INTRODUCTION

1. INTRODUCTION:

1.1 Global scenario of vector borne diseases:

Vector borne diseases impose a significant burden on public health and global socio-economic indices. The superiority of disease causing pathogens and transmitting vectors over the human population is well established causing the sustenance, resurgence as well as new emergence of various diseases of public health importance. Vector populations have efficiently adapted themselves for successful pathogen transfer. One such major adaptation is the evolution of hematophagy (blood feeding) in arthropod vectors enabling themselves transmission of numerous disease causing pathogens such as, viruses, protozoans, bacterias and helminths in humans (Gubler, 1998). Hematophagous arthropods ingest the disease causing pathogens from an infected individual and later inject the same into a healthy being causing the successful transmission of the infection. Some of the commonly occurring vector borne diseases are Malaria, Dengue, Chikungunya, Zika, Yellow fever, Lymphatic filariasis, Chagas disease, Onchocerciasis, Loiasis *etc.*

Of the total diseases suffered globally, around 16.6% are due to vector borne diseases (WHO, 2014). Globally, more than 1 billion people get infected by vector borne diseases and 1% die primarily by malaria, Dengue, leishmaniasis, chagas disease, yellow fever, filariasis and onchocerciasis (WHO, 2014). Since, the first discovery of vector mediated disease transfer in humans in 1877, these diseases have stood as a barrier to the development of countries present in the tropic areas (Gubler, 1998).

One of the most historically marked vector borne disease pandemic episode remains the 'Black death' *i.e.* plague in the 14th century followed by the yellow fever

epidemic (Gubler, 2009). Historically, these diseases remained confined to specific areas but later on the scenario changed drastically. Nowadays these diseases have become cosmopolitan owing to the easy transport of vectors mainly due to globalisation, increased human mobility throughout different parts of the world, rapid unplanned urbanisation *etc*. (WHO, 2014) creating opportunities for easy transfer and distribution of vectors and diseases (Figure 1).

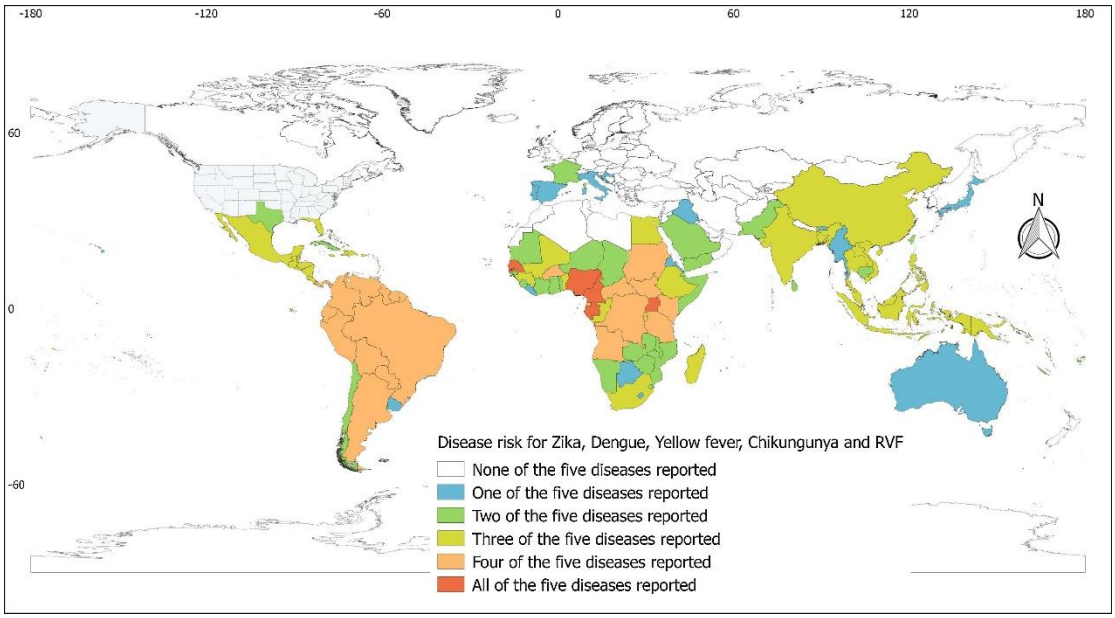

Figure 1: Global mapping of major vector borne diseases Zika, Dengue, Yellow fever, Chikungunya and Rift valley fever (Source: Leta *et al.*, 2018)

Globally, climatic changes are increasing the numbers and distribution of many disease vectors (WHO, 2014). Additionally, poverty related issues, *i.e.* lack of access to adequate housing, malnutrition, lack of proper sanitation and drainage and unavailability of safe drinking water also contribute towards the increased risk of such diseases. The efficiency of a disease agent transfer by a vector is dependent on many factors, particularly on the extent of contact with the host and on feeding behaviour (Gubler, 2009). In this regard, flies and mosquitoes may be assumed to be efficient vectors as evident from their close association with vertebrate hosts.

Mosquitoes, one of the most successful hematophagous arthropods are responsible for the transmission of numerous dreadful diseases, such as malaria, Dengue, Japanese encephalitis, Yellow fever, West Nile fever, Zika, Chikungunya, Filariasis causing several million deaths throughout the world annually (WHO, 2017). Mosquitoes are responsible for the greatest number of infections among other vector borne diseases (Figure 2).

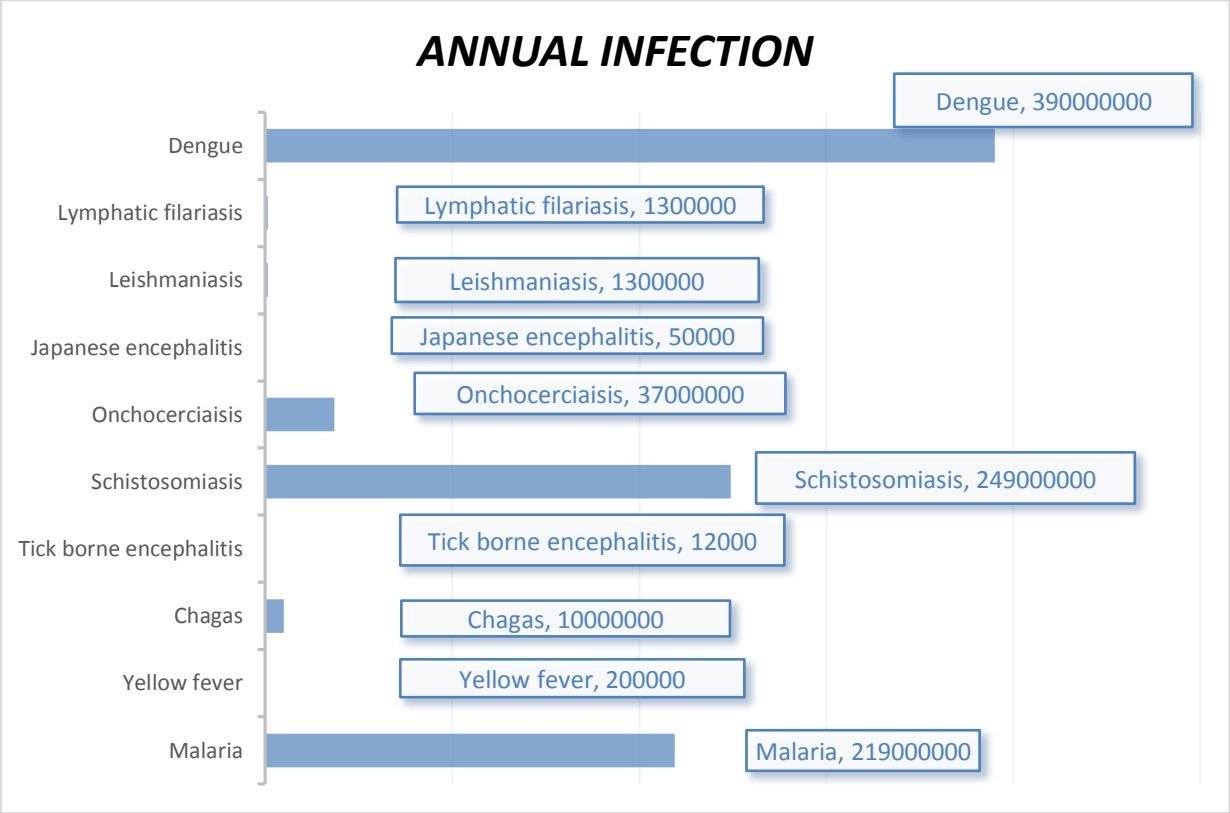

Figure 2: Vector borne diseases' proportion in global scenario

Majority of the tropical and subtropical countries are greatly affected by mosquito borne diseases. Globally, 97 countries have reported malaria transmission posing risk to 3.4 billion people (WHO, 2014). The most adversely affected region is the sub-Saharan region and the population at risk consist of young children, older aged individuals, pregnant women and non immune travellers to disease endemic regions (WHO, 2014). The most rapidly spreading mosquito borne viral disease is

Dengue which has expanded to previously unexplored regions increasing its incidence rate approximately 30 fold in the last 50 years (WHO, 2014). Another mosquito borne viral disease affecting Africa, Asia and the Indian subcontinent is Chikungunya. Recently this disease resurgence occurred in Asia, Africa, Europe as well as North America (WHO, 2014). India owing to its subtropical climate is subjected to regular infections of various vector borne diseases (Figure 3).

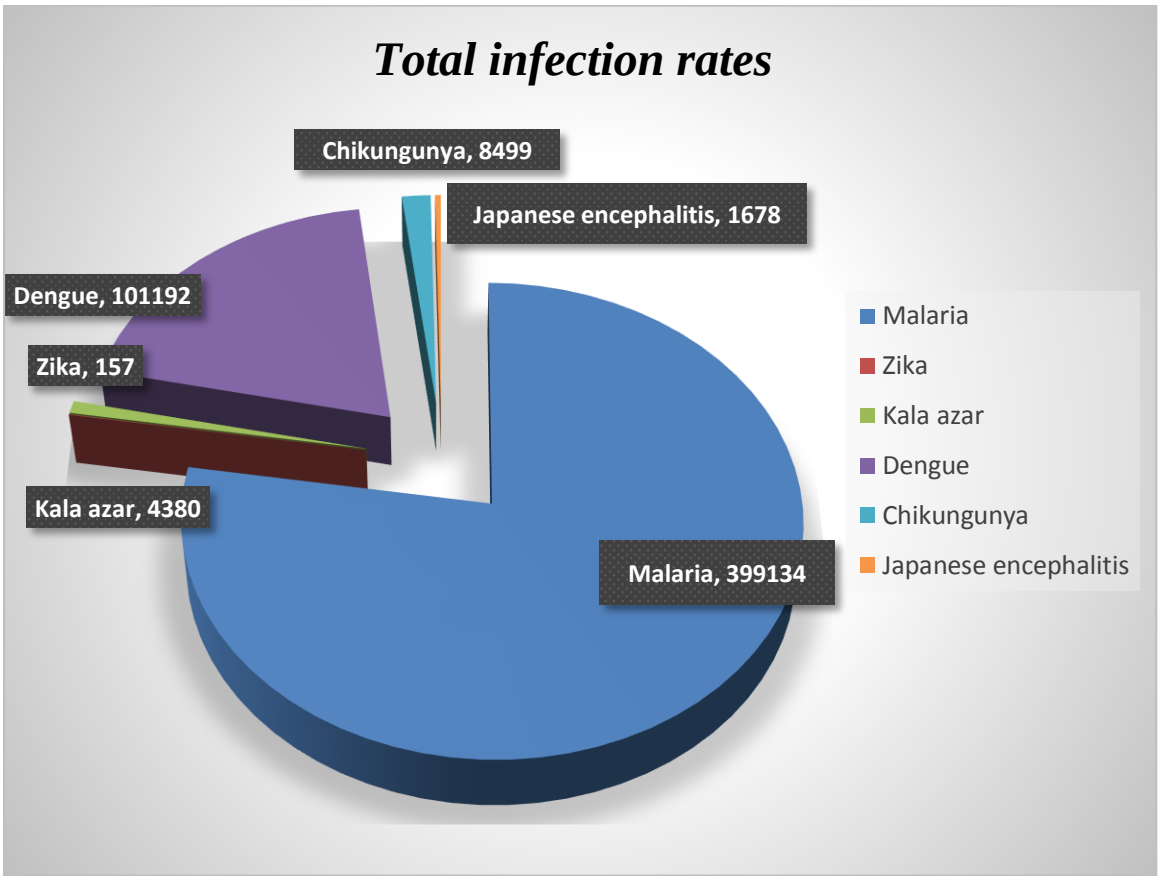

Figure 3: Annual proportion of vector borne disease transmitted by different vectors in India in 2018 (Source: NVBDCP, 2019a)

1.2 Dengue: a global burden

Dengue and Dengue hemorrhagic fever (DHF) are caused by virus belonging to family Flaviviridae, genus *Flavivirus*. *Flavivirus* is a medically very important genus of viruses, members of which can cause Dengue, yellow fever, west Nile

diseases, tick borne encephalitis and Japanese Encephalitis (Kuhn *et al.*, 2002). Dengue virus remains one of the most dreadful human pathogen transmitted by *Aedes* mosquitoes infecting humans worldwide (Figure 4).

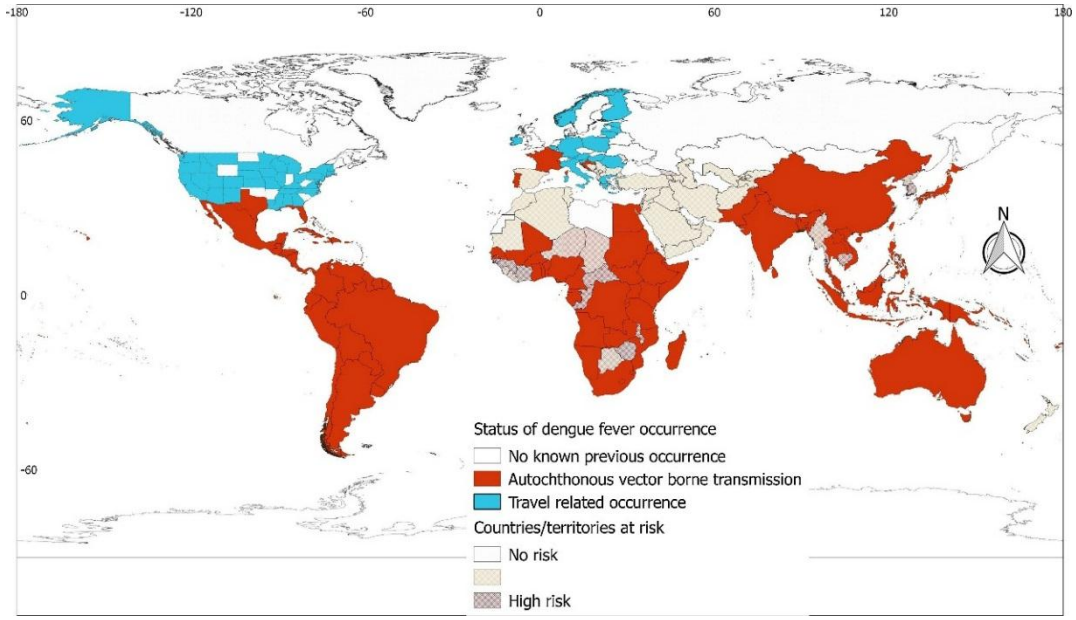

Figure 4: Dengue fever occurrence in global perspective (Source: Leta *et al.*, 2018)

There are four antigenically different yet closely related Dengue virus, generally termed as serotypes, *i.e.* DEN1, DEN2, DEN3 and DEN4. The presence of four different Dengue serotype creates additional complexity to the infection scenario and the serotypes only provide short term cross immunity against each other (Reich *et al.*, 2013). However, infection with one serotype does provide lifetime immunity against the specific serotype. DEN virus is endemic in around 100 countries of the world mainly situated in the tropical and subtropical regions (Sun and Kochel, 2013).The general DENV genome consists of a single chain of RNA (approx. 10,700 nucleotides) with its nucleocapsid embedded into three structural (core, membrane and envelope) and several non structural proteins (Kuhn *et al.*, 2002; Kurane, 2007). The interaction between the Dengue virus and the host immune system is mediated by

a non structural protein, known as NS-1, evoking an adaptive immunity mediated through T cell (John and Rathore, 2019).

The virus is transmitted to humans through the bite of *Aedes* mosquitoes namely *Ae. aegypti* and *Ae. albopictus*. The viruses circulates in the host blood and multiplies extensively for about 2-7 days. After this incubation period, the first clinical symptom is observed, *i.e.* fever (WHO, 2019a). Consequently more symptoms appear and the severity of the disease increases. Infected patients can transmit the virus via an *Aedes* mosquito vector to healthy person usually after the appearance of first symptom.

Reports also exist on the mucocutaneous mode of virus transmission (Chen and Wilson, 2004) as well as transmission from asymptomatic individuals or before the appearance of clinical manifestations (Duong *et al.*, 2015). They also report that asymptomatic individuals are more infectious to mosquitoes than people with symptomatic infections (Duong *et al.*, 2015).

Dengue fever is generally marked by high fever along with joint pain. The so called "breakbone fever" and frontal headache, however rash, nausea and lymphadenopathy may also develop (Kurane, 2007). The fever occurs after an incubation period of 2-7 days. More complex and severe is the Dengue haemorrhagic fever (DHF), that occurs in around 5,00,000 individuals (Gubler 1998). DHF arises due to the leakage of plasma into interstitial spaces leading to low platelet count and hemorrhagic symptoms (Kurane, 2007).

DHF is characterised by four main symptoms, fever, hemorhhages, hepatomegaly and failure of the circulatory system (WHO, 1997). DHF starts similarly as Dengue fever, but extreme weakening and collapse may occur within 2-5

days. The ultimate haematological symptoms of DHF are thrombocytopenia (low blood platelet count), increased RBC volume in the blood (hematocrit), elevated prothrombin time and bleeding time (Kurane, 2007). DHF has been characterised by WHO into four grades, of which most severe are the last two grades (grade 3 and 4) where plasma leakage is so extreme that onset of hypovolemic shock occurs, this is termed as Dengue shock syndrome (DSS) (WHO, 1997).

Dengue being one of the most rapidly spreading disease has increased its incidence rates in the past decades (WHO, 2019b). It is estimated that approximately 3.9 billion people residing through 128 countries inhabit Dengue risk areas (Brady *et al.,* 2012). An estimated 390 million infections of Dengue occur every year, out of which only 25% show clinical manifestations (WHO, 2019a). DHF is marked in tropical and subtropical countries of Asia and Latin Americas (WHO, 2019b). Annually around 2.5% of the infected individuals die due to the occurrence of severe form of Dengue (WHO, 2019a).

Severe Dengue epidemic was first noticed in 1950s in Phillipines (WHO, 2019a). However, now it has spread to almost every human inhabiting continent (Guzman and Isturiz, 2010). This is endemic in Africa, Southeast Asia, Eastern Mediterranean, America and the Western Pacific (WHO, 2019a). In 2015, around 2.35 million cases of Dengue infections were recorded in the Americas with 1181 deaths (WHO, 2019a). Similar epidemics were also noted in Portugal (2012), India (2015), Hawaii island (2015-2016) (WHO, 2019a). Worldwide severe Dengue outbreaks occurred in 2016 with the regions of Americas and western Pacific reporting the greatest disease burden rates (WHO, 2019a). In disease endemic countries, the Dengue burden is around 1300 DALY (Disability adjusted life years) per million population (Bhatt *et al.,* 2013).

1.3 Global burden of Chikungunya:

Chikungunya virus (CHIKV) belongs to the genus *Alphavirus*, family Togoviridae and is responsible for an acute fever along with joint pain and weakness symptom (WHO, 2019c). CHIKV was first identified in 1952 in Tanzania and its outbreaks are recorded in Africa and Asia since then (WHO, 2014) (Figure 5). A peculiarity about Chikungunya outbreak is that, their outbreaks are periodic, often recurring after a period above a decade (WHO, 2019c).

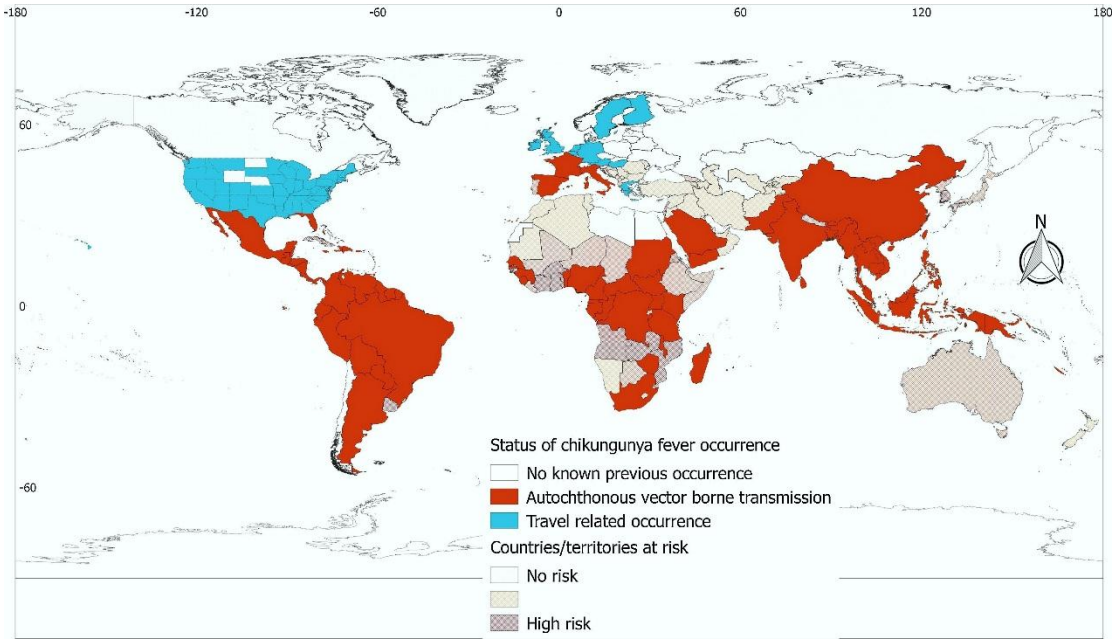

Figure 5: Global spread of Chikungunya fever (Source: Leta *et al.*, 2018)

Since 1982, Chikungunya epidemic have been reported from seven tropical Asian countries. Major epidemics have been reported to occur after 2000 in many countries, *i.e.* Congo (1999-2000), Gabon (2007), countries around Indian ocean (2005), India (2006-2007), Pakistan (2016-2017). Around 1.9 million Chikungunya infections have been reported to occur in five Asian countries viz., India, Indonesia, Maldives, Myanmar and Thailand (WHO, 2019c). Though Africa and Asia are the prime Chikungunya endemic continents, sporadic cases also occur in Europe, mainly

due to imported cases. Infections commonly localized in Europe have been reported in Italy (2007) and France (2013-2014) (WHO, 2019c). Over 43 countries of the Americas have been reported to have local Chikungunya transmissions. In 2015, approx 1.37 million CHIKV infection were reported in Carribean island, USA and Latin America countries (WHO, 2019c). In the Americas, highest infection rates were recorded for Brazil, Colombia and Bolivia in 2016 (WHO, 2019c).

The CHIKV consists of a genome of single stranded RNA (≈12000bp) with an icosahedral capsid (60-70 nm) enclosed within a lipid envelope (Thiberville *et al.*, 2013). Till now three distinct lineages of CHIKV have been identified each with specific genotype and antigenic determinants: i) Asian phylogroup, ii) East, central and southern African and iii) West African phylogroup (Powers *et al.*, 2000).

Two distinct cycles of transmission have been reported for CHIKV urban cycle and sylvatic/enzootic cycle (Singh and Unni, 2011). The transmission of CHIKV in forests (in Africa) with arboreal mosquitoes taking over the function of vector, mainly species of *Aedes* mosquitoes is termed enzootic cycle. In such cycles, non human primates basically serve as the virus reservoir (and thus serving as the virus amplification site). Such a cycle may sometimes infect human colonies inhabiting areas near the forest, initiating the urban cycle (Singh and Unni, 2011). Once the virus is introduced into the urban areas, the task of vectorial transmission of the virus is taken over by the urban anthropophilic *Aedes* mosquitoes, namely, *Ae. aegypti* and *Ae. albopictus* (Chhabra *et al.*, 2008). However, reports also suggest the inter-human transmission of CHIKV from sylvatic mosquito vectors (Tsetsarkin *et al.*, 2016). Common sylvatic vectors include *Ae. furcifer*, *Ae. taylori*, *Ae. luteocephalus*, *Ae. africanus* and *Ae. neoafricanus* (Chhabra *et al.*, 2008). So, in general the transmission can be summarised as:

Onset of CHIKV infection in the non human primates in the African arboreal forest > occasional spilling of the infection to humans dwelling nearby > anthropophilic mosquitoes carry on the viral transfer to more human > initiation of human – *Ae. aegypti*/ *Ae. albopictus*- human transmission in the urban areas > established Chikungunya epidemic

Since these two *Aedes* sp. lie in close proximity to human colonies they cause a high exposure rate of humans to the pathogen virus. No sylvatic transmission has been noted to occur outside Africa thus implying that Chikungunya arose in Africa (Chhabra *et al.*, 2008).

The clinical manifestation of Chikungunya starts 2 days after the introduction of the virus in the human body, *i.e.* when the patients have highest viraemia; however it declines afterward (Shah *et al.*, 1964), The symptoms start with fever, which may persist upto two weeks (Staples *et al.*, 2009). In majority of the patients, shortly after the fever, joint paint develops which mostly occurs in wrists, elbows, fingers, ankles and knees (Chhabra *et al.,* 2008). Sometimes maculopapular rash may develop spreading through the extremities and trunk, however, rashes on palms and the face have also been reported (Staples *et al.,* 2009). Although Chikungunya infections rarely result in deaths, yet elderly people (suffering from other medical conditions) and patients with co-infection generally acquire complications which may prove fatal (Economopoulou *et al.,* 2009). Due to similar symptoms and similar vectors, Chikugunya and Dengue is often confusing to differentiate, however Dengue is characterised by haemorrhages whereas Chikungunya is basically identified by the multiple joint anthralgias (Staples *et al.,* 2009).

1.4 Zika statistics and possible future scenario:

Zika is the first major infectious disease responsible for alarming rates of human birth defects (Petersen *et al.,* 2016). The Zika virus (ZIKV) also belongs to genus *Flavivirus* as DENV. It was first isolated in 1947 in Uganda from a rhesus macaque (Kirya, 1977). However, ZIKV causing human diseases was recognised for the first time in Nigeria 6 years later (Macnamara, 1954). Since Zika is vectored by *Aedes* mosquitoes, it poses an immediate danger to the regions suitable for *Aedes* habitation (Figure 6).

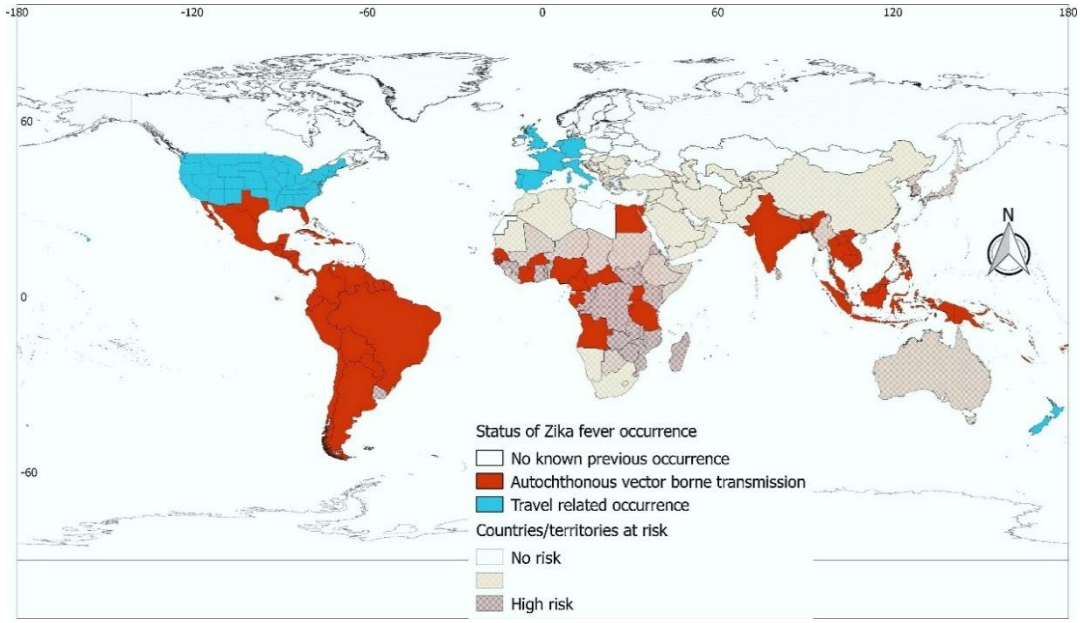

Figure 6: Global status for Zika fever incidence (Source: Leta *et al.,* 2018)

Though sporadic events of Zika were reported in Asia and Africa, the large outbreaks were reported in 2007 and 2013 (French Polynesia) (Musso *et al.*, 2014). Only 13 cases of Zika were reported till 2007, when a major outbreak took place in federated states of Micronesia causing an infection of 74.6% among the total residing population (Duffy *et al.,* 2009). Later outbreaks were reported from French Polynesia and other pacific islands (Petersen *et al.,* 2016). In India, 157 laboratory confirmed cases of Zika have been reported till November, 2018 caused by virus endemic to

Asia (Yadav *et al.,* 2019). Similar to CHIKV transmission, ZIKV has a sylvatic transmission cycle in Africa with similar reservoir and vectors. Moreover, the urban cycle is maintained by the same two *Aedes* species, *i.e. Ae. aegypti* and *Ae. albopictus* as CHIKV. However, *Ae. hensilli* and *Ae. polyniensis* were found to be the vectors of recent outbreaks in YAP and French Polynesia (Petersen *et al.,* 2016).

ZIKV has been found in many species of *Aedes* mosquitoes, *i.e. Ae. aegypti*, *Ae. albopictus, Ae. vittatus, Ae. luteocephalus, Ae. apucoargentus* and *Ae. furcifer* (Marcondes and Ximenes, 2016). Other Aedine mosquitoes have also been reported to carry ZIKV belonging to *Anopheles, Culex* and *Mansonia* genera (Benelli and Romano, 2017). Reports also point on the sexual mode of transmission (Foy *et al.,* 2011) and perinatal transmission of ZIKV (Besnard *et al.,* 2014).

The clinical symptoms associated with Zika is acute febrile illness with fever, conjunctivitis, arthralgia, rash or a combination of these. Other common symptoms include arthritis, headache, retro-orbital pain, vomiting, edema, hematospemia, subcutaneous bleeding, swelling of extremities, *i.e.* hands and ankles (Petersen *et al.,* 2016). However the most adverse effect of ZIKV is on the pregnant ladies causing microcephaly and other congenital malformations in the foetus (Petersen *et al.,* 2016). Microcephaly refers to reduced head size for the gestational size indicating the reduced growth of brain (Woods and Parker, 2013). In adults and children ZIKV can cause some neurologic complications such as myelitis, neuropathy and Guillain-Barre syndrome (WHO, 2019d). Central for disease control and prevention (CDC) has confirmed 69 countries and territories with active ZIKV transmission since 2007 (WHO, 2016).

`1.5 India's burden of Dengue, Chikungunya and Zika:

Dengue virus was first isolated for the first time in 1943 in Japan and in 1944 in India (Gupta *et al.,* 2012). The first virologically proved outbreak of Dengue occurred in during 1963, 1964 in the eastern coasts of India, however first Dengue like epidemic (not virologically proved) can be dated back to 1780s (in Chennai). In India, the onset of DHF was observed to occur in 1988 (Kabra *et al.,* 1992). However, since 1996, Dengue has become a more or less regular phenomenon causing substantial morbidity as well as mortality throughout the Indian country. All the four serotypes of Dengue have been noted to be present in India causing major epidemic episodes (Gupta *et al.,* 2012).

India is reported to acquire the largest number of Dengue infections annually with approximately 100 million asymptomatic and 33 million clinically manifested infections (Bhatt *et al.,* 2013). The presence of both the urban vectors and ideal climatic conditions aggravate the factors for major outbreak of Dengue. More than 0.1 million cases of Dengue occur every year in India, the trend towards an increase in infection rates every year (Table 1) (NVBDCP, 2019a).

Table 1: Infection rates of Dengue and Chikungunya in India during last five years (NVBDCP, 2019a,b)

Year	**Dengue**		**Chikungunya**	
	Case	**Death**	**No. of suspected cases**	**No. of confirmed cases**
2019	5504*	5	--	--
2018	101192#	172	47208	8499
2017	188401	325	67769	12548
2016	129116	245	64057	26364
2015	99913	220	27553	3342

*****Provisional data, #Except data from West Bengal

In India, major Chikungunya epidemic was reported in 1963 in Kolkata. Successively Pondicherry, Tamilnadu, Andhra Pradesh, Madhya Pradesh and Maharashtra reported Chikununya epidemic in 1965 and again in Maharasthra in 1973 (Sudeep and Parashar, 2008). The virus them had a resurgence in 2006 affecting 1.5 million people in thirteen India states (Cecilia, 2004). In 2011 again a major epidemic of Dengue affected every state and territory state of India except Punjab (in states) and Dadra-Nagar Haveli and Pondicherry (in territories) (Cecilia, 2004). The rate of mortality due to CHIKV is rare in India. Since 2015, around 1.39 million cases of infection have been reported throughout the country affecting approx this 213 districts in 15 states (Krishnamoorthy *et al.,* 2009).

Since majority of vector borne diseases adversely affect the Southeast Asian countries, it has been estimated that three region comprising of all the tropical and subtropical countries are at high risk of Zika infections (Messina *et al.,* 2016). Since eleven Southeast Asian countries have reported small/occasional Zika outbreaks, India is at a very high risk of this disease (Tilak *et al.,* 2016). It has been reported that co-circulation of ZIKV along with CHIKV and DENV is very likely in countries where both latter infections are common posing additionally risk on the country (Musso and Gubler, 2016). Moreover, a trade relation with ZIKV affected countries is also a risk factor in this context (Tilak *et al.,* 2016).

1.6 West Bengal and its burden of VBDs:

In 2017, among the Indian states the highest numbers of Dengue infections were reported in West Bengal (Table 2 and 3). Not only Dengue, West Bengal also records high incidences of other VBDs. The prevalence of these VBDs in W.B. may be pertained to many vectors. The warm and humid temperature and climatic

conditions, rapid urbanisation, high vegetation cover, lack of sanitation, hygiene and drainage in majority of the state together provide the ideal ambience for *Aedes* and other mosquito growth and proliferation. Additionally, the high population density of West Bengal (highest in India) along with above mentioned factors support the efficient circulation of disease causing pathogens.

Table 2: Prevalence of Dengue and Chikungunya in West Bengal (Source: NVBDCP, 2019a)

Year	***Dengue***		***Chikungunya***	
	Case	***Death***	***No. of suspected cases***	***No. of confirmed cases***
2019	*	*	*	*
2018	*	*	52	23
2017	37746	46	2103	577
2016	22865	45	1071	117
2015	8516	14	1013	61

*Data not available

Table 3: Statistics of major vector borne diseases and population at risk in northern districts of West Bengal (Source: State Vector Borne Diseases Control and Seasonal Influenza Plan, 2018)

District	***Dengue infection (2017)***	***Malaria infection (2017)***	***Population at high risk***
Alipurduar	74	1404	1024671
Coochbehar	217	129	1011047
Jalpaiguri	855	115	236588
Darjeeling	1266	58	286439
North Dinajpur	283	138	*

*Data not available

1.7 *Aedes*- the main culprit or "vector":

Aedes mosquitoes pose severe threat to human race because of its capability to transmit several arboviruses, *i.e.* DENV, CHIKV, ZIKV, Yellow fever virus, *etc* (Kraemer *et al.,* 2015). These diseases have increased severely in the past five decades and expanded itself many folds and spread throughout the globe. The total global burden put up by these mosquitoes is huge as calculated by the diseases they transmit. *Ae. aegypti* is also a key vector for the transmission of a serious emerging zoonotic disease, the rift valley fever (Pepin *et al.,* 2010). The habitat suitability range for these mosquitoes covers all the habitable continents of the world (Kraemer *et al.,* 2015). In a study, it was reported that of the 250 studied countries/territories 86% were suitable for the sustenance of *Ae. aegypti* and *Ae. albopictus* (Leta *et al.,* 2018). Furthermore the same was found to be more varied for *Ae. albopictus* than *Ae. aegypti*.

The most suitable regions for both the species were found to be the USA, South America, Indian subcontinent, Carribean, sub-Saharan Africa, Southeast Asia and few Pacific countries. In some regions of Asia and Western Africa, the distribution of both the *Aedes* species overlap, whereas in East Africa, Europe, United States, and Australia their distribution varies considerably (Kamal *et al.,* 2018). The ideal ambience for *Ae. aegypti* were mainly spread over the tropical and subtropical regions, whereas that of *Ae. albopictus* were greater str*etc*hing to the temperate regions too, *i.e.* Central USA and Southeast Europe (Leta *et al.,* 2018). This may be related to the ability of *Ae. albopictus* to survive in colder, dry and hardy environments unlike *Ae. aegypti* (Figure 7).

About 50% of the world has more than one arboviral disease (Leta *et al.,* 2018). Around 111 countries are endemic for Dengue, 106 for Chikungunya and 85

for Zika, 43 for yellow fever and 39 for rift valley fever (Leta *et al.*, 2018). Among the continents of the world, Autochthonous transmission of Dengue and Chikungunya have been reported from all the continents, Zika from all continents except Europe; yellow fever from Africa and America and rift valley fever from Africa and Asia only (Leta *et al.*, 2018).

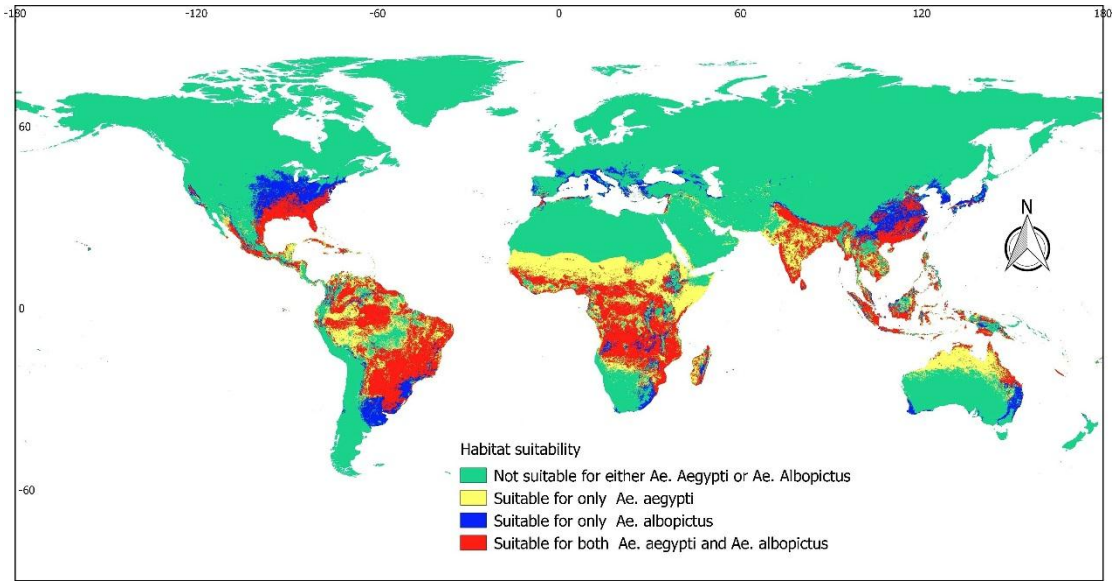

Figure 7: Habitat suitability for *Ae. aegypti* and *Ae. albopictus* throughout the world (Source: Leta *et. al.*, 2018)

Aedes mosquitoes are closely associated with the human colonies and occur near such dwellings, commonly laying eggs in and around human houses. Additionally, human made products, *i.e.* tyres, tanks, plastic containers *etc* serve as egg laying sites and becoming its breeding and proliferating site, thereby increasing their abundance and establishing colonies in human inhabiting areas. With the recent re-emergence of arboviruses, *Ae. albopictus* seems to become more efficient in virus transmission such as CHIKV because of minor mutations, the combination of which may increase the vector competence in this species (Tsetsarkin *et al.*, 2014).

1.7.1 Ae. aegypti:

Ae. aegypti, also known as yellow fever mosquito, is one of the most medically significant mosquito species responsible primarily for the transmission of Dengue, Chikungunya and yellow fever viruses worldwide (Powell and Tabachnick, 2013). *Ae. aegypti* originated in the African continent particularly the sub-saharan Africa region. Though, ancestral population of these mosquitoes were mainly non human feeders and tree holes served the main site of larval growth and habitation (Powell and Tabachnick, 2013). These population still represented by the sub species formosus is comparatively darker than the more recent subspecies adapted with human habitation *i.e. Ae. aegypti aegypti*. The latter lightly colored and domesticated species generally prefer feeding on human blood and breeding on man-made artificial containers. One more subspecies of *Ae. aegypti, i.e. Ae. aegypti queenslandensis* was present in Mediterranean region but doubt remains on its existence (Mattingly, 1967). Soon after domestication, *Ae. aegypti* expanded its distribution throughout different continents as a result of trade through ships. All the tropical and subtropical population of *Ae. aegypti* existing outside Africa is believed to form a monophyletic group (Brown *et al.*, 2014). However *Ae. aegypti aegypti* and *Ae. aegypti formosa* seem to show sympatry though they remain genetically separate yet they randomly mate to produce fertile offsprings when brought together (Moore, 1979). Moreover, these behavioural characters are not strictly maintained by these mosquito subspecies, since these are highly flexible and opportunistic and can respond quickly to environmental disturbances and changes.

Ae. aegypti are widely distributed in Asian and American continents with only five countries recording to carry 55% of the total *Ae. aegypti*, namely India, Thailand, United States of America, Mexico and Brazil (Kamal *et al.*, 2018). In Asian

continent, *Ae. aegypti* have been found to be distributed throughout major regions along with the Central and Southern part. This species has been recorded in Western Saudi Arabia, Western coasts of Arabian gulf, Lebanon, Israel and Syria (Kamal *et al.*, 2018). The *Ae. aegypti* is predicted to spread to Northern Australia, Eastern and Western coasts of Australia, Oceania and New Zealand. In Americas, Southeast USA, Caribbean islands, a minor region in the Pacific coasts of Canada and USA serve as the habitable regions for *Ae. aegypti* (Kamal *et al.*, 2018). In Europe, *Ae. aegypti* is believed to occur in Albania, Cyprus, Croatia, France, Greece, Italy, Spain and the coasts of Turkey and Portugal (Kamal *et al.*, 2018). In African continent, *Ae. aegypti* is maximally distributed across the sub-Saharan countries.

Domestication of *Aedes* mosquitoes determines its vector competence, as *Ae. aegypti aegypti* is more competent to transmit the disease carrying arboviruses than *Ae. aegypti formosus* (Tabachnick, 2013). This may be either due to the virus' adaptation to mosquito or the mosquito's bodily function increasing their competence (Powell and Tabachnick, 2013). The first instance of *Ae. aegypti* as disease vector in human, dates back to 1881 associated with yellow fever by Carbs Juan Fulay (Strode, 1951). Since then, these species have been documented to transmit human diseases throughout the world.

With an average flight range of 400 metres, most female *Ae. aegypti* are reported to spend their lifetime inside or in the vicinity of the human dwellings, where their development to adult has occurred, thus people are responsible for the rapid movement of the virus between the populations (WHO, 2019e). This indoor habitat increases the *Ae. aegypti* mosquitoes' lifespan, since these habitat are not generally susceptible to weather related variations (WHO, 2019e)

1.7.2 Ae. albopictus:

The second major disease transmitting *Aedes* species is *Ae. albopictus* commonly known as "Asian tiger mosquito". As the name goes, it has its origin in Asian continent, first identified in Kolkata as banded mosquito of Bengal by Skuse 1894 (Huang, 1968). Owing to its plasticity and elasticity it can invade regions throughout the world where other mosquitoes cannot thrive, thus known as one of the most invading mosquito species. It was originally a sylvatic mosquito species that became exposed to the human habitats as a result of deforestation, human habitat expansion in the vicinity of forests and ecotourism activities.

Unlike *Ae. aegypti* which is an anthropophilic mosquito, this species mainly prefers to feed on wild animals, however owing to its wide geographical distribution and behavioural plasticity now also efficiently adapted to feed on human blood and transmit diseases of public health importance, even more effectively in some instances than the primary vector, *Ae. aegypti* (Kraemer *et al.*, 2015). Spread of this species is also believed to be an effect of globalisation through trade of tyres, lucky bamboos (potted ornamental), containers carrying dormant mosquito eggs from one region to another, sometimes to previously un-invaded regions. As of now, *Ae. albopictus* has spread through Africa, middle east Europe to the Americas (Gratz, 2004).

Ae. albopictus, a native of Southeastern Asia, is now present in all the five habitable continents during the past four decades (Kamgang *et al.*, 2018). *Ae. albopictus* have been reported to be dominant over *Ae. aegypti* wherever both species co-exist owing to its higher mating competitiveness over *Ae. aegypti (*Bellini *et al.*, 2013). Moreover, wherever these two species show sympatry, *Ae. albopictus* shows preference to habitats/containers surrounded by vegetations.

In Asia, *Ae. albopictus* mainly occurs in rural and suburban regions, mainly surrounded with forests and vegetations. This species have been recorded to be distributed in Cambodia, China, India, Japan, Malaysia, Pakistan, Myanmar, Thailand, Vietnam *etc* (Gratz, 2004; Vontas *et al.,* 2012) In the Americas, the geographical str*etc*h of *Ae. albopictus* extends from Southeast to North USA and South Canadian border with its distribution widely in continental USA but low across the South American continent. Now, *Ae. albopictus* can be found in many American countries spread from USA upto Argentina; Hawaii, the Solomon Islands and Fiji among the Pacific Islands (Paupy *et al.,* 2009). In Africa, the suitable habitats for *Ae. albopictus* has been noted across the red sea coast and the mediterannean coast from Morocco to Egypt, and across the Eastern region. *Ae. albopictus* after its first detection in South Africa (in 1989) was later recorded from other African countries, namely Cameroon, Gabon, Equatorial Guinea and Nigeria. In Central Africa, *Ae. albopictus* was first reported in early 2000 and now it is present in much of the central African countries (Paupy *et al.,* 2009). In European continent, this species shows broad distribution through most of the Western countries and the Balkan region (Kamal *et al.,* 2018).With the first ever detection in Albania(in 1979) amongst the European countries, it has been since then reported in this continent in other countries too, *i.e.* Bosnia and Herzegovina, Croatia, France, Greece, Italy, Montenegro, Netherlands, Serbia, Slovenia, Spain and Switzerland. *Ae. albopictus* have also been reported in the Australian continent. The vector status of this species remained questionable till epidemic Dengue outbreaks in absence of *Ae. aegypti* but in presence of this species occurred (Gratz, 2004).

1.8 Available treatments for *Aedes* transmitted disease:

For none of the arboviruses transmitted by *Aedes* mosquitoes, any treatment or vaccination exist. So, the patients are treated of their symptoms not of the viral infection. Dengue fever is generally treated through early detection and proper case management, thereby reducing the deaths associated with the infection (NVBDCP, 2019a). For uncomplicated Dengue, administration of an analgesics and antipyretic such as paracetamol, oral rehydration and maintenance of body fluids and proper rest is advised (Khetarpal and Khanna, 2016). However, patients are monitored for adverse symptoms and different blood tests till three days of fever onset. Whenever the symptom of decreasing platelet count ($\leq$100,000/mm^3) or rising hematocrit is noticed, immediate hospitalization is recommended and the patients are treated with intravenous fluid, to maintain the plasma volume. However, other signs of Dengue shock syndrome are thoroughly monitored such as, hematocrit, platelet count, pulse rate, blood pressure, temperature *etc*. Mostly within 12-48 hours of fluid therapy, patients recover. Rarely, internal hemorrhage is noted, in such cases blood transfusion becomes necessary (Khetrapal and Khanna, 2016).

Patients infected with Chikungunya are basically advised to get proper rest and adequate hydration and nutrition. For management of the infection, analgesics, antipyretics and fluid supplementation is administered. For the peripheral arthritis, physiotherapy, short term corticosteroid administration or long term anti-inflammatory therapy is advised. However patients who develop complex symptoms such as renal failure, multiorgan system failure, refractory thrombocytopenia, encephalitis or acute infectious polyneuritis *etc* are generally hospitalised and treatment for the condition developed in a patient is provided (NVBDCP, 2019b). For

Zika symptoms are treated with acetaminophen for fever and pain and rest and rehydration is advised (CDC, 2019).

1.9 Disease prevention – the sole method:

In absence of specific medications and vaccines for all the above mentioned disease, the prevention becomes the only option to restrict disease transmission in humans. Disease prevention for *Aedes* transmitted arbovirus mainly involves vector control and personal prophylactic measures to minimise mosquito biting (NVBDCP, 2019a). Personal prophylactic measures consist of use of mosquito repellent tools and mosquito nets to prevent mosquito bites along with covering the whole body by wearing full sleeve clothes with socks (NVBDCP, 2019a).

The second part of disease prevention is vector control which can be done by the strategies mentioned below:

i) Environment management and source reduction strategy,

ii) Mechanical control,

iii) Chemical control,

iv) Health education and mass participation

Under environmental management, identification and subsequent deletion of mosquito breeding habitats, proper management and sealing of water storage containers *etc* are done to minimise mosquito breeding in these vessels. Use of biological agents to minimise the mosquito population is the aim of biological control, conventionally done with the use of larvivorous fish such as Guppy (*Lebistes reticularis*), Gambusia (*Gambusia affinis*) and recently with bacterial formulations *i.e.* *Bacillus thuringiensis* and *Bacillus sphaericus*.

Mechanical control of mosquito is done through fencing or screening the windows and doors, drilling of holes in artificial containers and disposed tyres (so that water is drained), safe disposal of scrap *etc*. The most common method of mosquito control through the use of larvicides and adulticides comes under the chemical control of mosquito. Adult mosquito control consists of fogging, aerosol spray, indoor residual spray, long lasting insecticide treated nets, outdoor barrier spraying *etc* (NVBDCP, 2019a). Moreover, oils or monomolecular films are also used to disrupt air breathing ion mosquito larvae.

Since the discovery of insecticides, they have been used heavily for mosquito control. In India, Organochlorines (DDT, Dieldrin, Aldrin *etc*), Organophosphates (malathion, temephos, dichlorvos, chlorpyrifos), Synthetic pyrethroids (Lambdacyhalothrin, Deltamethrin, Permethrin, cypermethrin and its derivatives, cyfluthrin) and Carbamates (Propoxur, bendiocarb) have been widely used for both mosquito control as well as agricultural pest control (NVBDCP, 2019c). The insecticides and larvicides recommended for mosquito control in India are provided in Table 4 and 5.

Mosquito control intervention makes the heavy use of insecticide at both household as well as higher levels. Insecticide treatment of bednet, curtains, windows, water storage cans *etc* have been reported to be highly effective at minimising the household *Aedes* mosquito infestation (Deming *et al.*, 2016). Similarly Ultra low volume (ULV) spray and thermal fogging have also been shown to be effective at reducing the risk of Dengue virus transmission throughout the world (Karunaratne *et al.*, 2013).

Table 4: List of insecticides recommended for indoor residual spray (IRS) for mosquito control in India (Source: NVBDCP, 2019c)

S. no.	*Name of insecticide*	*Insecticide class*	*Insecticide requirement per million population (MT)*
1.	DDT (50% WP*)	Organochlorine	150.00
2.	Malathion (25% WP)	Organophosphate	900.00
3.	Deltamethrin (2.5%WP)	Synthetic pyrethroid	60.00
4.	Cyfluthrin (10%WP)	Synthetic pyrethroid	18.75
5.	Lambdacyhalothrin (10% WP)	Synthetic pyrethroid	18.75
6.	Alphacypermethrin (5% WP)	Synthetic pyrethroid	37.50
7.	Bifenthrin (10%WP)	Synthetic pyrethroid	18.75

*WP: Wettable powder

However, use of any of the above vector control method is ineffective without the education of the community and their active participation. The knowledge about the vectors and their common breeding habitats along with its control/ management methods should be provided to common mob for efficient prevention of these diseases. Furthermore mass programmes aiming to detect *Aedes* breeding habitats and their elimination through proper disposal, sealing, drilling and proper drainage practices can help immensely in reducing the *Aedes* transmitted arboviruses transmission (NVBDCP, 2019a).

Table 5: Larvicide formulation and dosages recommended for mosquito larvae control in India (Source: NVBDCP, 2019c)

S.no.	*Name of insecticide*	*Class of insecticide*	*Frequency of application*	*Application on*
1.	Mixed larvicidal oil	--	Weekly	Shore of water body
2.	Temephos	Organophosphate	Weekly	Clean water
3.	*Bacillus thuringiensis* var *israelensis* 5% (strain- 164 Serotype H-14)	Biolarvicide	Fortnightly	Both clean and polluted water
4.	*Bacillus thuringiensis* var *israelensis* 5% WP (strain-ABIL Serotype H-14)	Biolarvicide	Weekly	Both clean and polluted water
5.	*Bacillus thuringiensis* var *israelensis* 12 Aqueous suspension	Biolarvicide	Weekly	Both clean and polluted water
6.	Diflubenzuron 25% WP	Insect growth regulator	Weekly	Both clean and polluted water
7.	Pyriproxifen	Insect growth regulator	3 Weekly	Both clean and polluted water

1.10 Constraints of vector control- Insecticide resistance:

The discovery of DDT initiated the use of chemicals in vector control. Shortly after its introduction the potency and efficacy of DDT as both larvicide and adulticide began a new era in vector control. However, soon was observed the ill effects of DDT including environment degradation and insecticide resistance in target species.

But due to the uncontrolled heavy use of these chemicals/ insecticides, both target as well as non-target species have evolved to resist the actions of those chemicals in their body through different mechanism. This phenomenon interrupting the chemicals to manifest their planned actions is known as Insecticide resistance (Corbel and N'Guessan, 2013). Insecticide resistance can be defined as the inability of the insecticide to manifest its planned action at the effective dosage against insects.

Insecticide resistance results in the failure of mosquito control programmes to achieve their planned targets, thereby increasing the risk of DENV infection even after insecticide spray during severe disease outbreaks (Corbel and N'Guessan, 2013). This phenomenon of resistance is widespread among majority of the insects exposed to insecticide in agriculture sector, public health sector or household region. Most of the insecticide used at household level are targeted against mosquitoes in the form of fumigants, coils, sprays, creams *etc*. Moreover when the breeding habitat of mosquito is situated around gardens, agricultural land, they get cross exposure to insecticide sprayed on those regions too thereby increasing the intensity of resistance in them.

Mosquitoes have developed insecticide resistance both as a direct effect of insecticides targeted on them as well as an indirect exposure of insecticide sprayed on agricultural field (Nkya *et al.,* 2013; Overgaard *et al.,* 2005). In tropical and subtropical countries, the high human population, congested area of living, small farming lands, poor sanitation practices, presence of farming land in the vicinity of living areas result in the cross contamination by agricultural run offs containing pesticide residue to mosquito breeding sites, thereby contributing towards the onset of insecticide resistance. Also, the household prophylactic measures *i.e.* use of mosquito repellent coils, creams, lotions, fumigants contain formulations of Synthetic pyrethroids (recent compounds contain transallethrin) also result in insecticide resistance development (Class and Kintrup, 1991).

Resistance to insecticides can be caused by an array of modifications within a mosquito, such as, behavioural alteration, physiological modifications within the cuticle reducing the insecticide penetration, biochemical changes within the activity of major insecticide detoxifying enzymes or structural modification within the target of the insecticide thereby blocking the insecticide binding and subsequent action (Yu,

2008). All the above mentioned mechanisms have been noted to occur in field populations of *Aedes* mosquitoes throughout the world. Also, varying degrees of insecticide resistance have been reported in both *Aedes*

Insecticide resistance in the insect body can be classified among four main mechanisms:

1.10.1 Behavioral resistance/ avoidance:

Behavioral avoidance also called as "deterrence" refers to the act of escape of an insect in response to insecticide treatment on an area. Mosquitoes have been reported to avoid DDTs and pyrethroid insecticides showing moderate to strong irritancy. It may be of two different types:

a. Contact excitation/irritancy: when an insect escapes the insecticide treated areas after making an initial contact with the insecticide.
b. Non contact/spatial repellency: when the insect moves away without making any contact with the toxic chemicals.

1.10.2 Cuticular resistance/ reduced penetration:

Insecticides manifest their action once they bind to their target site. However, if the toxic chemical fails to reach its target, its action will be hindered. In this type of resistance, there is a check on insecticide entry into the insect body. This may be brought by increasing the cuticular covering/ diameter or increasing the fat layer present immediately after the cuticle, thereby restricting the entry of the xenobiotic into the insect body (Yu, 2008).

1.10.3 Metabolic resistance/ insecticide detoxification:

Metabolic detoxification of insecticides refers to the degradation of the chemicals into non toxic and water soluble forms by the action of gut enzymes. During insecticide stress period, the production of these enzymes may get increased thus sequestering more and more insecticide residue. This enzyme production is generally mediated by up-regulation through mutation in trans or cis acting regulatory locus or through the duplication/amplification of the gene coding two techniques increases the quantitative levels of these enzymes. However, modification can also occur in the qualitative proportion of an enzyme, *i.e.* increased ability to detoxify the insecticide resulting from minor change in the coding sequence of the gene, thus in the amino acid sequence.

The enzymes carrying on the task of xenobiotic detoxification generally belong to large families of multigenes, the most notable being the carboxylesterases (CCEs), Cytochrome P450s (CYP450S) and Glutathione S-transferase (GSTs) (Corbel and N'Guessan, 2013; David *et al.*, 2013; Ranson and Hemingway, 2005; Hemingway and Karunartane, 1998). These enzyme families have been reported to provide resistance against different groups of insecticides throughout different insects. Over-expression of insecticide detoxifying enzymes have been found to confer resistance against insecticides in many mosquitoes.

Increased synthesis of one such enzyme, *i.e.* CCEs through gene amplification have been reported to confer resistance against organophosphate, carbamates and pyrethroid insecticides in dipteran insects (Bass and field, 2011). This may provide protection against insecticides by sequestration or slow turnover rate. Similarly, the elevation of isozymes of detoxification enzymes have also found to provide resistance. Metabolic detoxification has more severe outcomes than target site

resistance because these elevated enzyme activity may also provide cross resistance against insecticides belong to different classes unlike target site resistance which can provide resistance against only specific insecticide group.

Over expression of enzyme classes, Carboxylesterases (CCEs), Glutathione S-transferases (GSTs) and Cytochrome P450s (CYP450s) or Mixed Function Oxidases (MFOs) have been reported to confer insecticide resistance in many populations of insecticide resistant *Ae. aegypti* and *Ae. albopictus* population worldwide (Vontas *et al.,* 2012; Ranson *et al.,* 2010). Through advanced studies incorporating transcriptome studies and Detox chip analysis, all the three above mentioned enzyme classes namely, CYP450s, CCEs, GSTs have been implicated in conferring insecticide resistance against insecticides.

1.10.3.1 Carboxylesterases (CCEs):

Carboxylesterases enzyme, one of the major insecticide detoxifying enzyme belong to the Carboxylesterase gene family within the alpha/beta hydrolase superfamily which is one of the most commonly occurring protein folds in nature. This superfamily also contains lipases, dehalogenases, peroxidases, proteases *etc* (Montella *et al.,* 2012). These CCEs carry on the hydrolysis of esters, *i.e.* carboxylesters, splitting it into corresponding carboxylic acid and alcohol. These enzymes have been identified in all living species (Hatfield *et al.,* 2016). These esterases can hydrolyse a varied sort of substrates driving different actions. CCEs are omnipresent and play vital roles in the metabolism of several exogenous compounds, mainly ester carrying xenobiotics (Montella *et al.,* 2012). In insect life-cycle, these enzymes control a wide range of vital functions and behaviour, such as development, reproduction, digestion, odorant degradation, pheromone and other semiochemical hydrolysation (Montella *et al.,* 2012).

CCEs are mainly classified based on the interaction of esterases with organophosphates (Aldridge, 1953a; Aldridge, 1953b), those that hydrolyse them are categorized as esterases A (Est-A), those that are inhibited by them are termed esterases B (Est-B) and those that do not interact with organophosphate are categorized as esterases C (Est-C). The difference in the nature of Est-A and Est-B is due to the susceptibility of the serine residue present in the catalytic site of the enzyme to phosphrylation in Est-B but not in Est-A (Walker & Mackness, 1983).

CCEs are given much importance in insecticide chemistry since, many insecticides contain ester bonds rendering it susceptible to hydrolysis by enzyme activity (Sogorb & Vilanova, 2002). Of the commonly used insecticides, CCEs primarily detoxify OP insecticides, such as temephos, malathion, chlorpyriphos *etc* and secondarily carbamate insecticides such as bendiocarb, propoxur (Hemingway and Karunaratne, 1998). The mechanism of metabolic resistance involves insecticide hydrolysis or sequestration (Montella *et al.,* 2012).

Metabolic resistance to insecticides may arise by multifaceted mechanisms and insecticide resistant populations develop distinctive mechanisms for the efficient degradation of xenobiotic, *i.e.* insecticide (Saavedra-Rodriguez *et al.,* 2012, Poupardin *et al.,* 2008, Strode *et al.,* 2008). Insecticide detoxifying enzymes evolve rapidly by accumulation of mutations that doesn't affecting the original function rather may provide a selectivity advantage (Aharoni *et al.,* 2005). Resistance against OPs driven by over-expression of enzyme and/or insecticide sequestration resulting from CCE gene amplification have been reported in insects particularly, mosquito species (Grigoraki *et al.,* 2017).

Globally, very few CCEs gene have been reported to be amplified to confer resistance in insects. In *Culex* mosquitoes, resistance against OPs has been reported to be conferred by the elevated expression of two loci *i.e.* Est-2 and Est-3 as a result of gene amplification, which may be co-amplified as allelic pairs or amplified individually *e.g.* estβ1 gene in resistant mosquitoes (Bass and Field, 2011; Raymond *et al.,* 2001). Such amplified CCE alleles can get distributed to outlying regions by migration, as from different continents, the same common haplotypes have been noted in mosquitoes providing resistance against insecticides (Grigoraki *et al.,* 2017). Those alleles or combinations might get widely distributed than others owing to the higher fitness possessed by individuals carrying such alleles (Labbe *et al.,* 2009). This is the scenario for Ester2 which occurs in majority of the insecticide resistant populations, similar is true for the co-amplicon estα2-estβ2 (Li *et al.,* 2014).

CCEs have been also be implicated in conferring resistance against other insecticide groups, however the mechanisms of such action is not yet clear. Against temephos an OP insecticide, resistance has been shown to be conferred by the overexpression of CCE through amplification of *CCEae3a* transcript (Poupardin *et al.,* 2014). In other population co-upregulation of two transcripts belonging to different gene family, *i.e. CYP6Z8* and *CCEae3a* was found to confer resistance against OPs and synthetic pyrethroids suggesting the synergistic action by these transcripts (Marcombe *et al.,* 2009). In insects, the differences in the insecticide detoxification profile by enzymes might be due to the high rate of diversification as a result of species-specific evolution of detoxification gene families.

1.10.3.2 Cytochrome P450s (CYP450s) / monooxygenases:

The cytochrome P450-dependent monooxygenases (monooxygenases) are a vital group of enzymes involved in regulating the concentration of endogenous compounds as well as in the anabolism and catabolism of xenobiotics such as pesticides, drugs and plant derived allelochemicals. CYP450s monooxygenases are present in almost all aerobic organisms, ranging from bacteria to animals (Stegeman and Livingstone, 1998). These are the enzymes belonging to the largest superfamily of genes and bring upon the detoxification of xenobiotic compound as well as the metabolism of endogenous molecules such as hormones, steroids, fatty acids *etc* (Scott, 1999). These are found in all aerobic organisms ranging from bacteria to humans (Stegeman and Livingstone, 1998). These are basically heme proteins and can oxidise diverse substrates and this acts as the terminal oxidase in monoxygenase system (Scott, 1999). There appears to be 100 insect P450s (Nelson, 1998) however the first detection was made in 1967 (Ray, 1967). In insect apart from resistance, these play many crucial roles *i.e.* growth and development, tolerance to plant toxins, synthesis and catabolism of insect pheromones and hormones (Scott, 1999). Generally monoxygenase mediated resistance is provided against pyrethroids, imidacloprid, they are also crucial in activation of organophosphates (Hodgson *et al.,* 1991).

The main CYP450s associated with insecticide resistance are P450 reductase and b5 (Scott, 1999). Several P450s have been implicated in conferring resistance against synthetic pyrethroids, Over-expresssed P450s may also confer resistance against carbamates (Brooks *et al.,* 2001). In *Anopheles* mosquitoes CYP6Z1, CYP6Z2, CYP6M2, CYP6P3 and CYP325A3 have been shown to be involved in proving resistance (David *et al.,* 2013). In resistant wild mosquitoes, CYP6P3 and CYP6M2 have been noted to be over transcribed owing to their ability to degrade

permethrin and deltamethrin (Djouaka *et al.,* 2008). Similarly, CYP6Z1 has also been shown to detoxify DDT (Muller *et al.,* 2008) and CYP6M2 has also been found to be elevated in DDT resistance and CYP6Z1 in permethrin and DDT detoxification (Mitchell *et al.,* 2012; Chiu *et al.,* 2008). CYP6P9a and CYP69b have also been reported to be capable of detoxifying permethrin and deltamethrin (Stevenson *et al.,* 2011), thus providing resistance against them. *In Ae. aegypti,* CYP9J subfamily have the potency to metabolize pyrethroid insecticides (David *et al.,* 2013). CYP9J24, CYP9J28, CYP304C1, CYP6CB1, CYP6M10, CYP6M11 have been shown to provide resistance against permethrin, CYP6M6 and CYP6Z6 against deltamethrin and CYP9J32 against both permethrin and deltamethrin (Bingham *et al.,* 2011; Marcombe *et al.,* 2012; Marcombe *et al.,* 2009; Strode *et al.,* 2008). In *Culex quinquefasciatus,* mosquitoes, CYP450s have been implicated in permethrin resistance (David *et al.,* 2013).

1.10.3.3 Glutathione S-transferases (GSTs):

These soluble dimeric proteins are crucial in detoxification and subsequent excretion of many different endogenous as well as exogenous compounds. GST enzymes are encoded by genes belonging to two different supergene families (Hayes and Strange, 2000). These two families code for soluble and microsomal enzymes that provide protection against endogenous stress, *i.e.* reactive oxygen species and exogenous compounds *i.e.* xenobiotics, playing a key role in biotransformation of insecticides and drugs. GSTs drive the conjugation of electrophilic compounds with reduced glutathione (GSH) forming the thioester, rendering the resulting products water soluble which can be excreted out easily (Habig *et al.,* 1974).

In insects, two distinct classes of GSTs have been identified as class I and class II (Fournier *et al.,* 1992). A multigene family encodes the class I insect GSTs whereas a

single gene codes for all class II insect GSTs in majority of the insects (Enayati *et al.*, 2005). In *A. gambiae,* alternative splicing of the class II gene have been shown to produce two transcripts (Ding *et al.*, 2003).

Increased activity of GSTs have been shown to confer resistance against insecticides (Vontas *et al.*, 2012). Insecticide resistance can be conferred by elevation of one or more GST enzymes as a result of either increases in transcriptional rate or gene amplification; modification in qualitative properties of individual enzymes may also increase resistance levels but it less common (Ranson *et al.*, 2001).

GSTs have been implicated in resistance against organochlorines, such as lindane are conjugated to glutathione for detoxification and by dehydrochlorination of DDT moiety into DDE is catalysed by GSTs (Clark and Shamaan, 1984). Such dehydrochlorination of DDT have been shown to provide resistance to DDT in many insect species including mosquitoes belonging to *Aedes* and *Anopheles* genera (Enayati *et al.*, 2005). Amongst GST classes, Epsilon class have been found to be over-expressed in response to DDT selection pressure in *A. gambiae* resistant strain, with the highest dehydrochlorinase activity reported by *GSTe2* (Ortelli *et al.*, 2003). Reports also indicate on the involvement of GSTs in resistance against synthetic pyrethroids. GSTs have not yet been implicated in the direct metabolism of pyrethroid insecticides. Nevertheless, they may play an important role in conferring resistance to this insecticide class either by insecticide sequesteration (Kostaropoulos *et al.*, 2001) or detoxification of pyrethroid induced lipid peroxidation products (Vontas *et al.*, 2001). GSTs have also been implicated in resistance against organophosphates by conjugation of glutathione to organophosphate insecticides either by O-dealkylation (as in tetrachlorvinphos) or O-dearylation (in parathion and methyl parathion) (Hayes

and Wolf, 1988; Enayati *et al.*, 2005). No such detoxifications against carbamates have yet been noted by GSTs.

1.10.4 Target site resistance/ insensitivity:

Target site modification refers to the loss of sensitivity of the active site of the protein targeted by the insecticide. The most notable and commonly found is insensitivity of voltage gated sodium channel gene (VGSC) by synthetic pyrethroids (SPs) or organochlorines (OCs), thereby providing resistance against these insecticides, commonly called as knockdown resistance (kdr) (Kasai *et al.*, 2011). Modifications in the targeted site of insecticides are mainly brought upon by point mutations in the target gene. Several point mutations in VGSC gene have been reported in many mosquito vectors, *i.e. Anopheles, Aedes, Culex etc*. So far more than 50 sodium channel mutations have been identified in pyrethroid resistant insect pests and human disease vectors and many have been functionally confirmed to confer pyrethroid resistance (Du *et al.*, 2016).

Many point mutations conferring target site alteration in voltage gated sodium channel gene and acetylcholinisterase (AchE) gene have been identified in *Ae. aegypti* mosquitoes (Vontas *et al.*, 2012). In *Ae. aegypti* mosquitoes, around ten resistance related mutations have been identified in VGSC gene. Two of the most commonly detected point mutations in resistant *Ae. aegypti* are V1016G/I and F1534C in the IIS6 segment of VGSC (Hamid *et al.*, 2017; Li *et al.*, 2015). Other kdr mutations found in resistant *Ae. aegypti* population are G923V, L982W, I1011M, D1763Y (Du *et al.*, 2016). Double or triple mutations are also found in many insecticide resistant *Ae. aegypti* populations.

Knockdown resistance (kdr) mutation are widespread in different *Aedes* population. Presence of F1534C, V1016I/G *etc* have been shown to provide varying degrees of selective advantage under insecticide pressure in many populations of *Ae. aegypti* (Alvarez *et al.*, 2015; Plernsub *et al.*, 2016). Reports of kdr mutation in *Aedes albopictus* are very scanty as

compared to *Ae. aegypti*. However, there is a report of presence of kdr mutation (F1534C) in wild *Ae. albopictus* populations in Singapore resistant to permethrin (Kasai *et al.,* 2011). Till date, none of the examined *Ae. albopictus* population has been found to be positive for the presence of kdr mutations in India (kushwah *et al.,* 2015; Chatterjee *et al.,* 2018).