EVALUATION OF POTENTIAL ANTIVIRAL PROPERTIES OF TUALANG HONEY AGAINST IN VITRO CHIKUNGUNYA VIRUS INFECTION IN VERO CELLS

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by

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LIST OF SYMBOLS

g	Gram
mg	Milligram
ml	Mililitre
Pfu/ml	Plaque performing unit mililitre
γ	Gamma
±	About
µl	Microlitre
µg	Microgram
µg/ml	Microgram per mililitre
x	Multiplication
<	Less
≤	Less or equal to
>	More than
%	Percentage
℃	Degree Celsius

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BSC	Bio-safety cabinet class
CDC	Centre for Disease Control and prevention
CHIKV	Chikungunya virus
CHO	Chinese Hamster Ovary
CPE	Cytopathic effect
CMC	Carboxymethyl cellulose
CO_2	Carbone dioxide
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
ECSA	East Central-South Africa
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FBS	Foetal bovine serum
HEPES	4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid
HPI	Hour post infection
Ig	Immunoglobulin
MNTD	Maximum non-toxic dose
MOI	Multiplicity of infection
PBS	Phosphate buffer saline
PC	Positive control
PFU	Particle forming unit
SCV	Sementis Copenhagen Vector
SE	Standard error

SPSS	Statistical Package for the Social Sciences
VLP	Virus-like particle
WHO	World Health Organisation

KAJIANTENTANG POTENSI ANTIVIRAL MADU TUALANG TERHADAP JANGKITAN VIRUS CHIKUNGUNYA IN VITRO DI DALAM SEL VERO

ABSTRAK

Chikungunya adalah penyakit virus bawaan nyamuk yang ditularkan kepada manusia oleh CHIKV dan ia telah menjejaskan banyak negara di seluruh dunia. CHIKV disebarkan oleh dua spesies utama nyamuk Aedes, *Aedes aegypti* dan *Aedes albopictus* dan kebiasaannya menyebabkan penyakit akut dengan demam, ruam, dan artralgia. Pada masa ini, tiada antivirus atau vaksin yang tersedia secara komersil. Dalam kajian ini, kami menyelidik aktiviti antivirus madu Tualang terhadap CHIKV dalam sel Vero. Potensi sifat anti-CHIKV madu Tualang dalam assai yang berbeza, virusid, pra-rawatan, pasca rawatan, anti-kemasukan dan anti-penjerapan, menggunakan kepekatan madu Tualang yang tidak toksik pada waktu inkubasi yang berbeza. Kesan perencatan virus dinilai dengan memerhatikan perubahan morfologi sel Vero yang selanjutnya disahkan oleh assai plak. Hasil penyelidikan ini menunjukkan bahawa madu Tualang tidak menjadi toksik kepada sel Vero apabila digunakan pada kepekatan antara 20 mg/mL hingga 5 mg/mL. Kajian ini juga menunjukkan bahawa madu Tualang mempamerkan aktiviti antivirus yang signifikan terhadap CHIKV. Aktiviti virusid madu Tualang terhadap jumlah/kuantiti CHIKV yang berbeza menunjukkan perencatan yang signifikan pada titer virus. Menariknya, pra-rawatan madu Tualang terhadap sel Vero selama 12 dan 24 jam sebelum jangkitan telah memberi kesan perencatan tertinggi pada replikasi CHIKV terutama 48 jam selepas jangkitan, dengan kira-kira 90% perencatan titer virus dicatatkan ($P < 0.05$). Tambahan lagi, semasa assai pasca rawatan, replikasi CHIKV telah

direncatkan secara signifikan di dalam sel Vero selepas pasca pendedahan pada madu Tualang selama 8 jam. Rawatan pasca sel dengan madu Tualang menunjukkan kesan pengurangan titer virus adalah paling besar apabila dibandingkan dengan assai lain dengan peratusan perencatan sebanyak 98% ($P < 0.05$). Walau bagaimanapun, madu Tualang tidak merencat jangkitan CHIKV di dalam sel Vero semasa assai anti-penjerapan dan anti-kemasukan dengan peratus perencatan hanyalah 33 hingga 80%. Secara keseluruhannya, hasil dari kajian semasa menunjukkan bahawa madu Tualang mempunyai potensi untuk dikembangkan sebagai agen alternatif anti- CHIKV. Kajian masa depan adalah wajar untuk menjelaskan mekanisme tindakan madu Tualang dan sama ada kesan yang sama dapat ditunjukkan secara *in vivo*.

EVALUATION OF POTENTIAL ANTIVIRAL PROPERTIES OF TUALANG HONEY AGAINST IN VITRO CHIKUNGUNYA VIRUS INFECTION IN VERO CELLS

ABSTRACT

Chikungunya is a mosquito-borne viral disease transmitted to human by CHIKV which has affected many countries around the world. CHIKV is transmitted mainly by two species of *Aedes* mosquitoes, *Aedes albopictus* and *Aedes aegypti,* and causes typically acute illness with incapacitating arthralgia, fever and rashes. Currently, there is no antiviral or licensed vaccine commercially available. In this study, we explored the *in vitro* antiviral activity of the Tualang honey against CHIKV in Vero cells. The potential anti-CHIKV property of Tualang honey was determined in different assays including virucidal, pre-treatment, post-treatment, anti- entry and anti-adsorption, using the non-toxic concentrations of Tualang honey at different incubation hours. The viral inhibitory effect was confirmed by plaque assay after morphological changes of Vero cells were observed. The results showed that Tualang honey was not toxic to Vero cells at concentration between 20 mg/mL to 5 mg/mL. This study demonstrated that Tualang honey exhibited significant antiviral activity against CHIKV. The virucidal activity of Tualang honey against different amounts of CHIKV was observed by the significant inhibition noticed in the viral titre. Remarkably, the pre-treatment of Tualang honey on Vero cells for 12 and 24 hours before infection gave the highest inhibitory effect on CHIKV especially at 48 hours post infection, with about 90% inhibition of viral titres was observed ($P < 0.05$). Surprisingly, during post-treatment assay, CHIKV replication was significantly inhibited in Vero cells following post-exposure to Tualang honey for 8 hours. The post-treatment of cells with Tualang honey displayed the biggest reduction of viral titre effect when compared with the other assays with percentage of inhibition

98% ($P < 0.05$). However, Tualang honey did not significantly inhibit infection of Vero cells by CHIKV during the anti-adsorption and anti-entry assay with percentage of inhibition surpassed 33 to 80%. Overall, the results from the current study suggest that Tualang honey can be explored as an alternative anti-CHIKV agent. Future study is warranted to elucidate Tualang honey mechanism of action and whether similar effects could be demonstrated *in vivo*.

CHAPTER ONE

INTRODUCTION

1.1 Chikungunya virus

Chikungunya virus (CHIKV) was initially described by W.H.R. Lumsden and M. Robinson in 1955 after the outbreak along the border between Tanganyika and Mozambique (which is currently a part of Tanzania) in 1952 (Amar & Vilhekar, 2015). The virus was found in the serum of an infected patient in Makonde Plateau (Silva & Dermody, 2017). CHIKV belongs to the *Togaviridae* family, and the *Alphavirus* genus (Furuya *et al*., 2016). It has a capsid with 60-70 nanometres diameter, a phospholipid envelope and a single stranded positive sense RNA genome (Figure 1.1) (Cunha & Trinta, 2017). The CHIKV particle has icosahedral spherical structure with triangulation number (T) equals 4 symmetry structure of the virus. The structure contains 80 spikes including 20 icosahedral "i3" spikes that are situated on the icosahedral 3-fold axes and 60 quasi-3-fold "q3" spikes with quasi-3-fold axis (Nguyen *et al*., 2018). The CHIKV genome is approximately 12,000 nucleotides (Carletti *et al.,* 2017).

The first reading frame (ORF1) encodes for a polyprotein and acts as a precursor of the non- structural proteins (NS1, NS2, NS3 and NS4), while the second reading frame (ORF2) encodes the structural proteins (capsid proteins, assembly proteins E3, envelope glycoproteins E2, 6K protein and envelope glycoproteins E1 (Figure 1.2) (Jain *et al.,* 2017).

Structural proteins result from a cleavage of polyprotein by signalase and auto proteinase. Additionally, structural proteins such as envelope glycoprotein- E1 and E2 have been shown to mediate viral entry hosts (Wong & Chu, 2018). Similarly, non-structural proteins play crucial roles during virus replication, protein modification, and immune antagonism (Nguyen *et al.*, 2018). Mutations that occur in the E1 glycoprotein have received significant attention lately because it has been understood that the mutations modifies the virus's ability to infect mosquitoes and increase severity of the illness in affected persons (Bordi *et al.*, 2015).

The enhanced infectiousness and transfer of the virus by *Aedes albopictus* is due to the adaptative mutation of CHIKV to *Aedes albopictus* mosquito in the envelope glycoproteins E1 surface gene (Abdelnabi *et al.*, 2017). Berry *et al.*, (2018) observed that *Aedes aegypti* adaptive strain carrying the E1:K211E and E2:A264V mutations is capable of rapid spread in *Aedes aegypti* rich regions. These strains were found to be implicated in explosive outbreaks in some Asian and African countries. Therefore, mutated strains have the potential to completely replace the wild type strain and could further result in widespread outbreaks globally (Berry *et al.*, 2018).

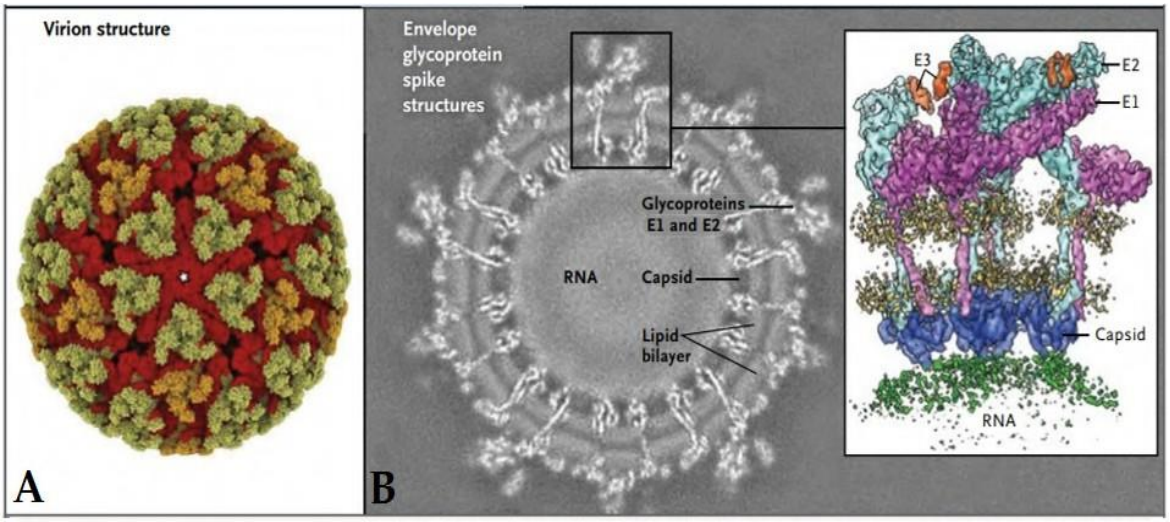

Figure 1.1 Viral morphology of CHIKV showing the orientation of the E2 and E1 envelope glycoproteins in the virion spikes (adopted from Herpan, 2019).

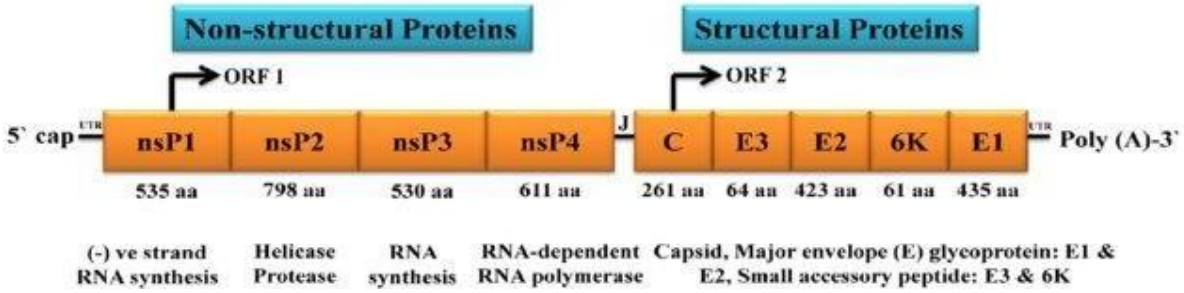

Figure 1.2 Arrangement of CHIKV genome (adopted from Jain *et al.*, 2017).

1.2 CHIKV replication

The positive-sense RNA genome of CHIKV encodes four nonstructural proteins (nsP 1-4) that allow CHIKV replication and transcription complex (Rausalu *et al*., 2016; Remenyi *et al.,* 2018). The genome's two open reading frame has a 5′ untranslated regions and 3′ untranslated regions (UTRs) with a non-coding intergenic region separating the two UTRs. The 5′ UTRs has a 5′ type-0 N 7-methylguanosine cap which allows cap-dependent translation to be initiated. According to Kendall *et al*. (2019) The 5′ UTRs has a novel element which is very important for CHIKV replication in host cells even though replication of positive-sense RNA virus genomes is initiated at the 3′ end of the molecule. On the other hand, CHIKV RNA synthesis occurs when distinct modules of the viral replicase complexes formed as a result of ORF-1 encoding nsP 1-4 (Kendall *et al*., 2019).

As replication of the genomic positive-sense RNA to full length negative–sense intermediates progresses, proteolytic processing of the non-structural protein precursors in the replicase complex occurs and therefore allows its association with the negative-strand and subsequent replication of positive-sense full-length genomic transcripts (Kallio *et al*., 2016). Meanwhile, ORF-2 transcripts encoding structural proteins (in the negative strand) are synthesised from a sub-genomic promoter in the negative strand (Kendall *et al*., 2019). When infection with CHIKV occurs, translation of genomic mRNA into non-structural precursor protein occurs with the aid of the cellular and viral proteases (Henss *et al*., 2018). Additionally, nsP 1-4 also plays crucial roles in the synthesis of a minus-sense RNA from the globin mRNA (gmRNA), resulting in double-stranded RNA intermediates.

These RNA intermediates then activate dsRNA-dependent protein kinase that phosphorylates while inactivating the initiation of factor 2 alpha (eIF2α) translation (Henss *et al*., 2018). Therefore, the minus-sense RNA is a template for the synthesis of subgenomic mRNA (sgmRNA) and the gmRNA. The structural proteins on the other hand are translated from the sgmRNA in an eIF2α-independent manner, whereas the synthesis of nsPs requires active eIF2α (Henss *et al*., 2018). The minus-strand RNA synthesis is the minus-strand is limited between 3 and 4 hours post infection and undetectable later while the positive-sense mRNA is synthesized.

1.3 Disease burden of chikungunya

The disease burden for chikungunya exists in both individuals and affected localities/countries (Fritzell *et al.,* 2018). Reduced productivity, extra health care costs and associated co-morbidities cause a huge burden on individuals, public healthcare systems and communities (Alvis *et al.,* 2018; van *et al.,* 2017). Severity of CHIKV infection has been observed in children and elderly (above 60 years) and mortality cases recorded maybe underestimated considering CHIKV confirmatory diagnosis are not carried in certain countries due to limited resources and similarities of chikungunya to dengue fever (Yapa *et al.,* 2019). Since chikungunya may present with cerebral disorders such as encephalopathy, altered mental status and disrupted behaviour, the severe cases of CHIKV infection affects quality of life of infected persons (Mehta *et al.,* 2018).

Burt *et al.* (2017) reported that two years after acute CHIKV infections, about 43% to 75% of infected persons experienced either late-onset of symptoms or prolonged symptoms which consequently meant that they suffered long-term health implications (as the disease burden can last up to 3 years).

Rahim *et al.* (2016) study also showed that persistent pain was experienced in some individuals 18 months after CHIKV infection. Additionally, Rodriguez *et al.* (2016) reported that post-chikungunya chronic arthritis was observed in about 14% of patients with CHIKV infection. Additionally, their studies revealed that the risk of developing chronic inflammatory rheumatism post chikungunya infection increases in elderly people, women and patients with multiple comorbidities. Therefore, disease burden as a result of CHIKV infection can have an impact on national development, business, public health, individual and even household costs (Alvis *et al.*, 2018).

An example of national development costs implications due to CHIKV infection is seen in the Caribbean countries where revenue loss from tourism was reported. Reduced workforce productivity can also affect business costs in relation to sick time or modified workload assignments. Individual costs as a result of CHIKV infection could be as a result of costs incurred from purchasing materials for personal prevention of vector-borne illnesses, loss of wages, cost of treating CHIKV infection or cost of morbidity management. This can lead to financial burden on individuals and their families and companies (Rezza & Weaver, 2019). Disease burden can be quantified in terms of Disability Adjusted Life Years (DALYs) (Paixão *et al.*, 2018).

In Rome, the economic burden was estimated at 322,000 euros (EUR) with a loss of 341 DALYs (Manica *et al.,* 2017). In 2014, the burden of chikungunya in Latin America was estimated to be about 151,031–167,950 DALYs lost, or 0.39 DALYs per case (as that 45.1–50.1% of 855,890 acute CHIKV cases developed chronic inflammatory rheumatism) (Bloch, 2016).

In Colombia, the estimated total costs of CHIKV infection was US$67 million. Median of direct medical cost was about US$258 for children while for adults it was about US$67. The productivity loss median expenditures were estimated to be up to US$81 for each adult patient. While the economic cost in adults was about US$153, of which over 50% was as a result of indirect costs. Similarly, out-of-pocket spending was estimated to be about 3% of all economic costs (Alvis *et al.,* 2018). Therefore, CHIKV infection is global problem that requires the attention of different stakeholders to prevent further disease burden.

1.4 Ecology and epidemiology of CHIKV

CHIKV infection was first identified in Africa during on outbreak in 1952 and its emergence recently in the Caribbean, Americas, Australia, Europe and the Indian subcontinent has raised serious concern about the disease (Sanyaolu *et al.,* 2016). Musso *et al*. (2018) reported that since the year 2000, the epidemiology of CHIKV has changed from hypoendemic (one serotype) to hyperendemic (multiple serotype cocirculation). Three CHIKV genotypes have been identified- Two African strains (i.e the West African and the East Central-South Africa (ECSA) strain), and one Asian strain (Mudurangaplar & Peerapur, 2016). However, Arankalle *et al*. (2007) studies showed that the West African, Asian and ECSA strains are closely related, with amino acid similarity of 95.2%–99.8%. An increasing number of cases from CHIKV infection has been reported globally (Ching *et al.,* 2017; Matusali *et al.,* 2019).

In the African continent, about 75% of the population was infected with CHIKV during the 2004 outbreak in Lamu Island, Kenya in 2004. In 2016, re- emergence of CHIKV was observed where about 1,792 cases of CHIKV outbreak was reported in Mandera, Kenya. Out of these cases, about 50% of health care workers were affected (Berry *et al.,* 2018).

At the same time, the neighboring Bula Hawa, Mogadishu, Somalia was also affected. This cross-border outbreak expands globally (Ching *et al.,* 2017; Leta *et al.,* 2018; Monaghan *et al.,* 2018). Increased spread of CHIKV to regions with lower temperatures have also been witnessed (Weaver & Forrester, 2015).

Similar to Mandera, Kenya, in Karachi, Pakistan, an outbreak of CHIKV occurred in 2011 with re-emergence in 2016 where an estimated 30,000 people were infected. As Karachi city lies on the coast of the Arabian Sea and houses South Asia's largest cargo port, serious concerns were raised that CHIKV infection might spread to other neighbouring countries through freight transport thereby increasing the virus abundance in the Asian continent (Rauf *et al.,* 2017). Similar to studies by Rauf *et al.,* (2017), Yapa *et al.,* (2019) also documented that chikungunya viral spread has expanded its geographic reach in the Asian region since 2005 onwards.

In Malaysia, outbreaks were limited until 2007 when over 10,000 cases were reported between 2008 and 2010 (Jesse & Benjamin, 2014). Furthermore, about 50 cases of CHIKV infection were identified between February and March in the year 2017 with infected patients exhibiting symptoms such as conjunctivitis, fever, joint pains, headaches and rashes. This makes CHIKV infection a significant health problem in Malaysia (Ali *et al.,* 2018).

According to a review by Yapa *et al.*, (2019) and Kaur *et al.,* (2017), India has experienced the highest burden high attack rate of CHIKV, accounting for the greatest number of reports as it affected billions and declared a major public health issue since the first incursion of the virus into South East Asia. In a study by Kaur *et al.*, (2017), 97% of patients out of 600 patient's samples experienced swelling, rashes, itching, restricted movement of the joints such as the hands, wrist, knees and feet and neurologic complications due to CHIKV infection.

In the Americas, explosive outbreaks and subsequent spread to several continental American countries have resulted in more than one and a half million suspected cases (Fox & Diamond, 2016) since the first case was detected in 2013 in Saint Martin in the Caribbean (Diaz *et al.,* 2015). Autochthonous transmission of the virus has been confirmed in 43 countries/territories in South America, North America, Central America and the Caribbean (Pham *et al.*, 2017). La Réunion island in the Indian Ocean experienced over 250,000 cases of CHIKV infection during a seventeen-month long outbreak in 2005-2006 and about 254 deaths were recorded (Tjaden *et al.*, 2017; Venturi *et al.*, 2017).

Additionally, in La Reunion Island, new clinical forms of CHIKV infection such as severe cutaneous effects and acute hepatitis, respiratory, kidney and cardiovascular failure, meningoencephalitis and other central nervous system (CNS) problems were observed in patients (Gerardin *et al*., 2016).

Italy for the first time experienced an outbreak in 2007 in the north east of the country, near the Adriatic coast, in the province of Ravenna and over 200 cases were reported (Marano *et al.*, 2017). Subsequently, in 2010 and 2014 autochthonous cases (linked to imported cases), were detected in the city of Montpellier and Var department in France (Venturi *et al*., 2017). In 2017, CHIKV outbreak also saw a cluster of locally acquired cases consisting of four confirmed and one probable case described again from the Var department in France (Vairo *et al.*, 2018).

Similarly, near Anzio, a coastal town in the province of Rome being a holiday resort, witnessed about 179 imported cases of CHIKV (ECDC, 2017). Ciocchetta *et al*. (2018) also reported that a new *Aedes* mosquito species, *Aedes (Finlaya) Koreicus* which had not been previously reported was observed in Europe during outbreaks. This brought to limelight the need to understand the vector potential of invading mosquitoes so as to prevent re-emergence of CHIKV infection.

In Oceania/Pacific Islands, CHIKV infection emerged in New Caledonia in 2011. This subsequently spread throughout the south and central Pacific (Musso *et al.*, 2018). Other Islands that were also affected by CHIKV includes Cook Islands, Tonga, Kiribati, French Polynesia, Papua New Guinea, Samoa, Tokelau, American Samoa and Federal States of Micronesia (Petersen & Powers, 2016). A summary of some epidemiological findings and implicated genotypes in different parts of the world is presented in Table 1.

Table 1: Epidemiological findings, implicated genotypes from different countries

Genotype	Country	Epidemiological findings
ECSA and Asian	Bangladesh	In 2011, first outbreak was reported (almost 30% prevalence rate). In 2014, six confirmed cases was witnessed (after re-emergence)
ECSA	Malawi	First outbreak between 1987-1989. Few cases in 2001 and 2015.
Asian and ECSA	Cambodia	The first case was in 1961. Re-emergence occurred in 2011 when about 24 patients had positive RT-PCR and ELISA. In 2012, about 45% seroprevalence rate was recorded during an outbreak in Trapeang Roka Kampong Speu. .
ECSA/Asian	Thailand	Over 40,000 suspected cases were recorded in the 1960s. In 1962, over 30% of the population was affected during the Bangkok outbreak. In 2008, 244 cases were confirmed. In 2013, Bueng Kan was severely

		affected during an outbreak.
ECSA/Asian	Singapore	In 2008, the first outbreak was reported (about 1000 cases) Re-emergence occurred in 2013.
ECSA	Laos	In 2012, 31 confirmed cases from Champassak Province An outbreak of CHIV and dengue was again reported.
Asian/ECSA	Aruba	An outbreak occurred in 2014 (estimated cases was over 203).
Asian	Cayman Island	In 2014 about 25 cases was reported. Re-emergence occurred in 2015 and 2016.
Asian/ECSA	Martinique	30,715 cases were confirmed in 2013 (with an incidence rate was 76 per 1000)
Asian	Colombia	In 2014, over 22,300 cases were recorded. In early 2015, 1,317 cases were documented.
Asian/ECSA	Venezuela	2303 and 3107 confirmed cases were reported in 2015 and 2016, respectively.
Asian/ECSA	Argentina	21 and 55 confirmed cases were reported in 2015 and 2016,

		respectively
Asian/ ECSA	French Guiana	Estimated cases in early 2014 was over 7,000 Local transmission also occurred in 2015, and 2016.
Asian	Japan	In 2010, an imported case was recorded In 2013, 14 confirmed cases were recorded (traced to autochthonous transmission from a traveller into the country)
Asian/ECSA	El Salvador	In 2014, about 123,339 cases were documented. In 2016, almost 6,000 cases was documented.

Adopted from Wahid *et al.,* 2017.

The different genotypes have spread drastically over the past 20 years (Smalley *et al.,* 2016). Globalisation of travel and trade has contributed to the spread of CHIKV infections (Therrien *et al*., 2016; Cella *et al.*, 2018). The sharp rise in the number of CHIKV infections from 2004 to 2008 indicated the ease of spread of this viral disease in humans (Faria *et al.,* 2016). The probability of global spread is thus alarming, increasing and challenging especially with the number of unpredicted cases that have emerged (Matusali *et al.,* 2019; Souza *et al.,* 2019). Therefore, measures must be taken to improve prevention, screening of travellers from endemic countries and control of the disease (Roiz *et al.*, 2018). Figure 1.3 shows the global distribution of CHIKV infection.

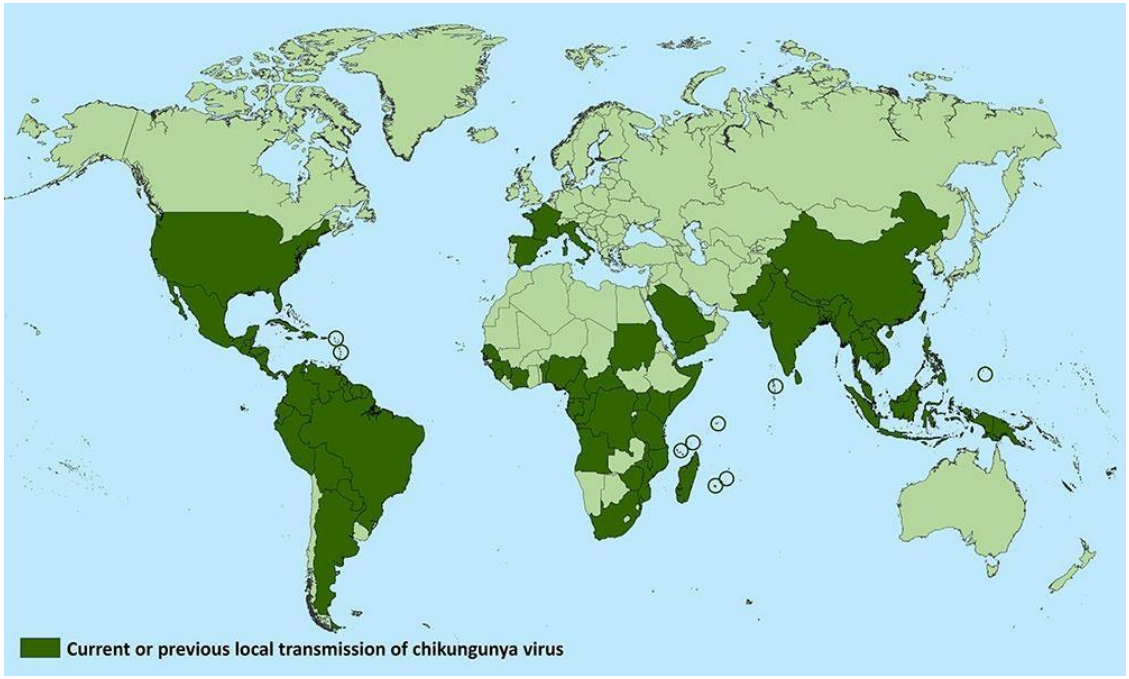

Figure 1.3 Global distribution of CHIKV infection as of May 2018
(Adopted from CDC, 2018)

1.5 Laboratory Diagnosis

Diagnosis of chikungunya infection is likely in individuals who have recently travelled to endemic countries and are exhibiting acute or abrupt onset of high fever (> 39 °C and lasts 3-5 days), joint pains (past weeks to months) and polyarthralgia (Johnson *et al.*, 2016; Wahid *et al.*, 2017). CHIKV infection is detected in addition to other clinical signs and symptoms through a blood test. Several important diagnostic tool used in CHIKV detection such as RT-PCR, Enzyme-Linked Immunosorbent Assays (ELISA) and lateral flow rapid test. RT-PCR is usually very sensitive and specific and hence very useful in early detection (Priye *et al.*, 2017). ELISA test is particularly important because antibodies in infected patients usually develop by the end of the first week, so if RT-PCR is negative, the infection may still be detected with the convalescent phase sample (Dinkar *et al.*, 2018). While rapid diagnostic test is better emergency testing and helps for detect both anti- CHIKV Immunoglobulin IgM and IgG antibodies in patient serum or plasma about in 15 minutes.

1.6 Pathogenesis of CHIKV

CHIKV infection transmitted by infected mosquitoes can cause different clinical manifestations. Viral load in patients can be as high as 10^{10} virus particles per milliliter of blood during the first days of infection and erythematous maculopapular or morbilliform eruption can occur (although may subside without any sequelae) in 3–4 days (Gasque *et al*., 2016). This eruption could then be a hallmark of an inflammatory response of the skin (the portal of entry of CHIKV after the infected mosquito bites) to mobilize resident cells such as dermal fibroblasts, melanocytes and keratinocytes (Gasque *et al*., 2016). CHIKV infection prompts immune response which leads to the production of interferon type I and interferon-stimulated genes, recruitment of innate and adaptive immune cells, and development of neutralising antibodies (Fox & Diamond, 2016).

High titres of CHIKV are present in blood samples of infected persons through the acute phase and remains high during the chronic phase as well. As a result, inflammatory response coincides with rise of immune mediators and infiltration of immune cells into infected tissues and joints (Burt *et al.*, 2017). Different concentration of chemokines and cytokines is observed in acute and/or chronic CHIKV infection (depending on the stage of the infection) (Michlmayr *et al.*, 2018).

High concentrations of several chemokines such as IP-10 and monocyte chemoattractant protein and proinflammatory cytokines such as interferon α, interferon γ, IL-6 and anti-inflammatory cytokines such as interleukin 1 receptor antagonist, IL-4, IL-Ra, IL-2R, IL-7, IL-8, IL-10, IL-12 and IL-15 have been reported (Goupil, 2016).

The alterations in cytokines during the acute stage of the infection have been observed (Goupil, 2016). While some cytokines are correlated with disease severity, others are correlated specifically with higher viral load (Piedra *et al.,* 2017). Lee *et al.* (2015) study showed that regulatory T cells (Tregs) (a distinct subset of CD4+ T cells) prevent exacerbated proinflammatory responses by maintaining tolerance and restoring immune homeostasis during inflammatory responses. In line with Lee *et al*. (2015) studies, Burt *et al.* (2017) showed that circulating activated and effector T cells also increased in patients with persistent chikungunya induced arthritis. Studies in mice using the established juvenile mice model revealed that depletion of regulatory T cells worsened disease pathology while acute loss of T cells resulted in severe immunopathology and increased viremia with long term sequelae (Wendy, 2016).

1.7 Control strategies of CHIKV

Because outbreaks caused by CHIKV has continued and expanded globally and there are no licenced vaccines or antiviral treatments for this debilitating infection, control strategies have been developed to treat infections (Chan *et al.,* 2016; Giancotti *et al*., 2018; Subudhi *et al.,* 2018). Current treatment has mainly been focused on symptomatic relief and hence the need for further investigation (Ganesan *et al.,* 2017). A discussion of some anti-CHIKV control measures and strategies of treatment reported in the literature is as follows:

1.7.1 Vaccines

CHIKV vaccines have been developed to serve as preventative and curative measures during epidemics (Erasmus *et al.,* 2016). Ramsauer & Tangy (2016) carried out an investigation on anti-CHIKV vaccines using the viral-vector technologies. They utilised vectors, for example altered immunization virus Ankara, complex adenovirus, alphavirus-based chimeras, vesicular stomatitis and measles immunization Schwarz strain in animal models as potential vaccines. They observed that full envelope gene cassette or the entire open reading frame expressing the structural genes yielded the most-effective and most-protective vaccine promising candidates and therefore concluded that virus-vector vaccines show promising results as potential CHIKV vaccine because they induce humoral and cellular immune responses using highly attenuated and safe vaccine backbones (Ramsauer

& Tangy, 2016). Aside from the viral-vector technologies, anti-CHIKV recombinant vaccines have been developed in modified Chinese Hamster Ovary (CHO) cells. Eldi *et al*. (2017) developed a Sementis Copenhagen Vector (SCV) vaccine against CHIKV (SCV-CHIK) and used in a range of human cell lines and in immunocompromised mice. In mice model, a single immunisation with SCV-CHIKV induced antibody responses specific for CHIKV thus neutralising the virus. In addition, SCV vaccine was also observed to prevent viremia and arthritis in mice model. Anti-CHIKV vaccine (MV-CHIK and VLP) that have advanced to phase I clinical trials have also been developed (Tharmarajah *et al.*, 2017).

MV- CHIKV vaccine is a recombinant measles virus vaccine that expresses CHIKV surface proteins from the ECSA CHIKV strain. During pre-clinical trials, mice models were protected from lethal doses of CHIKV upon being administered with the MV-CHIKV vaccine (Tharmarajah *et al.*, 2017). Similar to MV-CHIKV, the Virus-like particle (VLP) vaccine, VRC-CHKVLP059-00-VP also protected mice models and non-human primates from lethal doses of the ECSA strain of CHIKV during preclinical trials although this vaccine is not suitable for long term immunity due to the fact that it could enhance reactogenicity, impair tolerability of the vaccine and therefore increase production cost of the VLP vaccine (Tharmarajah *et al.*, 2017).