CHAPTER 1

INTRODUCTION

1. INTRODUCTION:

1.1. Mosquito-borne diseases:

The emergence and re-emergence of mosquito-borne diseases is a severe threat to the human society having a negative impact on both health and socio-economic status of mankind. Various mosquito-borne diseases vectored by three genera of mosquitoes *i.e., Aedes, Anopheles* and *Culex*, pose a substantial threat throughout the tropical and sub-tropical regions of the globe (Figure 1). Approximately, 16.6% of the total diseases in the world accounts to the vector-borne diseases (WHO, 2014). Vector-borne diseases, particularly those, transmitted through mosquito vectors infect more than a billion people every year globally with around a million deaths (WHO, 2014). People with a low socio-economic status and inhabiting the developing countries with poor sanitation are usually vulnerable to vector-borne diseases. The transmission of vector-borne diseases depends upon three requirements – disease pathogen, an established or newly introduced mosquito vector population and a suitable environment for proper survival of disease causing pathogen and the disease transmitting vector (Randolph and Rogers, 2010). As the growth and survival of both the pathogen and mosquito vector relies on suitable climatic conditions, preferably hot and humid environments. Therefore, mosquito-borne diseases are more prevalent in the tropics and sub-tropics. Moreover, mosquitoes usually breed in warm regions with high humidity and precipitation. This fact also provides a hint on the risk of higher prevalence of mosquito-borne diseases with an increase in temperature and precipitation associated with recent global climatic change (Servadio *et al*., 2018).

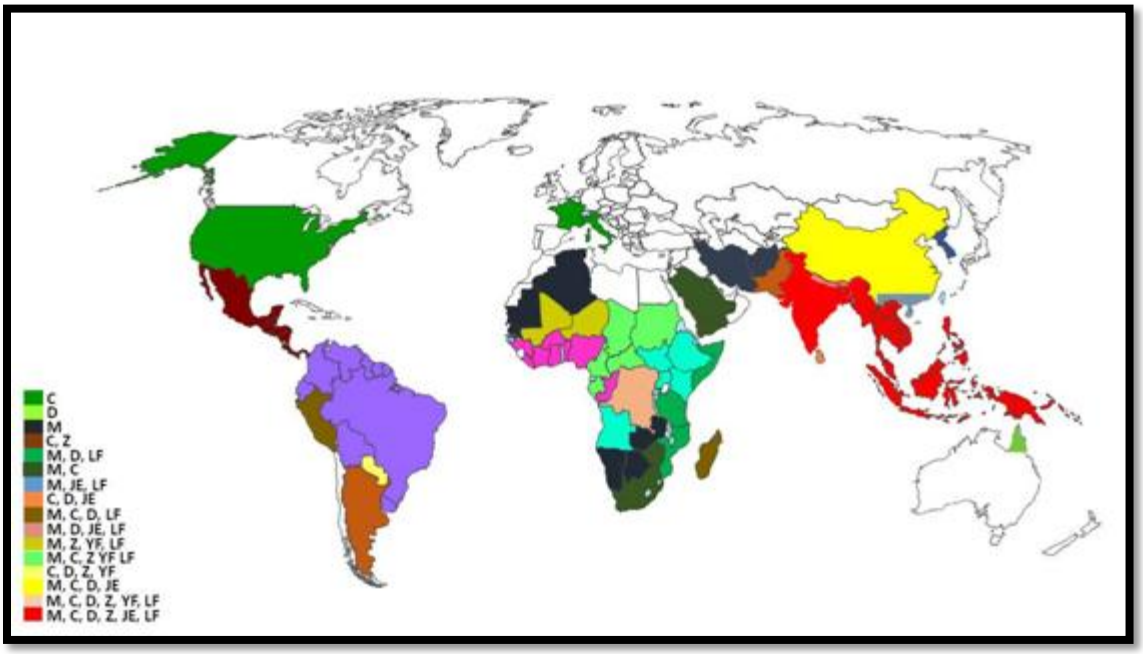

Figure 1: Global incidence of mosquito-borne diseases. C-Chikungunya; D-Dengue; M-Malaria; Z-Zika; LF-Lymphatic Filariasis; JE-Japanese Encephalitis; YF-Yellow Fever. (Source: WHO, 2020)

Mosquito-borne diseases are transmitted from its region of origin to other nations rapidly through the increasing trade and travel around the world. Globalization largely facilitates the spread of mosquito-borne diseases as air travel and sea route trade increases the risk of importation of invasive mosquito species to new regions where such species of vectors were not present earlier. A remarkable example in favour of the above statement is provided by the abrupt outbreak of Chikungunya vectored by *Aedes* mosquitoes in Italy in the year 2007 and 2017 (Rezza *et al.*, 2007; Venturi *et al.*, 2017). The Chikungunya vector in Europe – *Aedes albopictus* mosquitoes were introduced in the early 1990s along with the importation

of tyres from Asia (Semenza and Suk, 2018). As climatic condition of Europe was favourable for growth and proliferation of *Aedes albopictus* mosquitoes, the transmission of Chikungunya virus through air travel of infected people, led to the introduction of the virus into its vector and thereafter an outbreak of disease in human population of Europe.

1.1.1. Dengue:

Aedes mosquitoes are a vector to few arboviruses like Dengue, yellow fever, Chikungunya and Zika. Two species of *Aedes*, namely, *Aedes aegypti* (yellow fever mosquito, Stegomyia) and *Ae. albopictus* (Asian tiger mosquito) are reported as the primary vectors of dengue fever in humans globally. With approximately 30-fold increase in worldwide incidence in the last 50 years, dengue remains the most rapidly spreading mosquito-borne diseases with around 100 – 390 million annual infection reports globally (Caminade, 2019). Mutations of dengue virus into many serotypes present a major obstacle in the development of an effective vaccine against this disease. As such, studies are being conducted worldwide for vaccine development, though a fully effective vaccine for dengue has not been developed till date. Hence, the strategy to deal with dengue fever lies on the treatment of symptoms in infected person and precautionary control of mosquito vector – *Ae. aegypti* and *Ae. albopictus*.

As mentioned earlier, the spread of dengue infection into new areas occurred as a result of globalization and international trade and travel. There are several reports of dengue outbreaks worldwide in the past years. Dengue outbreaks were reported in France and Croatia during 2010, in United States during 2012, in Laos during the year

2010 and 2013, in Japan during 2014 with the largest dengue outbreak in China in the 2014 (Servadio *et al*., 2018). All adults over the age of 45 years in Thailand are seropositive for dengue virus (Servadio *et al.*, 2018).

In India, at the present scenario, dengue is endemic in all states and Union territories (NVBDCP, 2020) (Figure 2). Current status of dengue in India has been shifted to category A of public health from category B by the World Health Organization (WHO) in 2010 indicating it as a major health problem in the country. Infection rate of the disease increased from 0.13 million cases of infection in 2016 to 0.16 million in 2019 (NVBDCP, 2020). This increase in transmission rate might be because of increased mobility and travel and also the changing climatic conditions of our country. In the recent years, the southern and western parts of India have reported to face dengue burden throughout the year whereas, earlier the transmission in these regions were purely seasonal.

1.1.2. Chikungunya:

The re-emergence of Chikungunya fever in several parts of India in the year 2006 after about three decades of quiescent period was alarming as approximately 1.39 million cases of infection was reported from the country. The epidemiology continued post re-emergence event and in 2019 a total of 81,914 cases of Chikungunya infection was reported from India (NVBDCP, 2020). Though Chikungunya fever resembles dengue fever and is characterized by persistent joint pain in patients, mortality related to Chikungunya fever is not reported from any state. At the present scenario, Chikungunya is endemic in 32 states and Union Territories of

India (NVBDCP, 2020) (Figure 2). Following the unprecedented outbreak in 2006, Chikungunya outbreak was also reported from Italy and France in 2007, 2010, 2014, 2015 and 2017 (Semenza and Suk, 2018). The United States reported its first local transmission of Chikungunya in 2014 followed by Chikungunya epidemics in the regions of Central and South America (Caminade, 2019).

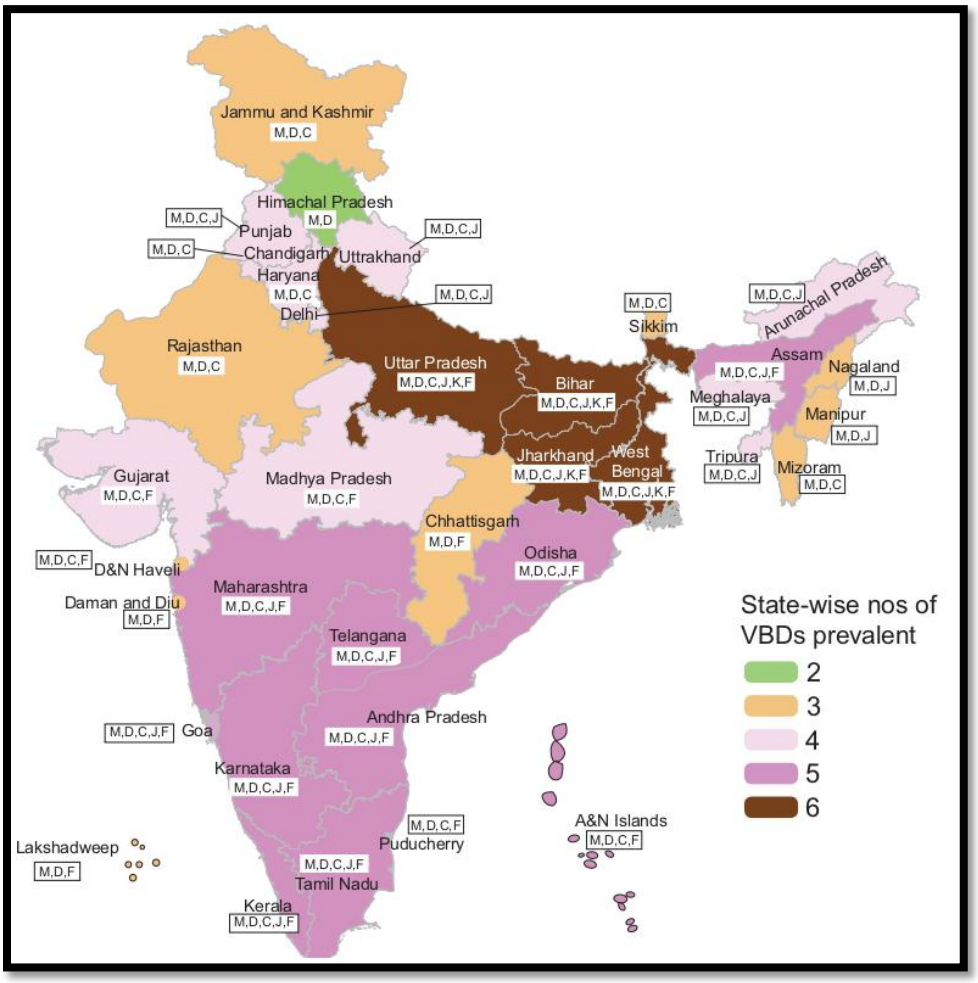

Figure 2: Prevalence of vector-borne diseases in India. VBDs: Vector Borne Diseases; M: Malaria; C: Chikungunya; D: Dengue; J: Japanese Encephalitis; K: Kala azar; F: Filariasis (Source: NVBDCP, 2020)

1.1.3. Zika:

After the occurrence of Zika epidemic in Brazil in 2015 (Faria *et al*., 2017), in February 2016, it was declared as a public health emergency of international concern by WHO (Caminade, 2019). Zika virus though primarily transmitted by *Aedes* mosquitoes can be sexually transmitted as well (Caminade, 2019). Zika outbreak subsequently spread to the Caribbean and South and Central American countries. Factors that aided in Zika virus pandemic in these regions were anthropophilic behavior of *Ae. aegypti* and storage of water in artificial containers during drought period in the urban slum areas (that facilitate mosquito breeding) along with other favourable parameters of disease outbreak. Subsequently, zika virus was reported from Africa and Southeast Asian countries (Cambodia, Philippines, Indonesia) also.

Zika virus causes severe neurological complications and Gullain-Barre syndrome – a paralytic autoimmune disorder. Though the number of zika infection cases decreased significantly in Brazil in 2016, the disease is still a burden on public health globally. In the year 2017, increase in the transmission rate of zika virus was reported from countries like Argentina, Peru and Ecuador (WHO, 2021a). In India, few cases of zika virus infection were reported in 2017 – 2018 from Gujarat, Tamil Nadu, Rajasthan and Madhya Pradesh (NVBDCP, 2020). Fortunately, there were no new cases of zika virus infection in 2019 in the country.

1.1.4. Malaria:

Malaria is a major public health issue because of the involvement of multiple vectors – *Anopheles culicifacies, An. fluviatilis, An. minimus, An. philippinensis, An. dirus, An. stephensi, An. annularis, An. varuna* and *An. sundaicus*. The malarial parasite *Plasmodium falciparum* is widespread in the tropics and sub-Saharan Africa and is responsible for 90% of the total malarial cases globally. Though the implementation of strict control measures has led to a decrease in global disease endemicity, yet complete eradication of malarial infection is still not accomplished (Figure 1). Drug resistance in the malarial pathogen – *Plasmodium* parasites and insecticide resistance in the mosquito vectors worsens the problem. Infection rates are increasing in sub-Saharan Africa (WHO, 2017a). Globally, an increase of 5 million cases in a year was reported in 2016 as compared to 2015 (WHO, 2017a). There are several reports on increasing malarial infection in tropical highlands of Nepal, Colombia and Eastern Africa as well (Dhimal *et al*., 2015; Siraj *et al*., 2014).

However, in India, the total number of infection cases per year is decreasing as evident from 10,87,285 cases in 2016 to 3,38,494 cases in 2019. Mortality related to malaria has also decreased in the subsequent years (NVBDCP, 2020). At present, malaria is reported from all states and Union territories of India (Figure 2). In 2016, the Government of India has formed a framework for elimination of malaria from India by the year 2030 with a milestone of zero indigenous transmission in the country by 2027 (NVBDCP, 2020).

1.1.5. Japanese Encephalitis:

Japanese Encephalitis Virus (JEV) is a flavivirus like dengue virus and is transmitted by *Culex sp.* particularly *Culex tritaeniorhynchus, Cx. vixhnui* and *Cx. pseudovishnui* (NVBDCP, 2021a). Apart from mosquito vectors, pigs and water birds act as amplifying hosts for JEV. Mortality rate of Japanese Encephalitis can be about 30% while 30 – 50% of the infected individuals show permanent neurological disorders. This disease is endemic in 24 countries in Southeast Asia, Western Pacific region and Northern Australia (WHO, 2019), thereby posing a risk of infection to approximately 3 billion people. The annual incidence of Japanese Encephalitis (JE) ranges from <1 to >10 per 0.1 million population with approximately 13,600-20,400 deaths per year (WHO, 2019) (Figure 3). JE cases are reported from Bangladesh, Bhutan, China, Japan, South Korea, Taiwan, Thailand, Russia, Pakistan, Nepal, North Vietnam and North India occasionally with seasonal outbreaks and follow the epidemic epidemiological patterns (Figure 1). However, other countries like Australia, Burma, Cambodia, Malaysia, Indonesia, Singapore, Laos, Sri Lanka, South India and South Thailand are endemic to the disease with incidences of JEV infection throughout the year (Figure 1). JE vaccines are available for immunization and the World Health Organization recommends vaccination drive in disease endemic areas and in regions where JE remains an important health issue (WHO, 2019). However, when infection with JEV occurs, there is no cure and the treatment procedures primarily focus on minimizing the symptoms.

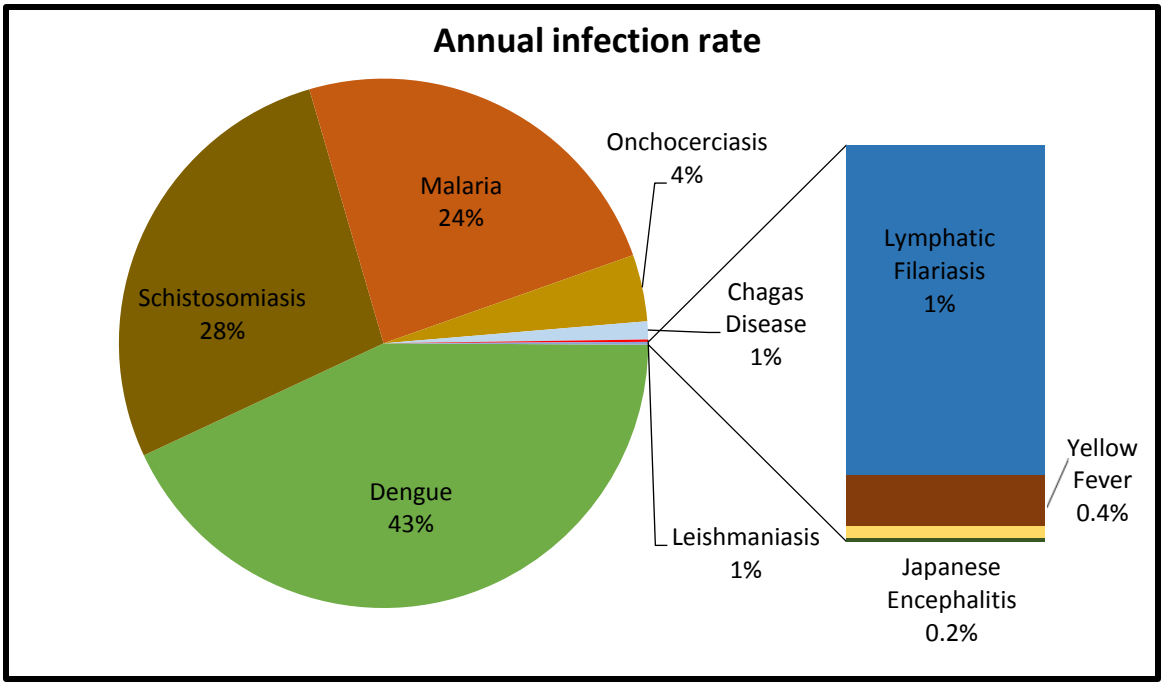

Figure 3: Annual infection rate of vector-borne diseases on a global scale. (Source: WHO, 2020)

In India, JE is one of the major public health problems and is endemic in 22 states with Assam, Bihar, Tamil Nadu, Uttar Pradesh and West Bengal showing highest endemicity comparatively (NVBDCP, 2020) (Figure 2). The first outbreak of JE in India was observed in West Bengal in 1973 and subsequently in the Southern and Western states with mortality as high as 34% in a hospital (Wang and Liang, 2015). In 2016, 1,676 cases of JE infection were reported with 283 deaths and the number of cases increased to 2,545 cases in 2019 with 266 deaths (NVBDCP, 2020). In 2020, infection rate was dramatically reduced to 691 cases with 79 deaths. Prime strategy to curb JE epidemiology incorporated by the Government of India is immunization of populations in the endemic region along with vector control measures.

1.1.6. West Nile fever:

West Nile Virus (WNV) is the most widely distributed flavivirus and infects birds, horses and other mammals apart from humans. WNV is transmitted by *Culex quinquefasciatus* and other species of *Culex* mosquitoes worldwide. West Nile fever is endemic in Africa and large outbreaks are also reported from Asian countries, Australia and Middle-East (Caminade, 2019). Frequent outbreaks since 2010 have been reported in eastern and southern Europe as well (Semenza and Suk, 2018). WNV infections though usually remain asymptomatic (60% of infection cases), but can be severe particularly in aged patients and those on immunosuppressive drugs. Similar to other flaviviruses, an effective antiviral treatment for WNV encephalitis has not been established at the current state. As such, primary focus still remains in the control of mosquito vectors with little success. In India, reports on West Nile encephalitis are recorded from Karnataka, Andhra Pradesh, Maharashtra and Assam since the 1970s (NVBDCP, 2020).

1.1.7. Lymphatic Filariasis:

Lymphatic Filariasis (LF) is the most common filarial disease in humans where the parasitic nematode worm – filariae infects the human lymphatic system. LF is a helminth disease and one of the most important Neglected Tropical Diseases (NTDs) transmitted through mosquito vectors. The filarial worms responsible for the disease are *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* that belong to family Onchocercidae. Among the three causative agents, about 90% of infection is caused

by *W. bancrofti* alone (WHO, 2016a) and results in damage of lymphatic system. Apart from human host, macaques and leaf monkeys are also known to be reservoirs of *W. bancrofti* and *B. malayi* in few regions of the world (Chandy *et al.*, 2011). The disease is prevalent and endemic in many tropical and sub-tropical countries affecting approximately 120 million people globally (Figure 4).

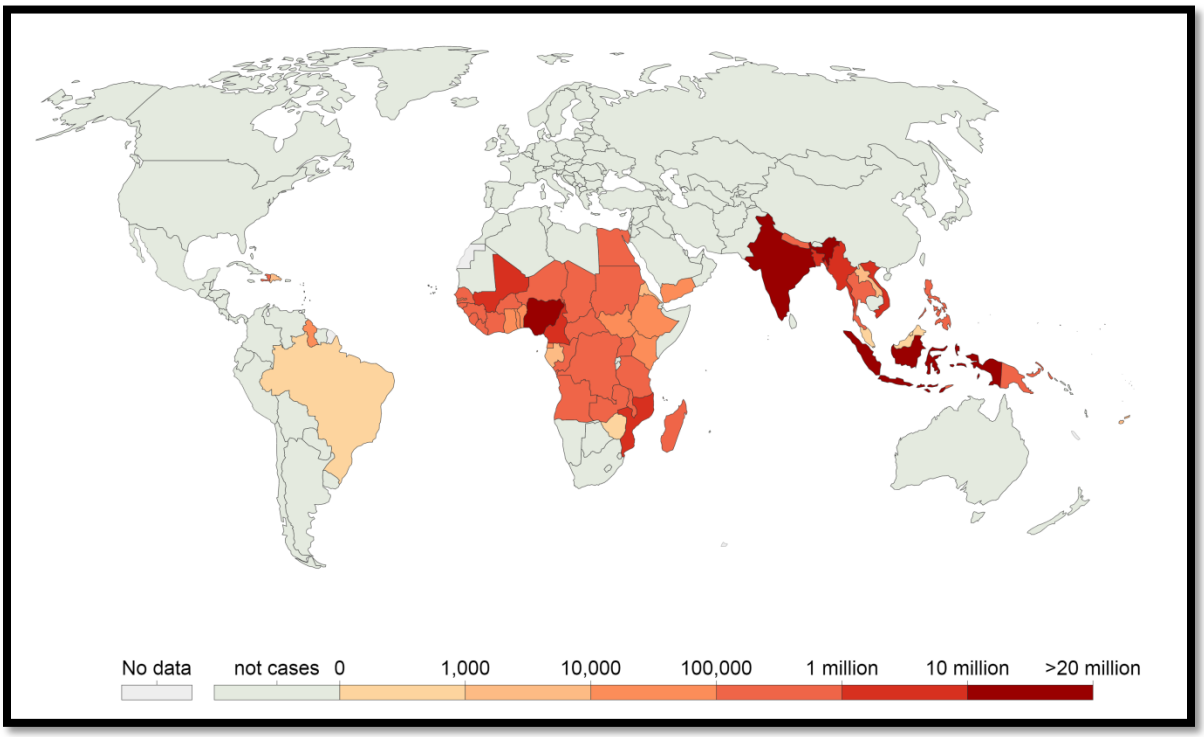

Figure 4: Infection rate of Lymphatic filariasis in the world. (Source: Institute for Health Metrics and Evaluation, 2018)

The filarial nematodes are carried and transmitted by various species of mosquito vectors – *Cx. quinquefasciatus, Anopheles gambiae, Aedes polynesiensis* and *Mansonia sp.* (NVBDCP, 2020) although *An. funestus, An. gambiae* and *An. arabiensis* are the primary vectors in Zambia (Chanda *et al.*, 2011). In the Southeast

Asian countries and many African countries, *Cx. quinquefasciatus* remains the predominant vector of LF.

LF does not develop with a single bite of an infected mosquito vector rather continuous exposure to several bites by infected mosquitoes for many years is essential for the disease to occur. Reason behind this long infection period is that the filarial parasite does not multiply inside mosquito vector and several bites are as such needed to have sufficient parasite load in humans. For a new LF infection to develop approximately 15,500 bites of infected *Cx. quinquefasciatus* is needed (Chandy *et al*., 2011).

On tracing the history of LF, first written document of the disease is provided from the Ancient Greek and Roman civilizations (Chandy *et al.*, 2011). However, the association of mosquitoes in transmission of LF was only known in 1877 when Sir Patrick Manson detected the filarial parasite in mosquitoes. This finding is important and needs mention because it was a first ever discovery of an arthropod acting as a vector of human disease. Following this discovery, mosquito vectors for other tropical diseases like malaria and dengue were identified. In 1900, the correct mode of filarial transmission was reported by George Carmichael Low with his discovery of the filarial pathogen in mosquito's proboscis.

Though LF affects individuals of all age groups and both genders, it is reported to be comparatively more common in males than females with predominant association of people having low socioeconomic status (Lenka *et al.*, 2017). The disease does not usually result in mortality. However, morbidity rate is high in the infected individuals due to clinical manifestations. Moreover, a severe psychological

and negative socioeconomic impact burdens the infected individual because of the economic stress posed by physical deformities post infection. Hence, the economic burden associated with LF is quite high.

Microfilariae – sheathed egg of filarial nematode is introduced into mosquito vector from the human peripheral blood when a mosquito bites a LF infected individual for blood meal. Along with human blood, microfilariae are also taken up by the mosquito vector and after 1 – 2 weeks of ingestion, the microfilariae shed its sheath in the midgut of mosquito vector and make its way to the thoracic muscles. In the thoracic muscle, microfilariae develop into first, second and third stage larva also known as filariform larva. Third stage larva is the infective stage of filarial worm and migrates to the mosquito proboscis from thoracic muscles through haemocoel.

During blood meal, the infected mosquito vector transmits the infective larva into human's body which ultimately reach the lymph glands where they mature into adults. This entire process is slow and might take 5 – 8 months. Male and female worm after maturation copulate to produce microfilariae which circulate in the peripheral blood usually at night. This periodicity of microfilariae is directly related to the feeding behavior of its vector as all LF vectors are nocturnal feeders except *Ae. polynesiensis* (Chandy *et al*., 2011). Periodicity is the time period during which microfilariae are more prevalent in human blood. Adult filarial worms live up to 10 – 15 years and a mature female produces microfilariae for about 5 years. Therefore, it hinders the smooth removal of microfilariae from human body and eradication of the disease is quite challenging.

Acute infection of filarial worms in humans causes local skin inflammation with sporadic and irregular inflammation of lymph glands termed as lymphadenitis and inflammation of lymph channels termed as lymphangitis (Addiss *et al*., 2010). Few individuals also experience extreme pain in the genital area along with the formation of pus-filled nodules. Chronic infection occurs when the adult filarial worms accumulate in the lymph glands and vessels obstructing the lymphatic flow. Accumulation of lymph in the affected areas result in enormous tissue swelling leading to a condition termed lymphoedema (Witt and Ottesen, 2001). With an increase in infection rate, the lymph vessels are invaded by plasma cells, eosinophils and macrophages which ultimately lead to lymphatic damage and lymph leakage into tissues. This condition is followed by thickening of the skin and underlying tissue with increasing bacterial and fungal infections ultimately developing elephantiasis. Elephantiasis is the most striking symptom of LF and commonly occurs in the lower limbs and genitalia (Chandy *et al*., 2011). Scrotal enlargement due to microfilarial infection is termed as filaricele and the accumulation of fluid in the scrotum and nearby areas is known as hydrocele (Mand *et al*., 2011). Sometimes, leakage of chyle occurs due to lymphatic blockage and this pathological condition is termed as chyluria (Kabatereine *et al*., 2010).

1.2. Lymphatic Filariasis – a global burden:

LF is endemic in 72 countries and is considered as one of the most important infectious diseases (WHO, 2021b). About 120 million people around the world are infected with LF and approximately 120 billion *i.e.,* 20% of the world's

populations are considered vulnerable to infection (Addiss *et al.,* 2010). The disease is endemic in central African countries: Madagascar, Turkey, Nile delta, Thailand; Southeast Asian countries: Malaysia, South Korea, Indonesia, Brazil, Philippines, Southern China *etc* (Utzinger *et al*., 2010) (Figure 4). Though LF is endemic in 72 countries, about 70% of the global infection is reported from India, Nigeria, Bangladesh and Indonesia. In Southeast Asian countries, 15 million people are infected with the nematode parasite causing LF (Sudomo *et al*., 2010). Among the 38 least developed countries of the world, LF is endemic in 32 countries (Chu *et al*., 2010; Utzinger *et al*., 2010) thereby posing a substantial threat of infection to people dwelling in these regions. A report from the World Health Organization (WHO) had estimated a considerable majority of LF cases from India on a global scale (Joshi, 2018).

Though highest number of LF cases in the world is reported from India with 4505 million cases and followed by sub-Saharan Africa (40 million cases), rate of disease prevalence is highest in sub-Saharan Africa (8% prevalence). India ranks second in the world with 5% disease prevalence rate (Chandy *et al*., 2011). In sub-Saharan Africa LF is one of the major public health problems as the region has highest number of LF endemic countries with moderate to high disease prevalence rate. Mosquitoes belonging to genus *Anopheles* are observed to be more efficient vector of LF parasites on comparison to *Culex sp*. As such, transmission efficiency of LF is higher in African countries than in Asia where *Cx. quinquefasciatus* is the predominant vector. Availability of different vectors to transmit parasites for LF

infection in two distinct geographical areas might be the reason behind differences in disease transmission efficiency (De Almeida and Freedman, 1999).

LF is considered as one of the leading cause of permanent morbidity and long-term disability. Approximately, 40 million people worldwide are either disabled or incapacitated due to LF infection (WHO, 2013). Though LF does not account for direct mortality, morbidity and long-term disability associated with the disease results in negative social stigma of the patient, reduced labour time, impact on daily income of an individual and associated burden of treatment cost (Keating *et al.*, 2014). Moreover, LF imparts a considerable economic burden on individual level, country level and at a global scale. With the advancement of infection in patients, painful swellings and hydrocele results in inability of the individual to perform daily domestic activity and economic activity to earn a living. This lost economic productivity and educational impairment in affected children gives rise to the economic burden of LF.

A case study conducted in India reported that LF patients with chronic symptoms spent 2.7 – 3.6 hour less per day on economic activities compared to healthy workers (Babu and Nayak, 2003). The study also documented absence of 88% of LF patient from work and inability to perform domestic activities by 55.1% of female patients (Babu and Nayak, 2003). Apart from economic burden in affected individuals, a bulk of cost related to LF infection is involved in its treatment and control (Keating *et al*., 2014). A study in South India reported an expenditure of US$ 1.81 – 5.63 per treatment of hydrocele on an average excluding the surgical expenses (Nanda and Krishnamoorthy, 2003). The treatment cost was estimated to be 1.5 - 4

times higher than the daily income of the patient. Another study in 2002 documents a cost of US$ 0.14 – 25.39 for non-surgical treatment of hydrocele in rural areas of India (Babu *et al*., 2002).

Prevention and control of LF transmission is executed under two categories or strategies – Mass Drug Administration (MDA) and morbidity management and vector control. MDA of anti-helminth drugs like albendazole, diethylcarbamazine (DEC) and ivermectin in the endemic areas initially for a period of 5 years is the principle approach in community control of transmission of LF. MDA is usually given annually or semi-annually to all individuals in LF endemic regions except pregnant women and children under 2 years of age. Morbidity management focuses on reducing suffering and disability with improved hygiene, proper skin care and surgery from hydrocele patients.

With a prime objective to eliminate LF as a major health issue by the year 2020, WHO launched Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000. GPELF works with the same two strategies of MDA and morbidity management along with vector control (WHO, 2010). WHO also launched water, sanitation and hygiene (WASH) campaigns for preventing LF transmission because apart from MDA and vector control, focus on improved water quality, sanitation, hygiene and general living standard were also required (Rebollo and Bockarie, 2013). Elimination of LF from Australia and significant reduction of LF transmission in Brazil were accounted to improved sanitation drives against *Cx. quinquefasciatus* (Bockarie *et al*., 2009).

GPELF resulted in a significant rise in MDA from 3 million people in 12 countries in 2000 to 466 million people in 2010 covering 53 countries (Centers for Disease Control and Prevention, 2011). However, efforts on morbidity management were not remarkable. WHO recommends preventive chemotherapy and control of disease transmission as prime strategy for curbing the spread of LF. Preventive chemotherapy includes MDA in endemic countries and control of disease transmission relies largely on vector control strategies and improved sanitation.

1.3. Current scenario of Lymphatic Filariasis in India:

LF is the second most important Neglected Tropical Diseases (NTDs) next to malaria to cause morbidity and is endemic in 257 districts in 21 states and Union Territories of India (NVBDCP, 2021b) (Figure 2). Approximately, 650 million people in India are at a risk of LF infection. Highest endemicity is reported from Bihar while Goa showed comparatively lowest endemicity (<1%) (NVBDCP, 2020). LF in India is caused by *W. bancrofti* transmitted by *Cx. quinquefasciatus* and by *B. malayi* transmitted by *Mansonia annulifera*, *Mn. uniformis* and *Mn. indiana* (NVBDCP, 2020). In Andaman and Nicobar islands *W. bancrofti* is reported to be transmitted by *Ae. niveus* (NVBDCP, 2020).

LF endemic states are Andhra Pradesh (10 districts), Assam (7 districts), Bihar (38 districts), Chhattisgarh (9 districts), Gujarat (11 districts), Goa (2 districts), Jharkhand (18 districts), Karnataka (8 districts), Kerala (11 districts), Madhya Pradesh (11 districts), Maharashtra (17 districts), Orissa (20 districts), Telangana (7 districts), Tamil Nadu (20 districts), Uttar Pradesh (51 districts) and West Bengal (12

districts) (Figure 5). Five union territories, namely Puducherry, Andaman and Nicobar islands, Daman and Diu, Dadra and Nagar Haveli and Lakshadweep islands are also endemic to LF (NVBDCP, 2021b). Apart from infections of *W. bancrofti*, *B. malayi* infection is reported to be prevalent in Kerala, Tamil Nadu, Andhra Pradesh, Orissa, Assam, Madhya Pradesh and West Bengal.

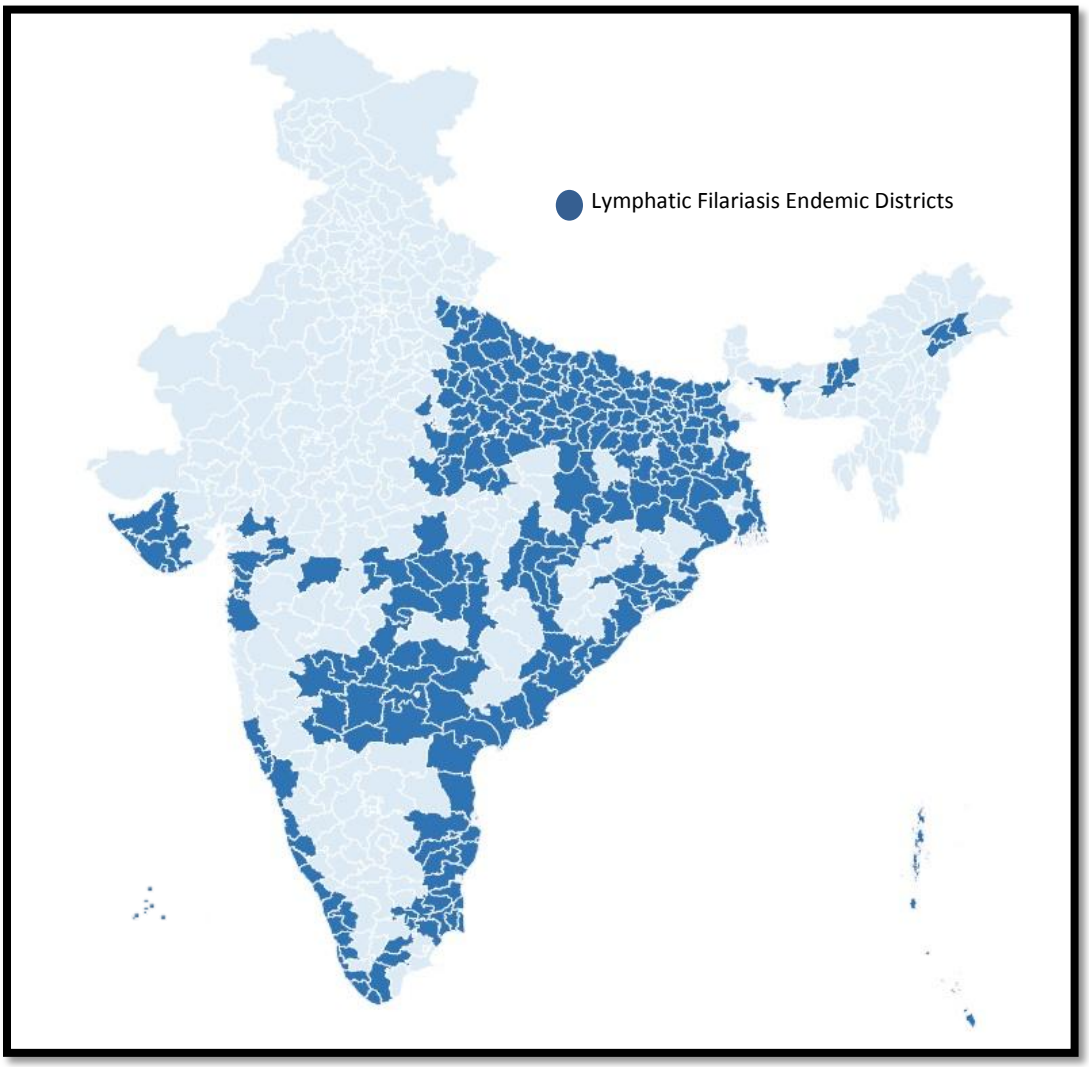

Figure 5: Lymphatic filariasis endemic districts in India. (Source: NVBDCP, 2021b)

India with highest number of infection cases (45.5 million) contributes significantly to the global burden of LF. The country stands only second to sub-Saharan Africa in terms of disease transmission rate. Around one third of the LF infected person is in India (Upadhyayula *et al.*, 2012). In West Bengal, 12 districts are endemic to LF (Figure 6). These districts are 24-Paraganas North, 24-Paraganas South, Bankura, Burdwan, Birbhum, Coochbehar, Malda, Medinipur East, Medinipur West, Murshidabad, Nadia and Purulia (NVBDCP, 2021b).

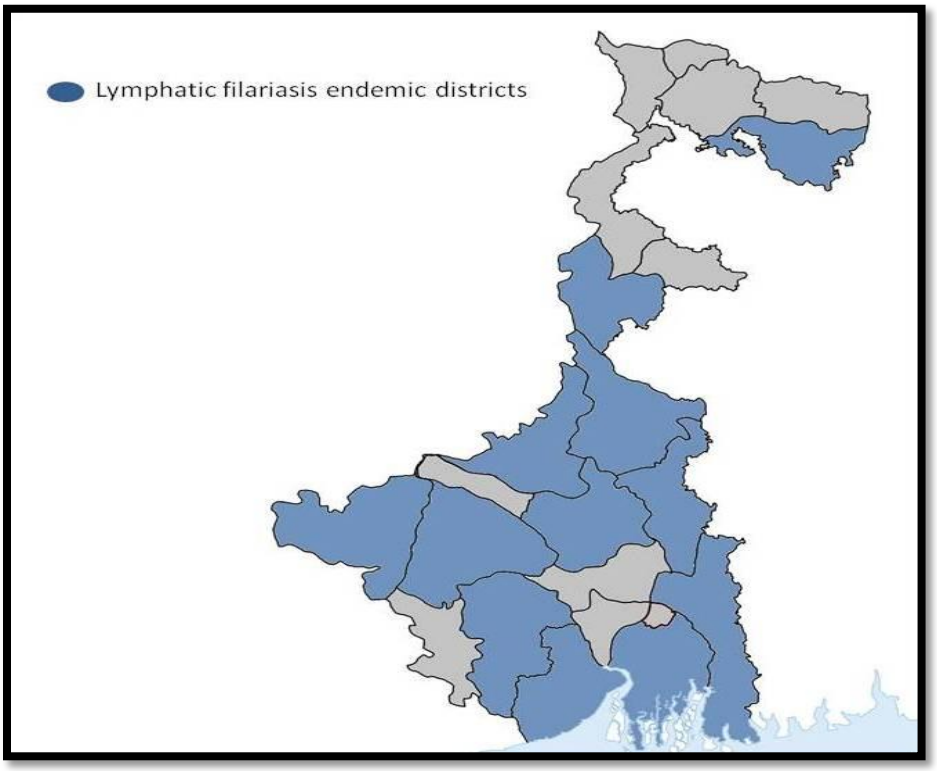

Figure 6: Map of West Bengal showing lymphatic filariasis endemic districts.
(Source: NVBDCP, 2021b)

Elimination of LF in India started after the formation of GPELF in 2000. In India, 'Twin Pillar strategies' of LF elimination *i.e.,* MDA for interrupting disease

transmission and Morbidity Management and Disability Prevention (MMDP) for taking care of the infected individuals is followed.

The Government of India started MDA in 2004 in LF endemic areas with a single dose of diethylcarbamazine (DEC). With a coverage rate of 72.6%, only 202 districts were covered in the initial year. In 2007, DEC was co-administered with albendazole for MDA and 256 districts were covered with drug administration. The tenth Global Alliance for Elimination of LF (GAELF) meeting held in New Delhi on 2018 launched an Accelerated Plan for the elimination of LF (APELF) with the introduction of Triple Drug Therapy, *i.e.*, Ivermectin, Diethylcarbamazine and Albendazole (IDA). IDA was approved by the Ministry of Health and Family Welfare, Government of India as an approach for MDA. IDA has been successfully implemented in five districts in 2019.

MDA coverage rate increased from 72.65% in 2004 to 87.33% in 2019, though cent percent coverage rate is yet to be achieved. In 2012, after MDA in Bankura – a district in West Bengal, a survey conducted in the district reported 98.8% drug distribution (Ghosh *et al.,* 2013). However, 94.8% individuals were reported to be drug compliant *i.e.*, took both DEC and albendazole. Remaining 4% took none of the drugs due to fear of side effects, lack of counseling for MDA in the area and absence at the time of drug delivery (Ghosh *et al.*, 2013).

Second strategy of LF elimination is Morbidity Management and Disability Prevention (MMDP) where the Government of India focusses on increasing hydrocele surgeries and home-based morbidity management. Various states have started awareness campaigns and demonstration programmes to manage morbidity

associated with lymphoedema through proper foot hygiene and personal care of LF patients. A total of 1,48,877 surgeries for hydrocele is documented to have been performed from 2004 – 2018 (NVBDCP, 2020).

Vector control strategies include the use of mosquito repellents in the form of creams, liquids, coils and insecticide treated bed nets (ITNs) for personal prophylactic measures along with the application of chemical and biological control of mosquito vectors. The Government also focuses on imparting knowledge on vector-borne diseases to common people through various awareness programmes and involving the community in control of mosquito vectors.

1.4. *Culex quinquefasciatus* – a primary vector of LF:

Culex quinquefasciatus commonly known as the Southern house mosquito is the principal vector of LF and one of the most widely distributed mosquito species globally. The southern house mosquito vectors a number of diseases affecting humans and animals. It is a potential vector of arboviruses like West Nile virus (WNV), Japanese Encephalitis virus (JEV), St. Louis Encephalitis virus (SLEV) in southern United States, Western equine encephalitis virus and Ross River virus (Bhattacharya and Basu, 2016; Lopes *et al*., 2019). This species is also considered as a potential vector of dogheart worm *Dirofilaria immitis* (Lai *et al.,* 2000) and known to be involved in the transmission of arbovirus responsible for Rift valley fever (Lindahl, 2012). *Cx. quinquefasciatus* were also detected with ZIKV and Mayaro virus infection in Brazil (Lopes *et al*., 2019) and is reported to transmit the protozoan *Plasmodium relictum* – causative agent of avian malaria.

Cx. quinquefasciatus was first described by Thomas Say in 1823 from a specimen in Southern United States. It is a member of the widely distributed *Cx. pipiens* complex and was earlier regarded as a subspecies of *Cx. pipiens* hence, named as *Cx. pipiens quinquefasciatus*. This is because the two species are morphologically similar and hybrids are also well documented. Later, with advancement in studies, it was found that *Cx. pipiens* and *Cx. quinquefasciatus* are two different sympatric species. These species showed remarkable differences in genetic constituent and was thus given the status of a distinct species (Bhattacharya and Basu, 2016).

Cx. pipiens complex is regarded as a controversial chapter in mosquito taxonomy because species belonging to this complex have similar morphology (Gopalakrishnan and Veer, 2018). Differentiation between *Cx. p. pipiens* and *Cx. quinquefasciatus* is based on the DV/D ratio which is the relative overlap and measurement of dorsal and ventral arms of mosquito male genitalia (Gopalakrishnan and Veer, 2018). Values obtained from DV/D ratio are used to distinguish the two species as *Cx. p. pipiens* will give a value of <0.2 and *Cx. quinquefasciatus* will have >0.4 value. Hybrids of *Cx. p. pipiens* and *Cx. quinquefasciatus* have a DV/D ratio value ranging from 0.2 – 0.4. PCR amplification of acetylcholinesterase gene is also used as a tool to distinguish the two species as both generate amplicons of different sizes specific to the species (Diaz-Badillo *et al.*, 2011).

Cx. quinquefasciatus was earlier believed to originate in Africa and thereafter spread to the tropical and sub-tropical regions through human activity. However, recent studies stated that *Cx. quinquefasciatus* originated in Southeast Asia and then

through merchant ships, military aircraft, ships and airline travel for trade spread to Africa, New Zealand, Hawaii islands, United States and islands in the Indian and Pacific oceans (Bhattacharya and Basu, 2016; Gopalakrishnan and Veer, 2018). At present *Cx. quinquefasciatus* is found throughout the tropical, sub-tropical and warmer temperate regions of the world confined from 36°N to 36°S lattitude. It is a dominant indoor resting mosquito species in India and in West Bengal this species is predominantly present in and around human habitation and cattle sheds (Gopalakrishnan and Veer, 2018).

Apart from its significant role in public health, *Cx. quinquefasciatus* is a major urban house hold pest because of its biting nuisance. The vector has a biting activity on humans in both indoor and outdoor environments with preferences of biting on the ankle and foot of humans (Oduola and Awe, 2006). *Cx. quinquefasciatus* is an opportunistic feeder and feeds efficiently on birds, cattle, sheep, pigs, horses, dogs and amphibians. In a study conducted in West Bengal, *Cx. quinquefasciatus* was found to be the predominant species constituting 97% of the nocturnal human-biting mosquitoes. The species fed upon several host with different preferences – ruminants (46.25%), humans (26.45%), pigs (14.19%) and birds (6.47%) (Bhattacharya and Basu, 2016). While in Southern India, the vector was highly anthropophilic with 50-76% feeding rate on humans. A high man-mosquito contact was evident with 63.56% anthropophilic index in West Bengal which is highly advantageous for disease transmission (Azmi *et al.*, 2015).

Feeding of *Cx. quinquefasciatus* on various mammalian and avian host makes this mosquito species an important bridge vector with significant role in the

transmission of zoonotic diseases (Uttah *et al.,* 2013). Moreover, biting at night causes discomfort along with allergic reactions in few individuals. Nocturnal biting habit of *Cx. quinquefasciatus* coincides with microfilariae periodicity. As such, probability of LF disease transmission remains quite high.

Cx. quinquefasciatus is usually observed to enter household at evening period of the day and feed on human blood during night time leaving the household subsequently in the morning. In urban areas, the mosquitoes have started to stay back and feed on humans even during the daytime. This change in the feeding behaviors even during day hours increases nuisance and irritation among human host (Gopalakrishnan and Veer, 2018).

Different geographical populations of *Cx. quinquefasciatus* have distinct feeding preferences and feed on different host thereby having a direct impact on disease transmission. In Southeast Asia, *Cx. quinquefasciatus* is the principal vector of LF and feeds predominantly on humans whereas birds are its preferred host in Hawaii therefore acting as an important vector of *Plasmodium relictum* that causes avian malaria and also as a vector of avian pox. This mixed feeding pattern is observed not only in *Cx. quinquefasciatus* but in entire *Cx. pipiens* complex thus acting as a bridge for transmission of zoonotic diseases to birds (Farajollahi *et al.,* 2011).

Cx. quinquefasciatus preferentially habituate stagnant water bodies rich in organic content like domestic water storage, flooded drains, cemented channels, ground pools, puddles, shallow wells, ditches and cesspools. Females lay eggs in polluted and organically rich water bodies, small containers and artificial containers

though it can breed in clean water as well (Mishra, 2014). Moreover, this species is an opportunistic breeder and might breed in any temporary or permanent water storage or collection (Lopes *et al.*, 2019). *Cx. quinquefasciatus* is an invasive species and found to co-occur with other mosquito species like *Cx. nigripalpus*, *Cx. australicus*, *Ae. polynesiensis etc* in peri-domestic habitats. This mosquito species is also observed to co-exist with *Ae.* a*egypti* and *Ae. albopictus* in water containers of household use (Bhattacharya and Basu, 2016).

After oviposition and hatching of the eggs, development from larvae to adult may take a minimum of 7 days at 30°C (Mishra, 2014). Hatching takes place post one day after egg laying. After emergence of adults from pupae, male and female feed on sugar diet from source and after 48 hours, females feed on blood meal and mate within 2 – 6 days of emergence (Bhattacharya and Basu, 2016). A gravid female lays single egg raft in a gonotrophic cycle and an egg raft contains 155 eggs on an average. The eggs are loosely attached to one another to form oval raft and are whitish when freshly laid which darkens within few hours of egg laying. Blood source, amount of blood feed and age of mosquito affect the quality and quantity of eggs laid in the egg raft (Day, 2016). In India, during summer 2 – 3 gonotrophic cycles are completed in a lifetime and in winter the cycle number is increased to 4 – 8 (Bhattacharya and Basu, 2016). A single female lays up to 5 egg rafts in a lifetime. Females feeding on birds lay comparatively more number of eggs than those feeding on humans which is an evidence that mosquito is primarily a bird feeder (Gopalakrishnan and Veer, 2018). There are four larval stages or instars in the mosquito life cycle and larvae feed on biotic material in the water. *Cx.*

quinquefasciatus are reported to host 83 bacterial species belonging to 31 genera in the midgut. This gut bacterial diversity may be one of the reasons for variation in vector competence in this mosquito vector (Chandel *et al*., 2013).

Cx. quinquefasciatus larvae have a stout head with eight segments in the abdomen each with a unique pattern of setae arrangement. Dorsal side of abdomen has a siphon with multiple setae tufts. Head and thorax is fused in the pupal stage. Adult *Cx. quinquefasciatus* is brown in colour with light brown head. Proboscis, thorax, wings and tarsi are darker than rest of the body parts (Tyagi *et al*., 2015). Pale, narrow, rounded bands are present on the basal side of abdominal tergite and narrow, curved scales are present in the thorax.

On molecular level, *Cx. quinquefasciatus* has three metacentric chromosomes with increasing length from chromosome 1 to chromosome 3 (Bhattacharya and Basu, 2016). *Cx quinquefasciatus* has 18,883 number of protein coding genes which is 22% more than *Ae. aegypti* and 52% more than *An. gambiae*. Studies have shown that this mosquito species among all dipterans has the highest number of olfactory receptors related to preferences of host and oviposition site (Arensburger *et al.*, 2010). Diverse host preference of *Cx. quinquefasciatus* suggests the presence of large number of proteins for digestion of blood intake from various hosts (Arensburger *et al*., 2010).

1.5. Vector control in combating the mosquito-borne diseases:

One of the key components of managing the mosquito-borne diseases and other vector-borne diseases is vector control. Vector control programme is

implemented to reduce the risk of disease transmission by keeping in check the growth and proliferation of disease causing vectors. Efficient strategies of vector control aims to i) prevent disease outbreaks, ii) check mortality and morbidity associated with vector-borne diseases, iii) reduce the rate of disease transmission and iv) avoid resurgence of vector-borne diseases in near future.

Vector control programmes are region specific and execution of control actions depend on the current status of disease causing vectors in that particular area and also the type of vector that is to be targeted. The strategies undertaken at one region with positive result may not be effective enough in another region even if the same disease vector is being targeted. As such, while designing vector control strategies for a particular site, factors like disease epidemiology, ecology of the region along with housing conditions and proper sanitation facilities, vector biology and behavior, agricultural practices and trend of insecticide application history are to be considered.

Vector control measures are usually divided into two categories. Active measures include the use of chemical and biological control strategies to kill mosquito vectors in larval or adult stage and habitat destruction by elimination of probable mosquito breeding sites. Passive control however, involve prophylactic measures like the use of mosquito repellents, insecticide-treated bed nets, screening of windows and ventilators with mosquito nets and use of clothing with minimum skin exposure to reduce man-mosquito contact.

Control of mosquito larvae is done by the application of chemical insecticides and oils in mosquito breeding sites, water containers in coolers and areas with

stagnant water. The use of Mosquito Larvicidal Oil (MLO), 50% emulsifiable concentration (EC) temephos and 1% granules of temephos, *Bacillus thurengiensis israeliensis* (Bti) and Insect Growth Regulators (IGRs) like diflubenzuron and pyripoxyfen are recommended by National Vector-Borne Disease Control Programme (NVBDCP, 2020), Government of India for the control of larvae belonging to *Culex, Anopheles* and *Aedes* genera of mosquitoes (Table 1). Use of larvivorous fishes like *Gambusia affinis* and *Poecillia reticulata* especially against *Ae. aegypti* and *An. stephensi* larvae are also practiced in many parts of India.

Table 1: NVBDCP recommended strategies for mosquito larvae control (NVBDCP, 2020)

Insecticide	Insecticide class	Frequency of application	Applied substratum
Mosquito Larvicidal Oil (MLO)	----------	Weekly	Edge of larval habitat
Temephos 50% EC*	Organophosphate	Weekly	Clean water
Diflubenzuron 25% WP	Insect growth regulator	Weekly	Clean and polluted water
Pyriproxifen	Insect growth regulator	3 weeks interval	Clean and polluted water
Bacillus thuringiensis var *israelensis* 5% (strain- 164 Serotype H-14)	Biolarvicide	Fortnightly	Clean and polluted water
Bacillus thuringiensis var *israelensis* 5% WP (strain- ABIL Serotype H-14)	Biolarvicide	Weekly	Clean and polluted water
Bacillus thuringiensis var *israelensis* 12 Aqueous suspension	Biolarvicide	Weekly	Clean and polluted water

*EC- emulsifiable concentration; WP- wettable powder

For the control of adult mosquitoes, Indoor Residual Spray (IRS), use of insecticide treated bed nets (ITNs), long lasting insecticide treated nets (LLINs), indoor space spray and fogging are usually applied methodologies of insecticide use. In India, chemical insecticides belonging to three classes *i.e.*, synthetic pyrethroids, organophosphates and organochlorines are used for mosquito control (Table 2).

Table 2: NVBDCP recommended insecticides for control of adult mosquito vectors (NVBDCP, 2020)

Sl. No.	Insecticides	Insecticide class	Vector control techniques	Recommended dose
1	DDT	Organochlorine	Indoor Residual spray (IRS)	50% WP*
2	Malathion	Organophosphate		25% WP
3	Deltamethrin	Synthetic Pyrethroid		2.5%WP
4	Cyfluthrin	Synthetic Pyrethroid		10%WP
5	Lambdacyhalothrin	Synthetic Pyrethroid		10% WP
6	Alphacypermethrin	Synthetic Pyrethroid		5% WP
7	Bifenthrin	Synthetic Pyrethroid		10%WP
8	Cypenothrin	Synthetic Pyrethroid	Indoor space spray, Outdoor fogging	5% EC
9	Pyrethrum extract	----------	Indoor space spray	2% WP
10	Technical Malathion	Organophosphate	Outdoor fogging	-------
11	Synthetic pyrethroids		Long lasting insecticide treated mosquito nets (LLINs)	------

*WP- wettable powder; EC- emulsifiable concentration

Apart from the chemical and biological control of mosquito vectors, new tools of vector control include the use of bacteria – *Wolbachia sp.* against *Aedes* mosquitoes, the release of sterile male mosquitoes to reduce vector mosquito proliferation and vector traps to attract the gravid female mosquito vectors. However, further research and implementation plan is needed for the involvement of these new tools in vector control programme.

1.6. Insecticide resistance in mosquito vectors:

Vector control remains a primary strategy of managing vector-borne diseases for most of the diseases including mosquito-borne diseases. Combating mosquito vectors through the use of chemical insecticides is the most important component of global strategy for managing vector-borne diseases. Lack of proper medication and vaccination for mosquito-borne diseases has forced our dependence on the application of synthetic insecticides for controlling mosquito vectors of dreaded mosquito-borne diseases like dengue, chikungunya, lymphatic filariasis, malaria, West Nile fever, Japanese Encephalitis and Rift Valley fever. Insecticides belonging to six classes – organophosphates, carbamates, synthetic pyrethroids, organochlorines, pyrroles and phenyl pyrazoles are recommended for application against mosquito vectors mainly *Aedes*, *Anopheles* and *Culex* sp (WHO, 2016b).

Continuous and uncontrolled exploitation of these synthetic insecticides in the form of indoor residual spraying, ITNs, LLINs, mosquito repellents, creams, oil, coil and sprays over many decades to avoid man-mosquito contact and disease outbreak

has led to the development of resistance in mosquito vectors against these insecticides. After the first report of resistance in *Aedes* mosquitoes against DDT in 1952 (Giullin and Peters, 1952), there have been several such reports of insecticide resistance in mosquito vectors to all classes of insecticides from various regions of the world.

Though in past years, the application of chemical insecticides were helpful in successfully controlling mosquito-borne diseases, the development of insecticide resistance on a global scale in mosquito vectors is now a serious issue of concern (Hemingway *et al*., 2002). Recently, there are even reports on disease outbreaks related to the development of resistance in mosquito vectors to commonly used insecticides (Hemingway *et al*., 2002; Kelvin, 2011).

Resistance development in mosquito vectors is of prime concern due to lack of proper medication for mosquito-borne diseases. This further makes insecticide application the most reliable source of vector control even today particularly during major outbreaks. Likewise, resistance to synthetic pyrethroids puts an enormous pressure on vector management as synthetic pyrethroids are the only class of insecticides recommended for use in ITNs and LLINs due to its efficacy and safety towards humans. ITNs and LLINs forms an important tool in checking the man-mosquito contact for indoor resting mosquito vectors especially the malarial vector Anopheline mosquitoes and lymphatic filariasis vector *Cx. quinquefasciatus* (WHO, 2007; WHO, 2009a).

The re-emergence of mosquito-borne diseases in various regions around the globe is associated with resistance to synthetic pyrethroids and related cross

resistance to other insecticide classes (Zaim and Guillet, 2002; Butler, 2011). The increasing trend of insecticide resistance to all classes of insecticides have been reported from more than 60 countries in the world with resistance development in all known species of mosquitoes acting as vector for one or more than one of the mosquito-borne diseases (Liu, 2015).

Insecticide resistance in mosquitoes is defined as a mosquito's ability to endure the toxic effects of an insecticide either through adaptive natural selection or mutations (Hemingway *et al*., 2004).

Insecticide resistance in mosquito vectors is a pre-adaptive phenomenon (Liu, 2015). Prior to insecticide exposure few individuals with resistant genotype are present in a mosquito population that survives insecticide application because of the presence of resistant allele. With continuous use of insecticides, the mosquito population undergoes a selection pressure and mosquitoes with resistant allele increase in number. Offsprings of resistant mosquitoes will have more survival rate in insecticide exposed environment and gradually mosquitoes with resistant alleles form the predominant population. Inheritance of insecticide resistance in mosquito vectors is highlighted in a study of reciprocal crosses in permethrin-selected *Cx. quinquefasciatus* populations. The study revealed that inheritance pattern of permethrin resistance in *Cx. quinquefasciatus* is autosomal and incompletely recessive (Li and Liu, 2014).

1.6.1. Mechanisms of Insecticide Resistance Development:

Development of insecticide resistance in mosquitoes occurs through four mechanisms –

i) behavioral resistance,

ii) cuticular resistance,

iii) metabolic detoxification and

iv) target-site insensitivity.

1.6.1.1. Behavioral resistance:

Behavioral resistance refers to the avoidance behavior in mosquitoes to insecticides on contact with prior exposure history. The ability of mosquito vectors to recognize chemical insecticides on second or third encounter makes the mosquito avoid further contact with insecticide sprayed surfaces like curtains, bed nets *etc*. This resistance mechanism makes the commonly used insecticides inefficient for mosquito control thereby causing hindrance in vector management strategies and in turn in combating the vector-borne diseases.

1.6.1.2. Cuticular resistance:

Cuticular resistance is the thickening of mosquito cuticle which in turn affects the entry of chemical insecticides through the thickened cuticle. This results in more time taken by insecticides in entering the mosquito's body, less probability of insecticide intake by the vector and inefficiency of the insecticide to tackle the vector control issues. Cuticular resistance though less studied is reported from the malarial

vector *An. gambiae* where mosquitoes with less permeable cuticle were found to have more survival rate in insecticide exposed environment than the mosquito population with normal cuticle (Yahouedo *et al*., 2017).

These two mechanisms of resistance in mosquitoes are less studied while the latter two mechanism *i.e*., metabolic detoxification and target-site insensitivity are studied worldwide and are thought to be the main mechanism of insecticide resistance development in not only mosquitoes but other arthropod vectors as well. There are several reports on involvement of major detoxifying enzymes and target-site insensitivity in the development of insecticide resistance in all three prime vector genera of mosquitoes – *Aedes*, *Anopheles* and *Culex* (Ishak *et al*., 2015; Soko *et al*., 2015; Scott, 2019). Insecticide resistance in the mosquito vectors is the result of either metabolic enzymes or target-site insensitivity or a combination of both mechanisms.

1.6.1.3. Metabolic detoxification / Insecticide detoxifying enzymes:

In mosquitoes, insecticide detoxification involves three major detoxifying or metabolic enzymes or gene families – Carboxylesterases, Cytochrome P_{450} monoxygenases (CYP_{450}s) and Glutathione S-transferases (GSTs) (Liu, 2015). These enzymes are involved in the detoxification of xenobiotics and other endogenous compounds in the insect's body. Insecticide resistance associated with the major detoxifying enzymes occurs either due to an increased production of these enzymes as a response to insecticide exposure or an increase in the enzyme activity leading to enhanced detoxification of insecticides and plant toxins. Transcriptional up-regulation

of the enzyme gene family results in production of detoxifying enzymes in a comparatively higher amount with increased enzyme copy number than the normal production rate. Amplification or duplication of genes that encode the three major detoxifying enzymes – esterases, CYP_{450}s and glutathione S-transferases (GSTs) resulting in increased production of enzymes and enhanced activity are reported to be one of the prime mechanisms of insecticide resistance development in mosquito vectors (Liu, 2015; Soko, 2015; Scott, 2015).

The increased number of detoxifying enzymes along with enhanced activity, actively and efficiently metabolizes the insecticide thereby preventing it from reaching its target site through rapid hydrolysis and transformation. The three major metabolic enzymes are reported to either detoxify a specific class of insecticide (Strode *et al*., 2008) or associated with all insecticide classes showing diverse specificity (Hemingway *et al*., 2004; Soko, 2015).

1.6.1.4. Target-site insensitivity:

Apart from rapid detoxification of insecticides by metabolic enzymes, another important mechanism of insecticide resistance development in mosquitoes and other arthropod vectors is the insensitivity of insecticide's target site. This occurs due to certain structural modifications in the target protein because of point mutation(s) occurring in the gene encoding the target proteins (Casida and Durkin, 2013). Structural modification of target proteins hampers the normal binding of insecticides to its target and such reduced binding affinity affects the effect of insecticides on mosquito physiology thereby resulting in resistance development.

1.6.1.4.1. Voltage-gated sodium channel:

Voltage-gated sodium channel (vgsc) are target of synthetic pyrethroids and DDT which upon binding to channel protein prolongs the channel opening leading to continuous discharge of nerve impulses in mosquitoes, resulting in paralysis and ultimately death. However, mutation in the vgsc gene with structural modification in channel protein causes reduced binding affinity of DDT and synthetic pyrethroids to vgsc with normal neurophysiology of mosquito vector. Moreover, the inability of insecticides to bind with channel proteins and disrupt nerve impulse flow results in resistance of the mosquito vectors to DDT and synthetic pyrethroids. Resistance to synthetic pyrethroids and DDT in mosquitoes and other arthropod vectors because of insensitive vgsc is referred to as knockdown resistance (kdr) (Scott, 2019). Mutation from Leucine to Phenylalanine in 1014 codon (L1014F) of segment II of vgsc is the first reported target-site mutation in insects and is also the most widely studied mutation. In mosquitoes, apart from L1014F mutation, Leucine to cysteine / serine / tryptophan (L1014C/S/W); isoleucine to methionine or valine, valine to glycine and phenylalanine to cysteine are reported to occur in one or more than one of the mosquito vector species (Liu, 2015). Co-occurrence of multiple mutations in vgsc gene is regarded as a key factor in conferring resistance development in mosquitoes with synergistic effect (Scott, 2019).

1.6.1.4.2. Insensitive acetylchlolinesterase (AChE):

Resistance to organophosphate and carbamate insecticides is related to insensitivity of acetylcholinesterase enzyme encoded by the acetylcholinesterase

gene. Mutation from glycine to serine (G119S) codon of acetylcholinesterase gene results in reduced affinity of the enzyme to insecticides and associated resistance development reported in *An. gambiae*, *Cx. vishnui*, *Cx. pipiens*, *Cx. quinquefasciatus* (Wondji *et al*., 2011). However, in *Cx. tritaeniorhynchus*, substitution of phenylalanine by tryptophan (F455W) of ace gene is related to organophosphate resistance (Nabeshima *et al*., 2004).

1.6.1.4.3. γ – amino butyric acid (GABA) receptors:

Cyclodiene insecticides like dieldrin and phenyl pyrazoles like fipronil target the γ – amino butyric acid (GABA) receptors that act as receptor for the inhibitory neurotransmitter GABA and is involved in the opening of chlorine channel (Liu, 2015). Mutation from alanine to glycine in 296 codon of GABA receptor gene is related with dieldrin resistance in *An. gambiae* and A296S (alanine to serine) substitution is associated with dieldrin resistance in *Ae. aegypti*, *An. stephensi* and *An. funestus* (Du *et al*., 2005). Furthermore, in *Cx. quinquefasciatus* and *An. stephensi*, fipronil resistance is linked to insensitive GABA receptor (Davari *et al*., 2007).

The mostly studied target-site insensitivity in mosquito vectors is the voltage-gated sodium channel conferring resistance to synthetic pyrethroids and an organochlorine DDT. In *Cx. quinquefasciatus*, the presence of L1014S mutation in voltage-gated sodium channel has not been reported earlier while there are several reports on L1014F kdr mutation in this vector (Corbel *et al*., 2009; Sarkar *et al*., 2009; Kudom *et al*., 2015; Yadouletan *et al*., 2015; Yanola *et al*., 2015; Lopes *et al*., 2019).