active surveillance may not just help to detect positive rodents, but also prevent the possible zoonotic transmission of the virus with early detection. For instance, the Wesselsbron virus was detected in both asymptomatic black rats and febrile humans carrying the same strain of the virus, but the virus strain is not detected in the mosquitoes inhabiting the area. So, the serological survey should be further extended by taking a larger sample of mosquitoes to also detect the presence of the virus in the mosquitoes. Cross-sectional study can be performed by sampling rodent population in an endemic area that have higher risk of exposure to the mosquito-borne flavivirus. This help to study the prevalence rates of rodents for the mosquito-borne flavivirus. This study can also help in the early detection of the mosquito-borne flavivirus in rodents.

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