VECTOR-BORNE DISEASES & TREATMENT

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Published in: Nov 2018

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Chapter 1

Filariasis: its Manifestations, Epidemiology and Control Strategies

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1. Introduction

Filariasis is a helminth disease, caused by parasitic worms known as filariae and transmitted through mosquito vectors. Filariasis presents a threat to public health as it causes severe long term disability and hampers one's socio economic status. Filariasis is endemic in many tropical and subtropical regions of the world. Lymphatic filariasis, a major type of the disease alone puts about 120 million people at risk of disease infection. When we trace back the history of the occurrence of this disease, though the first written document is from the Ancient Greek and Roman civilizations [1] yet the confirmation was made only many centuries later in 1877, when Sir Patrick Manson detected microfilaria causative agent of lymphatic Filariasis in mosquitoes. This was the first ever discovery of an arthropod acting as a vector of human diseases which was later found to be the case for other tropical diseases such as malaria, dengue *etc*. Even though it can affect individuals of all age groups and both genders, it is predominantly found to be associated with people of low socio economic status [2]. Moreover, filarial infection in general has been found to be more common in males than females. Although mortality is not associated with the disease, morbidity rate as a result of clinical manifestations is very high [3] and economic burden posed by the physical deformities resulting from infection have a severe psychological and socio economic impact [4].

A single bite of the infected vector does not establish the disease instead many years of continuous exposure to bites of hundreds of infected mosquitoes is required. This is because

Citation: *Luigi Falsone (2018) Vector-Borne Diseases & Treatment, Vol. 2, pp. 1-85.*

inside the mosquito vector, multiplication of the filarial parasite does not occur. Approximately, 15,500 bites of infected *Culex quinquefasciatus* is essential for a new infection to occur [5].

2. Types

Depending on the site of occurrence of the parasite and the types of parasites causing the disease, filariasis has been found to be of four different types [1]. These are:

2.1. Lymphatic filariasis

Lymphatic filariasis is one of the most important neglected tropical diseases (NTDs) and is caused due to the infection with nematode parasites known as filarial worms. These worms belong to the Onchocercidae family and their infection results in the damage of one's lymphatic system. The causative agents of lymphatic filariasis are *Wuchereria bancrofti, Brugia malayi* and *B. timori*. About 90% of the infection is caused by *W. bancrofti* alone [6]. Although *Wuchereria bancrofti* and *Brugia malayi* live almost exclusively in humans, macaques and leaf monkeys in some parts of the world are said to be reservoirs of the parasites [1]. There is no other known natural animal reservoir of lymphatic filariasis, making man the only reservoir. Several species of mosquitoes serve as vector for these microfilarial worms. The vectors include *Culex quinquefasciatus, Anopheles gambiae, Aedes polynesiensis* and *Mansonia sp.* [7]. In many regions of Africa the *Anopheles* vectors of lymphatic filariasis is similar to those of malaria [8,9]. In Zambia, *An. funestus*, *An. gambiae* and *An. arabiensis* are the predominant species [10,11]. Periodicity of these microfilariae is directly related to the feeding habits of the above mentioned vectors. Almost all of these mosquito vectors feed during night hours except *Aedes polynesiensis*.

2.1.1. Transmission/life cycle

In 1877, Patrick Manson proposed that mosquito vector deposited microfilariae in water and human consumption of this contaminated water or direct skin penetration through contact led to the infection. However, it was George Carmichael Low who paved the correct mechanism of transmission of microfilariae in 1900 when he discovered the presence of pathogenic microfilariae in the mosquito's proboscis. When a mosquito bites an infected human, the microfilariae present in the circulating peripheral blood of human is also taken up by the mosquito vector along with the human blood. After 1-2 weeks of ingestion by the intermediate host, the microfilariae in the midgut of the vector shed their sheaths and make their way to the thoracic muscles. Here the microfilariae develop into first stage larvae, second stage larvae and finally to the third stage larvae which is also known as filariform larvae, which is infective to man. Then the third stage larvae migrate from the thoracic muscles of the vector to the proboscis through haemocoel. The infected vector introduces these larvae into a human host during another blood meal and larvae enter the body of the host through the bitten wound

and reach the lymph glands where they mature into adults.

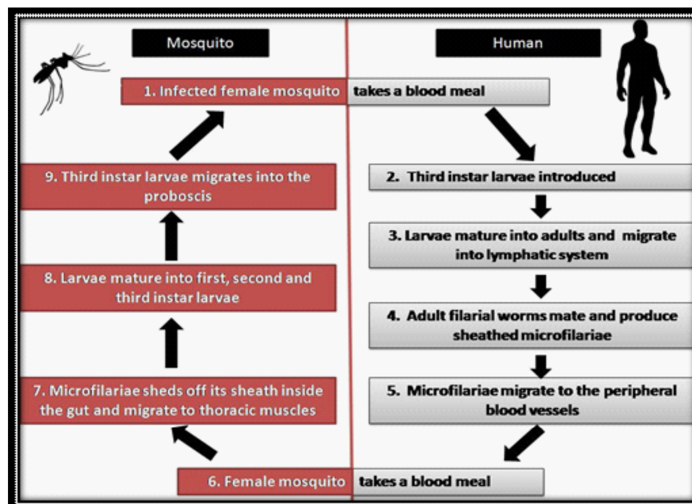


Figure 1. life cycle of *Wuchereria bancrofti*

This is a slow process and generally takes 5 to 8 months [12]. The mature male and female worms copulate to undergo sexual reproduction and produce sheathed eggs known as microfilariae. These circulate in the peripheral blood of the host in turn to be picked up by a mosquito and the cycle continues. Lifespan of adult worms is quite long and can live up to 10-15 years [1]. A mature female filarial nematode can produce microfilariae for up to about five years of maturation.

2.1.2. Symptoms

A light infection does not produce serious effects but causes filarial fever, headache and mental depression. A large number of pathological symptoms are observed during heavy infection of the parasites. Symptoms of lymphatic filariasis can be grouped into three categories such as asymptomatic infection, acute infection and chronic infection.

Asymptomatic infection*:* Some of the patients with lymphatic filariasis show no symptom of infection. Though these patients appear clinically asymptomatic, the parasites cause damage to the host's lymphatic system, kidneys and gradually alter the immune system.

Acute infection*:* In acute infection, microfilariae circulating in the human blood stream cause acute manifestation of lymphatic filariasis. The symptoms include episodic local inflammation of skin along with irregular and sporadic occurrence of lymphadenitis (*i.e.* inflammation of the lymph glands) and lymphangitis (*i.e.* inflammation of lymph channels), the latter two being characteristic of infection either by *W. bancrofti* or *B. malayi* [13]. Some of these inflammations are due to the action of host's immune response against the microfilarial parasites. Rest results from bacterial infection of the protective skin barrier of the host which becomes susceptible to such infections due to underlying lymphatic damage. During this sporadic attack, the distal end of the affected limb of the host becomes swollen and may remain so for several days. In lymphadenitis, the parasites essentially take over lymph nodes in the body causing immune reaction and inflammation [14]. Inflammations related to acute infection

results in immense pain and red streaks on the affected skin. Along with these symptoms, sometimes a patient may suffer from extreme pain in the genital area followed by formation of pus-filled nodules. These nodules keep on swelling until they rupture to discharge bacteria and dead adult worms.

Chronic infection: When adult worms deposit themselves in the lymphatic vessels and glands it results in lymphatic obstruction that restrains the back flow of lymph into the circulatory system. This results in the accumulation of lymph in the affected areas leading to enormous swelling in tissues of those areas thereby producing a condition known as lymphoedema [15]. But there are experimental evidences which propose that simple lymphatic blockage may not cause lymphoedema until and unless it is associated with certain inflammations. Later as infection increases there is invasion of plasma cells, eosinophils and macrophages resulting in chronic lymphatic damage and leakage of lymph into the tissues, thickening of the skin and underlying tissues and bacterial and fungal infections. All this leads to elephantiasis which is the most spectacular symptom of lymphatic filariasis and is more common in the lower limbs and genitalia than the upper extremities [1]. Elephantiasis due to the infection of *B. malayi* affects the upper and lower limbs with no genital pathology and infection with *B. timori* causes more swelling as compared to that of *B. malayi* and *W. bancrofti* [16]. Accumulation of fluid in scrotum and nearby areas of the host is termed as hydrocele and all types of scrotal enlargement due to the infection of microfilariae are termed as filaricele [17].In some cases, lymphatic blockage leads to the leakage of chyle and produce certain pathological conditions like chyluria, chylus diarrhoea and chylorrhagia [18].

2.2. Occult filariasis

Depending on whether or not the microfilariae can be found in the peripheral blood of the host, infected individuals may be termed as either microfilaraemic or amicrofilaraemic respectively. This amicrofilaraemic condition is termed as Occult filariasis. Though not found in the peripheral blood, microfilariae may be found in the tissues and other body fluids. Occult filariasis is believed to result from hypersensitivity reaction to filarial antigens. In a community where filariasis is endemic, only a small proportion of the population develops occult form of filariasis. The term occult filariasis embrace a number of pathological conditions such as, Tropical Pulmonary Eosinophilia (TPE), Glomurelopathies, filarial arthritis and filarial infections of the breast [19].

Tropical Pulmonary Eosinophilia (TPE) is the most common example of occult filariasis and is found mainly in the Indian subcontinent. It can be seen in people belonging to all age groups and symptoms of the disease include cough, fever, chest pain, breathlessness and occasional abdominal pain. After infection, the microfilariae lodge in the lungs and pulmonary arteries of the host causing pulmonary lesions and is frequently accompanied by filariatic fever.

TPE is characterized by high eosinophil level in the blood and asthma-like symptoms which is due to hyperresponsiveness of the host's immune system to the circulating microfilariae. If treatment is not provided for a long period of time the condition progresses to pulmonary fibrosis and respiratory insufficiency followed by impairment of lung function.

Glomerulopathies is associated with the production of typical lesions in the glomerulus and diffuse mesengial proliferation on the basement membrane. Filarial antibodies have been reported from patients with glomerulonephritis [20].

Filarial arthritis is usually common in the filariasis endemic areas and affects the knee joints. Though microfilariae may not be detected in the circulating fluid, however filarial antibodies may be detected in antibody test. It is important to differentiate filarial arthritis from rheumatoid arthritis as their respective treatment is quite different. The disease may be caused by other species excluding *W. bancrofti* [21]. In filarial arthritis, only the large joints are affected and majority of the patients have a painless swelling in the knees.

Filarial infections of the breast results in hard breast lumps attached to the overlying skin and at times are difficult to distinguish from malignant tumours [22]. Both adult worms and microfilariae have been found in the breast granuloma of patients through histological examinations.

The occult form of filariasis is generally caused by microfilariae but the symptoms are sometimes very much similar to other well known clinical conditions and are impossible to distinguish. The diagnosis of these occult manifestations can be done with ELISA test using specific antigens [19,23].

2.3 Onchocerciasis

Onchocerciasis also known as river blindness is caused by a parasitic microfilarial worm *Onchocerca volvulus*. It is also a NTD widespread in different countries of world. The parasite *O. volvulus* is transmitted by blackflies (*Simulium sp.*) that breed along fast flowing rivers and streams. An infected black fly introduces third stage filarial larvae into the human skin. The larvae then develop into adults and reside in the subcutaneous tissue nodules for up to 15 years. The adult worms produce microfilariae that migrate mostly to the skin and eyes. Symptoms include severe itching, disfiguring of the skin and eye lesions which sometimes can lead to permanent blindness [24]. Studies reveal that patients suffering from Chronic Onchocerciasis show increased eosinophil and high levels of serum immunoglobulin E (IgE) [1].

2.4 Loiasis

Loiasis also referred to as Loa loa filariasis is a skin and eye disease caused by a filarial nematode *Loa loa* commonly known as the African eye worm. The nematode is transmitted

in human through the bites of deer flies or mango flies of the genus *Chrysops sp*. Two of the most important vectors include *Chrysops silicea* and *C. dimidiate* [25] that are generally found in the rain forest region of West and Central Africa. Adults harbour the subcutaneous tissue of the human host where the male and female mate and produce microfilariae that have diurnal periodicity probably due the day feeding habit of their vector. Clinical symptoms include localized swellings (popularly called Calabar swellings owing to the place of its first reported incident) most commonly in the limbs and rarely in the face. The adults often migrate into the eyes where it is externally visible for a short duration hence securing the name 'eye worm'. Loa loa infection generally does not affect normal vision but its movement through the tissues have been reported to be very painful [26,27]. Though infection with *L. loa* is usually asymptomatic microfilariae may sometimes be found in the blood, lungs, urine, spinal fluid and sputum [27].

In certain regions of West and Central Africa, loiasis is reported to be co-endemic with onchocerciasis. The first case was reported during the 1990s in Cameroon where patients with high intensity of Loa loa infection developed severe adverse neurological reactions after treatment with ivermectin for onchocerciasis [28,29]. This co-endemicity is of great concern because mass drug therapy for onchocerciasis with an anti-filarial drug ivermectin has an adverse effect on patients with high densities of Loa loa infection [28]. Probable explanation for the fore lying sentence is encephalopathy that results from massive killing of microfilariae near the optics and brain region in patients having high microfilarial loads [27]. Therefore, in communities with a high level of loiasis endemicity, there is a significant risk of severe adverse reactions to ivermectin treatment [30]. As a result, loiasis has recently evolved as an important public health issue.

3. Epidemiology

The World Health Organisation (WHO) considers lymphatic filariasis as one of the only six eradicable diseases and in order to achieve this goal proper information regarding disease prevalence should be considered. Lymphatic filariasis is endemic in tropical and sub tropical areas of the world and includes 32 of the world's 38 least developed countries [31,32] thereby developing a higher risk of infection to people living in those regions. Lymphatic filariasis endemic regions are Central Africa, Nile delta, Madagascar, Turkey, South East Asian countries, Thailand, Malaysia, Vietnam, South Korea, Indonesia, Philipines, Timor, Southern China, Guinea and Brazil [32,33]. Lymphatic filariasis affects approximately 120 million people in the world and 120 billion people are considered to be at a risk of becoming infected [34].

Approximately 15 million people with lymphatic filariasis live in Southeast Asian countries [35]. Earlier WHO estimated that on a global scale, a significant majority of filarial

infections and disease cases occurred in India [36]. It was later reported that most number of cases around the world occurred in India (45.5 million) and Sub Saharan Africa (40 million) with India having 5% and Sub Saharan Africa having 8% of disease prevalence [34,37]. Sub Saharan Africa has the largest number of countries with moderate to high prevalence of filariasis and due to lack of current data on incidence of the disease in many of these countries, Sub Saharan Africa pose as the region where the disease is of immense public health significance [34]. Transmission efficiency of these diseases is also known to be higher in Africa than in Asia which may be due to the availability of different vectors that are responsible for transmission of filarial worm in these two distinct geographical locations [37]. In general, *Anopheles sp*. transmits the disease much more efficiently than *Culex sp*., although with a few exception [38].

Infection with *Onchocerca volvulus* is prevalent mainly in the tropical areas. Though most of the infected people are found living in 31 countries of sub-Saharan Africa [39], occasional reports on cases with onchocerciasis have also come to the limelight from Yemen and the United states.

Loiasis is an African disease that is restricted to the rain forest region of West and Central Africa [30,40,41] limiting its distribution to Benin in the West, Uganda in the East and Zambia towards the South [42]. Highly endemic regions for loiasis are the Equatorial Guinea, Gabon, Cameroon, Democratic Republic of Congo, Central African Republic, Chad and Sudan [30]. Endemicity of the disease is closely linked to the habitats of its vectors *Chrysops silicea* and *C. dimidiate*. An estimated 12-13 million people in the endemic area are disease affected [43]. As co-endemicity of loiasis with Onchocerciasis possesses a great hurdle towards control of filariasis, knowledge relating to the co-endemic regions is important. Loiasis was once prevalent in Ghana, Mali and Ivory Coast but has now been completely and successfully eradicated [43]. Cases of Loa loa infection have also been occasionally reported from the United States but only in those who have returned from endemic areas [43-45].

4. Diagnosis

For implementation of effective control programs at community levels, an accurate diagnosis of filariasis should be of prime concern. The first and foremost step involves collection of information regarding the exposure of patient in endemic areas whether currently or in the past and thereafter laboratory tests can be carried out like:

Serology test to detect circulating microfilariae in the peripheral blood. However, i. the periodicity of the pathogen should be kept in mind [46]. This is by far the most widely used diagnostic technique due to its simplicity and low cost.

For detection of ii. *Onchocerca volvulus,* skin biopsy is usually performed.

PCR tests using species-specific primers to detect DNA of the pathogen in human iii. blood and also in the infected vector.

Immunochromatographic test holds advantage in being independent of periodicity of iv. the pathogen [47].

Ultrasonography to locate filarial worms in the genitals of asymptomatic males. This v. is a prime diagnostic technique to distinguish between cases requiring immediate surgery and cases that can be dealt with drugs [17].

Recently, many advanced techniques and methodologies have been developed for the diagnosis off ilariasis worldwide like filariasis strip test [48,49], antibody rapid test, molecular xenomonitoring to detect filarial DNA using reverse transcriptase PCR (RT-PCR) and loop mediated isothermal amplification for rapid detection of filarial DNA in mosquitoes [50,51].

5. Anaphylactic Treatments

5.1 Drug therapy

Several drugs are used for the treatment of filariatic infection. Most important and the commonly used ones are Diethylcarbamazine (DEC), Ivermectin, Suramin, Albendazole, Mebendazole, Flubendazole and Doxycycline [1]. DEC is both micro-filaricidal and macro-filaricidal thereby being a drug of choice for patients with active Lymphatic filariasis. It is a potent micro-filaricidal drug and also has moderate macro-filaricidal effect [52]. The most important action of DEC appears to be the alteration of microfilariae, which are readily phagocytosed by tissue fixed monocytes but not by the circulating phagocytes [53].Recommended dose for DEC is 6mg per kg body weight per day for 12 days [37]. However, recent studies also report that a single dose of DEC (300 mg) in combination with albendazole (400 mg) is equally effective [54]. Ivermectin and Suramin are efficient only against microfilariae and not the adult worms. The filarial nematodes when exposed to these two drugs develop tonic paralysis. Ivermectin can be used to treat onchocerciasis but has to be administered only in areas where co-endemicity of loiasis does not occur as the drug has an adverse effect on patients infected with high intensities of Loa loa infection [55]. Studies show that Albendazole works by decreasing the ATP production in worms thereby resulting in energy depletion, immobilization and death of the filarial worm [56,57]. Albendazole can also be used in combination with DEC and Ivermectin to increase the anti-helmintic property [58]. The combination of Albendazole with DEC and Ivermectin has shown to reduce the prevalence of angioedema in a study conducted in South India [59], and the same in Nigeria has shown to reduce mosquito infection rates [60]. The triple drug combination of Albendazole, DEC and Ivermectin represents a potentiality to significantly reduce the number of doses of anti-helmintic drugs when used singly [61]. Mebendazole and Flubendazole acts by blocking the glucose uptake of nematodes. This results

in glycogen depletion and reduced ATP generation but the blood glucose levels of the infected human remains unaffected. Doxycycline is a drug that ultimately hampers the embryogenesis of the filarial nematode [62] leading to sterilization or reduced reproduction, but is used not directly against the nematode but against its endo-symbiont a bacteria *Wolbachia*. Doxycycline, alike Ivermectin can also be administered in Onchocerciasis and loiasis co-endemic areas. Ivermectin is also contradicted among pregnant women, nursing mothers and small children [63].

Hydrocele can be treated by frequent excision of the overlying skin following the traditional procedures and thorough cleaning of the skin. Surgical treatment for lymphoedema of the limb can be of two major types *i.e.* drainage and excision. In drainage procedure the lymph flow of the infected individual is improved by either bypassing the blocked portion or addition of new lymph channels. Excisional procedure is the trimming off of the extra large limb volume.

Herbal treatments: For centuries, people used and still use several herbs against filarial infection. Some of the herbs being used for treatment of filariasis in South Africa are *Elephantorrhiza elephantine, Eucomis autumnalis, Ganoderma sp., Solanum aculeastrum, Hermannia geniculata, Datura stramonium, Ricinus communis* and *Pentanisia prunelloides* [64].These herbs can be used individually or in a combination to enhance their effect against the disease. Some of the herbs like *Vitex negundo, Butea monosperm a*and *Aegle marmelos* have also been reported to show antifilarial activities [65].

5.2 Targeting Wolbachia an endosymbiont of filarial nematodes

Wolbachia, a gram-negative proteobacterium is an endosymbiont in all human filariae belonging to family Onchocercidae except *Loa loa* [66,67]. Studies on the symbiotic relationship between *Wolbachia* and Onchocercidae show that *Wolbachia* promotes normal development, fertility and survival in the filarial worm. Till date, relationship between *Wolbachia* and Onchocercidae is considered to be mutualistic [68] as evident from the complete genome analysis of *Wolbachia* in *Brugia malayi* [69]. The bacterium is vertically transmitted to the filarial progeny through the female germline [70]. *Wolbachia* till now has not been detected in any other nematode groups [71,72] excluding Onchocercidae [73].

On contrary to the endosymbiont nature of *Wolbachia* in Onchocercidae, this bacterium is highly parasitic in arthropods. As a result, in mosquitoes, it inhibits the transmission of certain viruses like Dengue, Chikungunya, Yellow fever, West Nile and also of malarial parasite *Plasmodium* and filarial nematodes [74].

Most anti-filarial drugs currently in use are effective only against the larval forms of filariae, *i.e.* microfilariae and development of resistance against those has also been reported

[74]. The adult worms can survive in the human host for 10-15 years and has the ability to fecund for almost their entire lifetime. Keeping this in mind drugs must be administered for a long period of time. Targeting the adult worm is the need of the hour. This can be achieved through targeting *Wolbachia* whose depletion may in turn result in stunted embryogenesis [62] and death of the adult worm.

Wolbachia is present in all larval stages of filarial nematode and also in the adults [75,76] being mainly localized in the hypodermal cells [77]. It is also found in the ovaries and uterus of the female but has never been reported in the male reproductive system [78]. *Wolbachia* plays an important role in triggering pro-inflammatory response in the patient and also enhances the survival rate of the nematode. Therefore, targeting *Wolbachia* as a filaricidal seems to hold great potentiality for treatment of filariasis. Doxycycline has already been recommended as an anti-Wolbachia therapy for the treatment of lymphatic filariasis and onchocerciasis [79,80].

Electron microscopy study has shown the absence of *Wolbachia* in microfilariae [81,82] and adults of *Loa loa*[83], this has further been confirmed by PCR analysis. Agreeing to which Helen *et al.,* reports that the neurological consequences following ivermectin treatment of individuals with *Loa loa* are not associated with *Wolbachia* [82]. In co-infected individuals, post treatment reactions may be due to adverse events induced by *Wolbachia* derived from either *O. volvulus* or *W. bancrofti* [82].

6. Control and Prevention of Filariasis

The principal approach in community control of filariasis is the mass administration of anti-filarial drugs known as Mass Drug Administration (MDA) in the endemic areas. MDA consists of annual or semi-annual drug administration initially for 4-5 years. The use of anti-Wolbachia drug doxycycline may also be considered for MDA but as the required treatment course being six weeks, makes its large scale implementation very difficult [84].

Secondarily, focus has to be made on vector control strategies in order to sustain the advantages of MDA. Lack of vaccine against filariasis makes vector control and management through insecticides, one of its prime strategies to eradicate the disease. However, the widespread developments of insecticide resistance in vector populations pose a great threat to vector control. Moreover, prolonged vector control, do contribute to subsidence of parasite transmission though recently it is widely accepted that vector control should complement chemotherapy [85]. Vector control when used with DEC administration reduced transmission rate significantly when compared to drug administration alone [86]. Studies in Tanzania and India have reported reduced transmission through the use of vector control strategies. The use of insecticide treated bed nets (ITNs) or long lasting insecticide treated bed nets (LLINs) and untreated nets in combination with chemotherapy has documented a reduction in prevalence of lymphatic filariasis in countries like Kenya, Nigeria and Papua New Guinea [86-87]. Senkwe *et*

al., reported a significant decline in lymphatic filariasis when use of ITN scaled up through the entire nation in Zambia [88]. Habitat destruction of the vector has also been one of the targeted steps. Application of insecticides and biological agent *Bacillus thuringiensis israeliensis* in the breeding grounds of the vectors help control vector population to some extent. As man is the only host of *Wuchereria bancrofti*, its transmission can be interrupted efficiently by implementation of MDA and vector control strategies.

6.1 Control programmes worldwide

In the year 2000, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched by World Health Organisation (WHO) with a prime objective to interrupt transmission of the parasite [89]. GPELF aims to eliminate lymphatic filariasis as a public health problem by 2020 through two strategies mentioned below.

i. Interrupt the transmission of disease following four sequential steps.

a. Mapping areas to determine the geographical distribution of the disease and identify endemic areas.

b. MDA is then implemented to the entire populations living in the disease endemic areas. It includes single dose of DEC or ivermectin combined with albendazole initially for a period of five years to the populations at risks.

c. After the end of MDA programme, infection levels are monitored through post-MDA surveillance of the endemic areas in order to identify areas of ongoing transmission.

d. Verification of the absence of transmission is the final step to check whether a country succeeded in interrupting transmission or not.

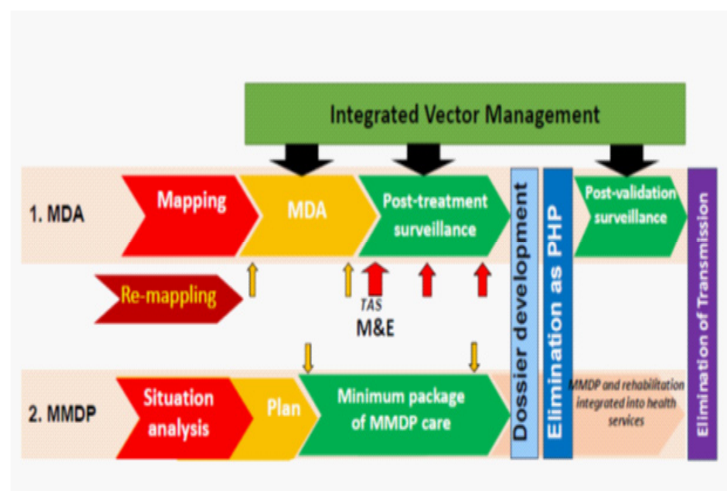
ii. Reduce suffering and disability of the infected people by introducing measures ike improved hygiene and skin care for lymphoedema patients and provision of surgery for hydrocele patients. Morbidity management is considered as an integral step in the eradication of lymphatic filariasis. Therefore, managing mobidity to relieve sufferings related to the disease is one of the primary motive of GPELF. Morbidity management basically includes providing lymphoedema management, urogenital surgery for affected males, improving hygiene and skin care on the affected portion and to promote improvements in the quality of life of people infected with lymphatic filariasis.

After the launch of GPELF, the rate of mass distribution of anti-filarial drugs significantly rose up. During the first 10 years, the number of people treated by MDA increased from 3 million in 12 countries in 2000, to 466 million in 53 countries in 2010 [90] but the efforts to provide mobidity management was not up to the mark. WHO then recommended the preventive

chemotherapy and transmission control as a primary strategy to interrupt the transmission of lymphatic filariasis. Preventive chemotherapy is executed through MDA in the endemic areas and transmission control approach focuses on vector control techniques.

Along with MDA and vector control, emphasis should also be given to improve water quality, sanitation, hygiene and general living standard [91]. As an alternative strategy, WHO has now launched water, sanitation and hygiene (WASH) campaigns for interrupting the transmission of the parasite. Through sanitation campaigns against *Culex quinquefasciatus*, lymphatic filariasis has been eliminated from Australia and reduced significantly in many parts of Brazil [92].

As mentioned earlier, WHO has recommended the following four steps that should be followed in order to make the Filariasis elimination campaign fruitful. a. Mapping the area to



a. determine the geographical distribution of the disease.

b. MDA initially for 5 years and thereafter decision should be made whether to stop MDA or not based on the researches carried out on the recent transmission rate of the disease.

c. The area should be kept under surveillance even after completion of MDA.

d. Decrease in transmission rate should be checked during short time intervals.

In 2012, many organizations from around the world joined together against NTDs and signed the London Declaration with the aim to control and eradicate the NTDs. Since then, lymphatic filariasis has been targeted to be eliminated from the world by 2020 [51]. To achieve this goal, in combination with the various strategies earlier mentioned in this chapter, increase in funding and donations from government and other organizations are equally important.

Control of onchocerciasis is executed with the help of three programs in Africa, West Africa and the Americas [39]. In Africa, from 1995-2015, the African Program for Onchocerciasis Control (APOC) was implemented and mainly focused in controlling onchocerciasis through sustainable community-directed treatment with an anti-filarial drug ivermectin. It also supported

the vector control program using environmentally safe methods. APOC in Africa has now been replaced by the Expanded Special Project for the Elimination of Neglected Tropical Diseases (ESPEN).

In West Africa, onchocerciasis has been brought under control by the WHO Onchocerciasis Control Program (OCP). This program mainly focuses on the vector control strategies through use of insecticides against the black flies supplemented by MDA of ivermectin in the endemic regions. The Onchocerciasis Elimination Program of the Americas (OEPA) operated through MDA with ivermectin twice a year. All of the combined effort against the disease led to the eradication of onchocerciasis first from Colombia (2013) then followed by Ecuador (2014), Mexico (2015) and Guatemala (2016).

7. Conclusion

Lymphatic filariasis and onchocerciasis forms a major portion of NTDs in tropical and subtropical countries. Though steps both at the community level and global level have been implemented for successful eradication of these diseases, yet they still persist and seriously affect the socio-economic status of a country. Along with the therapeutic treatment, much importance should be provided to the follow up thereafter to prevent related secondary infections. In lymphatic filariasis, avoidance of secondary bacterial and fungal infection in the affected portion of the patient is a must for proper management of the disease. Much scientific studies should be directed to Loiasis, which has recently come into focus because of the hindrance provided by its causative agent in the MDA against onchocerciasis with ivermectin. Eradication steps therefore, should also involve ways to tackle such associated problems through improving the current tools and techniques and the methods of assessment. Vector control is a promising tool against filariasis and also other vector borne diseases. Prior information regarding insecticide resistance status and the degree of resistance towards a particular group of insecticides has to be in mind before the application of an insecticide against a vector. Survey of the endemic areas and research relating to insecticide susceptibility/resistance status of different vectors provides a baseline data for designing of an efficient vector control program. Therefore, such surveys and researches should be encouraged and promoted at the regional levels. Lastly, the involvement of mass/community should be encouraged for the efficient implementation as well as proper management for the eradication for the eradication of these diseases.

8. References

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Chapter 2

Salient features of Trypanosoma Congolense in Animal African Trypanosomiasis in the Sub-Saharan Africa

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Abstract

Despite the multiple causes, contribution of *Trypanosoma congolense* in Animal African Trypanosomosis (AAT) in the sub-Saharan Africa is great. More than 80% of AAT and losses in domestic animals (cattle, goats, sheep, horses, pigs and dogs) in South, East and Central Africa are due to *T. congolense* infections. In the West, *T. congolense* remains one of the major causes of AAT in livestock. This chapter discusses the biology and disease caused by *T. congolense*, challenges and opportunities for control are highlighted.

Keywords: Trypanosoma congolense, Animal African Trypanosomosis

1. Introduction

African Animal Trypanosomosis (AAT) or "nagana" is one of the vector-borne diseases that have impact on economic growth and a threat to food security in the sub-Saharan Africa. The disease is caused by salivarian trypanosomes mainly *Trypanosoma congolense*, *T. vivax* and the lesser extent *T. brucei brucei*. *T. evansi* and *T. equiperdum* are responsible for other forms of the disease, Surra and Dourine respectively. The disease is spread by the bite of infected tsetse flies (*Glossina species*). Its distribution in the sub-Saharan Africa corresponds to geographical boundaries of tsetse flies within the latitude 14° N and 29° S. The possibility of mechanical transmission by Tabanidae and Stomoxynae has enabled the disease to spread beyond the tsetse belt of sub-Saharan Africa [1,2]. *T. vivax* is now established cause of the disease in cattle causing high morbidity and mortality in South America [3,4] and to the lesser extent in Asia and Europe [5]. Nevertheless, *T. evansi* is a threat to livestock production particularly cattle, water buffaloes and camels across Asia and South America [5,6].

It is estimated that about 40 million cattle are at risk and 3 million die every year, leading to economic loss of US$ 1.0 – 1.2 billion annually [7]. The total domestic product lost is estimated at US$ 4.5 billion per annum when secondary losses such as reduced manure and draft power are included [7]. Despite the multiple causes, contribution of T. congolense in AAT in the sub-Saharan Africa is enormous. More than 80% of AAT and losses in domestic animals (cattle, goats, sheep, horses, pigs and dogs) in South, East and Central Africa are due to T. congolense infections [8]. In the West, T. congolense is second to T. vivax in causing AAT morbidity in livestock [8]. The characteristic features of T. congolense to such high prevalence and losses is probably due to host susceptibility, intrinsic factors, virulence and vectorial capacity of vector tsetse flies to the parasite [9]. This chapter discusses the biology and the various features of the disease caused by T. congolense, challenges and opportunities for control approach are discussed.

2. Biology of *Trypanosoma Congolense*

2.1. T. Congolense as Intravascular Parasite

Trypanosoma congolense was first discovered by Broden in 1904 in the blood of sheep and donkey from then "Leopoldville" which is currently known as Kinshasa in the Republic of Congo [10]. It is a monomorphic (12.1–17.6 μm) salivarian parasite (development take place in the mid-gut and mouthpart of tsetse flies) and lacks a free flagellum at any stage of development [11], and can grow in mice [10]. Unlike *T. vivax* and *T. brucei*, *T. congolense* occurs in the blood vessels only [12] except during development of infection at the site of inoculation where the parasite is found in the skin, extravascularly and localized draining lymphatics [13,14]. In established infection, studies have shown unevenly distribution of *T. congolense* in the host circulation, but mostly localized to the walls of capillaries and small

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vessels particularly of brain, heart and skeletal muscles [12,15] and therefore, providing the possibility of passively damaging the attached cells in response to anti-trypanosome antibody and complement fixation [16].

2.2. Trypanosoma Congolense Types

T. congolense has been found to comprise three different types that are morphologically identical but genetically heterogeneous types infective to livestock and other mammalian hosts [17,18]. They have been classified as Savannah, Riverine-Forest and Kilifi [19,20]. Savannah and riverine-forest are genetically closely related than do Kilifi with the other two [21]. However, all three varies in their virulence, pathogenicity, drug resistance, vectors and geographical distribution [22]. Studies have associated Savannah type with a number of *Glossina* species (morsitans, forest and fusca groups) and affect a wide range of hoofed mammals and carnivores across savannah ecosystem of sub-Saharan Africa [23]. In contrast, *T. congolense* riverine-forest type is largely restricted to the *palpalis* group of tsetse mainly affecting pigs, goats, cattle, and dogs in the humid forest ecosystem of West, Central and to the lesser extent in East Africa [24–26]. *T. congolense* Kilifi type is restricted to East Africa and to the small extent in South Africa; it is associated with tsetse of morsitans group and mainly reported in cattle, sheep and goats [20,22,27,28].

However, field investigations in many parts has frequently found co-infections of T. congolense types in livestock and tsetse flies [23]. For instance, whereas Savannah and riverine-forest co-infection are common in West and Central Africa [25,26,29,30], Savannah and Kilifi co-infections occur in East and South Africa [31,32]. Nevertheless, Zambia, Kenya and Tanzania are the only countries which have reported co-infections of all three types [28,31,33].

3. The Life Cycle

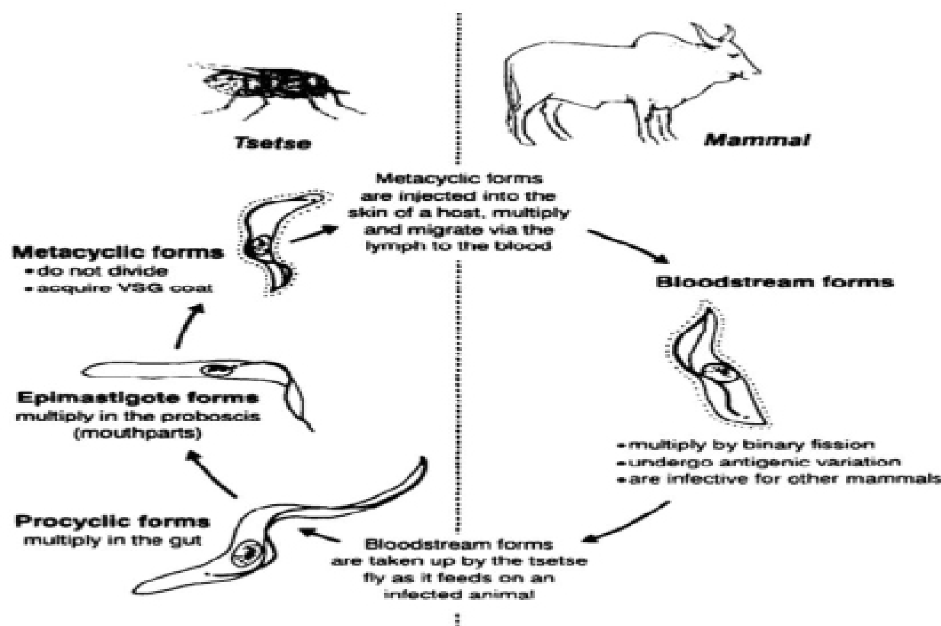
Trypanosomes have a complex lifecycle **(Figure 1)**. The bloodstream forms that proliferate in the blood of infected mammalian host are ingested by the insect (tsetse fly) during the blood meal. They differentiate into procyclic forms in the mid-gut and migrate to the salivary glands and proboscis where they attach as epimastigotes forms. They then differentiate into infective metacyclic forms that are transmitted to a new mammalian host during the next blood meal.

More elaborate of the life cycle of T. congolense has been described by [10]. And the recently developmental cycle in tsetse fly outlined by [35]. In fact, four stages are described in tsetse fly ranging from procyclics, trypomastigotes, epimastigotes and the infective metacyclic trypomastigotes. The bloodstream forms taken up by the fly differentiate into procyclics in the fly-midgut and grow in length. The procyclics through peritrophic matrix penetrate the prov-

entriculus where they cease division and become uniform in size and shape, and hence the name trypomastigotes. These trypomastigotes migrate to the cibarium and proboscis where they differentiate to epimastigotes and eventually metacyclic trypomastigotes, some of these forms have extremely long or truncated posterior ends [35]. The infective metacyclics are very small and do not divide.

Transmission of *T. congolense* into susceptible host is mainly through infected tsetse bites, the reported mechanical transmission have only based on controlled environment [1] and therefore, there is no evidence of mechanical transmission under natural conditions. Following the tsetse bite, few numbers of metacyclic variable antigenic types (M-VATs) will be at the inoculation site leading to raised nodule known as "chancre". Not all isolates of *T. congolense* will lead to formation of chancre. For instance, certain forms of *T. congolense* Kilifi did not induce chancre while isolates from Serengeti where observed to induce chancre formation [36]. Nevertheless, a tremendous multiplication of the parasite do occur with subsequent increase in M-VATs that is essential for survivorship of the parasite and establishment of the infection in the host [13]. After four or five days post infection the trypanosomes will get into the circulation through lymphatic vessels [13].

In the circulation, the parasite continues to multiply until a certain density level where multiplication ceases by the process known as density dependent quorum sensing. Meanwhile, trypanosomes covered by the dominant Variable Surface Glycoprotein (VSG) will be eliminated by antibody mediated immune response. However, residual variable antigenic type (VATs) will give rise to another wave of parasitaemia. This is the mechanism that ensure survivorship and transmissibility by the tsetse fly vector [37].



Source: [34]

Figure 1: The general summary of the lifecycle of Trypanosomes showing the stages involved in tsetse and Mammals.

4. Virulence

There is a marked variation in the virulence between and within *T. congolense* types. A study by [38] showed that cysteine proteinases (CP1 & CP2) were responsible for the pathology and hence virulence in *T. congolense* infection. Later, CP2 was found to be specific for *T. congolense* and was characterised as congopain [39]. Rodrigues and collegues [23] observed a significant variability of congopain among *T. congolense* Savannah, Riverine-forest and Kilifi types with extensive polymorphism within Savannah, moderate polymorphism within Riverine-forest and relative homogeneity within Kilifi. Interestingly, virulence in isolates of the same species is enhanced by sexual recombination (mating) that gave rise to genetic diversity even within a population from a single endemic focus [9].

Throughout the literature, studies have identified *T. congolense* strains within the Savannah type to be the most pathogenic whereas riverine-forest and Kilifi types were indicated as moderate and low pathogenicity respectively [40].

For instance, variations in the virulence have been detected in *T. congolense* Savannah strains isolated from cattle in eastern Zambia, including identification of extremely virulent strains [41]. In another study, all three genetically distinct types produced acute infection in the Balb/c mice but chronic in Clun sheep and large white pigs [42]. In addition, the riverine-forest type resulted in a more severe disease in mice as compared to the two indicating that pathogenicity is attributed to strain variability within *T. congolense* types [42], and the susceptibility of host species. For instance, riverine-forest type is considered to be refractory in cattle. While riverine-forest type was only present in the vectors, savannah type was predominant in tsetse flies as well as in cattle in the epidemiological survey in the West Africa [43]. Furthermore, cattle that were infected with *T. congolense* riverine-forest and kilifi strains were able to clear the parasites without receiving treatment as opposed to those infected with savannah strain [29,44].

Therefore, it can be concluded that virulence within *T. congolense* types could be attributed to polymorphism of congopain, host species susceptibility and genetic recombination of the parasites within a defined population. The higher the virulence in *T. congolense* strains the higher the chances for its transmissibility and hence its maintenance in the field [41]. However, experience has shown that, the virulence of extremely virulent *T. congolense* strains decreases when co-infected with a less virulent strain [45]. Such phenomenon could be attributed to genetic recombination and diversity of the parasite. Interestingly, the severity and infection outcome in animals may depends on the variant circulating in a particular population that is rendered highly pathogenic upon interactions with different hosts and vector [22]. More precisely, at the wildlife-livestock interface where livestock near the park are severely affected than those at distant [46].

5. Transmissibility

It is clear that virulence of the parasite is positively associated with increased transmissibility [41]. Based on such phenomenon, there is no doubt that *T. congolense* Savannah type has high transmissibility than other types when picked up by tsetse fly [47]. Generally, not all trypanosomes picked up by tsetse fly can successful develop to metacyclic trypomastigotes. It is shown that, despite of high incidence of trypanosomosis in mammals in sub-Saharan Africa; tsetse captured in the wild population have shown to possess a relatively low midgut trypanosomes infection in the range of 2–20 % in different tsetse species and sample sites [48]. In one hand, studies in *T. b. brucei* have shown that stumpy forms can successful survive and grow to infective stage in tsetse whereby the slender forms are digested with proteases [49,50]. Furthermore, stumpy forms are more abundant at peak parasitaemia in *T. b. brucei* infection, indicating that more transmissible forms exist at peak parasitaemia [50]. On the other hand, *T. congolense* being monormophic does not possess the stumpy forms. Likewise, higher *T. congolense* parasitaemia did not correlate with increased transmissibility in tsetse flies [51]. Interestingly, in contrast to *T. b. brucei* and *T. vivax*, the transmissible forms of *T. congolense* have shown to occur in both ascending and peak population [37]. With the exception of few studies, several studies within sub-Saharan Africa have indicated high prevalence of *T. congolense* in tsetse vector [9,31,52]. While *T. vivax* is more transmissible than *T. congolense*, it is hereby suggested that availability of *T. congolense* transmissible forms in ascending and peak parasitaemia could account for its high prevalence in tsetse flies. However, other contributing factors include levels of trypanosome resistance to trypanocidal drugs, stage of infection in the host and bloodmeal type [48]. For instance, it was shown that high infection rates of isogenic clones of *T. congolense* in *G. m. morsitans* was attributed to high level of resistance to isometamidium chloride [53]. On the other hand, a significantly higher infection rate of tsetse midgut was observed when tsetse were fed from mice with acute than in chronic phase independent of parasitaemia level [54]. Nevertheless, *G. m. centralis* fed with infected goat or pig blood had higher rate of infected midgut in contrast to flies fed with blood from other mammals [55]. Therefore, it is suggested that, in a particular ecological environment where different susceptible host species exist for example goats and cattle there could be high transmissibility of *T. congolense* in tsetse flies, however, it might depend on tsetse type. *Morsitans* - group tsetse are more susceptible to *T. congolense* infection than *palpalis* group. Reifenberg and colleagues [56] showed that cyclical development of clones of both *T. congolense* savannah and riverine-forest types were arrested in the midgut of most tsetse belonging to the *palpalis* group whereby completed the developmental cycle was acquired in morsitans group.

6. Clinical Presentation

In contrast to other species of trypanosomes, *T. congolense* exhibit different clinical manifestation in infected hosts with severity of disease varying depending on the number of factors. These include host and trypanosomes factors. The host factors include the type of animal species, breed and the immune status [57]. For instance, N'Dama breed were shown to develop less severe form of disease than Boran after sequential challenge with *T. congolense* [58]. Very few studies have been done in small ruminants, however, a study in Sudan showed that *T. congolense* developed a chronic form of disease in goats and in some cases became a source of acute infection in cattle [59].

Nevertheless, the trypanosome factors may include strain virulence and genetic variability within a defined population, and whether co-infected [45,60]. The virulent isolates of T. congolense were shown to induce acute disease and high mortality while less virulent strains caused benign and chronic infection [60].

Generally, the clinical signs of T. congolense infected animals are specific and include intermittent fever, abortion, cachexia, anaemia, lymphadenopathy, lethargy, anorexia, oedema of the throat, ventrum and forelimbs, ocular discharge and eventually death [8]. Other symptoms includes the history of premature birth and prenatal losses as such was observed in goats [61]. In some cases, distension of the abdomen in dogs may be a prominent feature particularly as a result of ascites and probably hepato-splenomegaly in advanced stage of the disease [62,63].

On the other hand, the disease caused by Forest type in cattle is of low pathogenicity with mild symptoms, anaemia is present at the earlier stages of the disease but cattle reported to self-cured the infection after three months [44] which means cattle have the ability to eliminate this trypanosome. The same applies for Kilifi type that caused asymptomatic disease and no major alteration in pack cell volume (PCV) and leucocytes count [44].

7. Diagnosis

Precisely, diagnosis is defined as methods for detecting infection through identifying the aetiological agent or interpretation of reactions of immunological tests. Normally as rule of thumb, initial diagnosis is based on clinical signs and symptoms, and through demonstration of the causative agent or reactions to diagnostic tests. The demonstration of *T. congolense* in the peripheral blood is readily important during the early infection than in chronic or latter stages of the disease [64]. It is already known that *T. congolense* is an intravascular parasite with much of the parasite occupying the blood capillaries. Therefore, the blood smear should be taken from small veins preferably early in the morning as it may increase chances of detecting the parasites [65] . This is because, the average concentration of *T. congolense* in the ear vessels was found to be inversely proportional to the amount of blood passing through in unit time to

the temperature [65]. On the other hand, although diurnal had little variation, *T. congolense* was detected easily from blood collected from ear than jugular veins [66].

In addition, trypanosomes can be detected through aspiration of chancre several days post tsetse bite [14]. This can be possible if animals are examined in earlier days after introduced in the area known to be tsetse infested. However, this kind of diagnostic procedure may not be reliable in areas where forest type is circulating as some forest strains do not induce chancre formation.

On the other hand, examination of lymph is not a promising efficient tool although in the chronic cases has regarded by some as useful means of diagnosis [64]. More importantly, anaemia is a major clinical sign in AAT caused by T. congolense infection, when correlated with ecological conditions might provide a tentative diagnosis.

Immunological methods includes enzyme linked immunosorbent assay (ELISA) which is a very reliable method, and easy to use in the field. However, can only detect anti-trypanosomal antibody and does not determine whether infection is the current or past, this is because antibodies can persist longer even after the parasite has been removed [67].

On the other hand, the molecular method is based on the detection and or amplification of nucleic acid, such technique include polymerase chain reaction and loop-mediated isothermal amplification (LAMP) [24,68–70]. These methods exploit the existence of a 177 bp repetitive sequence in the trypanosomes genome, a set of six primers have been used for differentiation of members of Nannomonas subgenus [68].

Other diagnostic methods that have been developed include restriction fragment length polymorphism (RFLP) [71], randomly amplified polymorphism DNA (RAPD) and amplified fragment length polymorphism (AFLP) [72]. Recently, ITS (Internal Transcribed Spacer) of ribosomal DNA repeating units have been used for the species-specific diagnostics of trypanosomes including T. congolense. It allows with one set of primers to distinguish most of African trypanosome species in a single PCR reaction based on the size polymorphism [73].

8. Pathological Findings

Pathological changes of the animal died of AAT caused by *T. congolense* are however not pathognomonic. There is paucity of knowledge on the changes induced by each particular *T. congolense* types in animals. The pathological lesions presented here are from infection due to savannah type in cattle since the other two have low pathogenicity and in most cases undergo self-cured especially in cattle [44]. The carcass is emaciated as evidenced by sunken eyes, prominent vertebrae and ribs and the tuber ischii become prominent with the wastage of the gluteal and crural muscles. The haircoat is lustress and there is starry enlargement of

all body lymph nodes, haemorrhages of superficial lymph nodes particularly prescapular and the mandibular. There is haemorrhagic fluid in the plural cavity and the heart has shown to consistently lose its parenchyma tissue. Other lesions are enlarged liver which may be accompanied with congestion and some signs of necrosis. The kidney size was normal with some necrosis in the renal cortex. There might be thick fluid in the bronchus and the trachea.

9. Treatment

Chemotherapy and chemoprophylaxis has been a central component of AAT control and hence *T. congolense* infection for many decades [74]. Two drugs are currently available for control of AAT which are diminazine diaceturate and isometamedium chloride. Diminazene diaceturate has been used therapeutically only due to its rapid metabolism and excretion [75]. The recommended therapeutic dose is 3.5 mg/Kg body weight (7 mg Kg^{-1} may be recommended for resistance strains) administered intramuscular or subcutaneous injections [74]. On the other hand, isometamidium chloride is rapidly cleared from the plasma to very low concentrations and accumulates in tissues from which it can be released slowly to the circulation to exert its activity [76]. The drug is used both therapeutically and prophylactically. Depending on the dosage of drug, species and strains of trypanosomes; it can offer prophylaxis for the period of about 1–5 months [76].

10. Prognosis

T. congolense was reported to became refractory to diminazene treatment when issued on day 19 rather than 24 hours post-infection [75]. Therefore, early detection of the disease and prompt treatment could normally lead to good recovery.

11. Challenges and Opportunities for *T. Congolense* Control

Privatization of veterinary services in 1980s and 1990s in most African countries left many livestock keepers to administer chemotherapy in absence of veterinary professionals [77]. This, in some instances has lead to misuse of trypanocidal drugs by farmers and contributing to wide-spread treatment failure [78]. Considering the fact that drug to treat AAT are over than 50 years in the market coupling with the highly needed safe and effective drugs for HAT, the need of new trypanocidal agents are urgently needed. One of the opportunities is that *T. congolense* expresses specific surface proteins, lectin-like glycoproteins (TcoClecs) that are involved in its parasitic lifestyle which have shown to be suppressed with trypsin [79], drugs that could target this protein is a most welcome. Nevertheless, plants have always been a frequent source of medicaments either in form of traditional preparations or as active principles. In the recent past, pioneering screening work on various plants [80–82], have shown that many have promising *in vitro* and or *in vivo* trypanocidal activity potential. It is now more than 20 years of anti-trypanosomal research from plant sources in Africa. There is yet a realistic molecule(s)

that is to be subjected to vigorous clinical trials leading to useful agent that can help fight trypanosomosis. Most studies have ended either *in vitro* and or *in vivo* studies. There is a need of conducting more research to turn the promising anti-trypanosomal compounds into useful product(s).

On the other hand, most livestock keepers in the affected regions have limited access to quick diagnostic methods for early detection of the disease. They frequently rely on clinical signs that are often not pathognomonic. Therefore, simple and easy to use field diagnostic tools could play an important part in the control of disease and minimize risks associated with AAT.

12. Conclusion

Although there is distinct variability between genetic types of *T. congolense* with some known to cause moderate to low disease phenotype. *T. congolense* savannah type is the most important pathogenic trypanosome species in the sub-Saharan Africa. Much is needed in order to control this parasite.

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Chapter 3

West Nile Virus: An Emerging and Reemerging Infectious Disease

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Abstract

West Nile Virus (WNV), a flavivirus of the *Flaviviridae* family, is maintained in nature in an enzootic transmission cycle between birds and ornithophilic mosquito vectors, although the virus rarely infects other vertebrates. WNV causes disease in horses and humans, which develops febrile illness, meningitis, encephalitis and flaccid paralysis. Until recently, its medical and veterinary health concern was relatively low; however, the number, and severity of outbreaks with neurological consequences in humans and horses have increased in European countries and the Mediterranean basin. Since its introduction in the America, the virus spread across the continent with worrisome consequences in bird mortality and a considerable number of outbreaks among humans and horses, which have developed in the largest epidemics of neuroinvasive WNV disease ever documented. Even great advances have been obtained lately regarding WNV infection, and although efficient equine vaccines are available, no specific treatments or vaccines available for human use. This review updates the most recent investigations of WNV particularly pathogenesis, transmission dynamics, host range, clinical symptoms, epidemiology, diagnosis, control, and prevention, and highlights some approaches that certainly require further research.

1. Introduction

West Nile virus (WNV) is a neuroinvasive human pathogen that is the causative agent of WNV in human and horses [1,2]. In 2000, the WNV spread out in 12 states and the Columbia District of U.S. WNV infect many avian and mosquito species throughout North America [3,4]. More than 2.5 million people affected by West Nile fever and encephalitis between 1999 to 2010. The outbreaks of West Nile virus occurred in U.S, Israel, Egypt, India, France, and South Africa. West Nile virus belongs to the family *Flaviviridae.* The family *Flaviviridae* comprises 3 genera: the flaviviruses, which contain WNV, dengue Virus (DENV), and yellow fever virus (YFV); the hepaciviruses, which contain hepatitis B and C viruses; and the pestiviruses, which causes encephalitis in hoofed mammals. Within the *Flavivirus* genus, which contains more than 70 viruses, viruses can be further classified into tick-borne and mosquito-borne viruses. The mosquito-borne viruses classified into the encephalitic clade, or the JE serocomplex, which contain WNV and Japanese Encephalitis Virus (JEV), and the nonencephalitic or hemorrhagic fever clade, which contain DENV and YFV, and there are 10 serologic/genetic complexes [5,6,7]. The global distribution of the mosquito-borne flaviviruses depends on the habitat of the mosquito vector. *Culex* mosquitoes transmitting encephalitic flaviviruses mostly in the Northern Hemisphere.

2. Structure of the Virus

WNV is an enveloped virus having a single-stranded, positive- sense RNA genome. The genome comprises of a single open reading frame of about 11 kb length. Both the 5' and 3' noncoding regions of the genome synthesize stem-loop structures that help in replication, transcription, translation, and packaging [8,9,10]. The West Nile RNA directly translated in to a single polypeptide and then cleaved by host and viral proteases. Followed by the formation of three structural (capsid, envelope, and premembrane) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins. Structural proteins are essential for entry of virus in to host cell and also aid in fusion encapsidation of the viral RNA during assembly **[Figure 1]**.

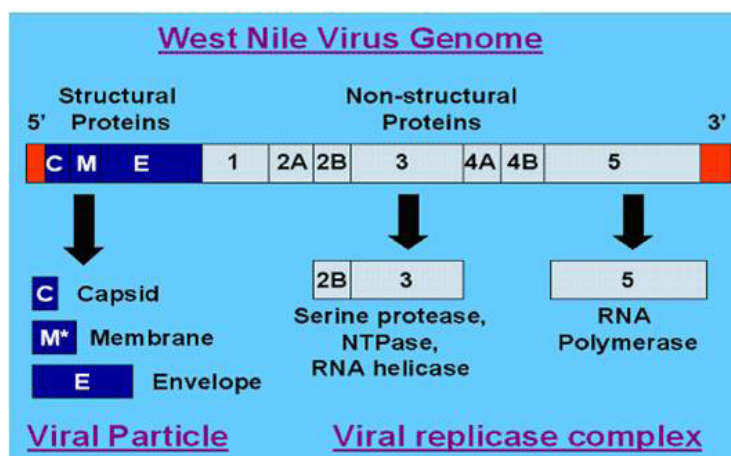

Figure 1: Schematic of genomic Organization of West Nile Virus .The author acknowledge the support from; http://cme.cwru.edu/brochures/2003/WESTNILE

www.openaccessebooks.com

The nonstructural proteins play important role and perform several functions. NS1 protein has "cellular" as well as secreted form and has highly immunogenic but do not play important role during encapsulation and packaging, but it plays significant role in replication of viral genome [11,12,13,14]. NS3 protein suggested to play important role in cleaving of viral polyproteins with the aid of the viral proteases and encodes enzyme activities. The NS5 protein is essential for viral replication because it has viral polymerase activity and encodes a methyltransferase. Other nonstructural proteins, including NS2A, NS2B, NS4A, and NS4B, have been shown to prevent several components of the innate immune system against viral infection [15,16,17]. West Nile virion is an icosahedral particle. The capsid protein associating with the viral genome to form the nucleocapsid and lipid bilayer is present outside of the nucleocapsid. A high ratio of capsid protein localizes to the nucleus, while viral assembly always occurs in the cytoplasm. Budding is takes place in the endoplasmic reticulum (ER) [18,19,20]. Although inside the nucleus functions of capsid are not fully known, recent researches suggests a role in gene regulation. During virus assembly, the envelope protein enclosed firmly in the lipid bilayer of the virus and display to the virion surface [21,22,23,24]. The envelope protein is necessary for binding to the receptor on the cell surface for viral entry. The prM protein is also known to enclosed firmly in the lipid bilayer. At the time of infection, the virus population comprises both mature and immature virion particles [25].

3. Life Cycle

WNV enters in to the host cell through receptor-mediated endocytosis process. Several molecules have been serves as receptors for West Nile virus, like DC-SIGN, mannose receptor, and several glycosaminoglycans etc. Endosome of the virus matures during internalization from the cell surface, with the pH decreases down from neutral to slightly acidic in the early endosome and having more acidic at the time of maturation in to the late endosome. Inside the late endosome, the envelope protein changes their conformation and then viral lipid membrane fuse with the endocytic membrane and viral RNA genome releases into the cell cytosol. After capsid disassociation, the viral genome is replicated and assembled. The viral polyprotein is translated and processed on intracellular membranes of cell organelles and expression of the 10 viral proteins occurs. The viral genome is replicated with the help of viral and cellular proteins [26].

Immature flavivirus particles also play significant role during infection. Thes immature flavivirus particles form during inefficient cleavage of the prM protein at the time of maturation and budding. Immature flavivirus particles were traditionally thought to be noninfectious, several reports have shown that immature WNV particles may be potentially immunogenic and infectious *in vitro* and *in vivo* when linked by antibodies against the E or prM protein. These antibody-linked immature virus particles penetrate the immune cells via the Fc receptor of the antibody resulting infection occurs [27] **[Figure 2]**.

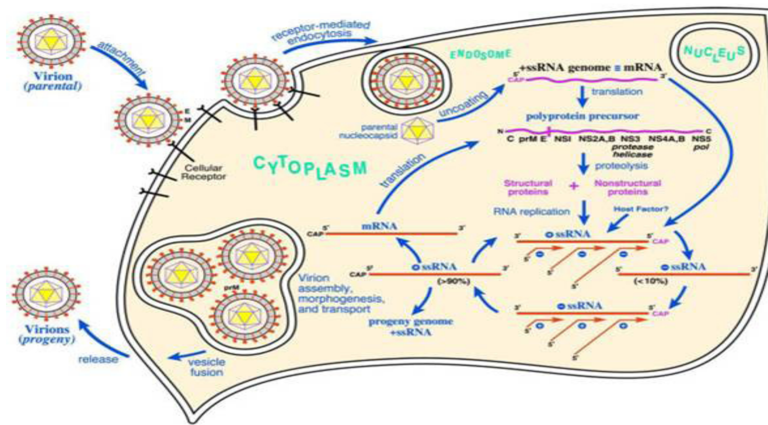

Figure 2: The West Nile virus life cycle. During viral entry, the E protein interacts with one or more cell surface receptor (s). It is not completely clear which cellular receptors are involved in WNV binding, however DC-SIGN, alphaVbeta3 integrin and laminin-binding protein have been reported as potential receptors. After binding to the cell, the virus is taken up via clathrin-mediated endocytosis and in the acidified endosome the E protein undergoes conformational changes resulting in fusion between the viral and cellular membranes. After the fusion event the positive-stranded RNA genome is released into the cytoplasm of the cell. The viral RNA is translated into a single polyprotein, which is proteolytically processed to yield three structural proteins (the envelope protein E; the membrane precursor protein prM; and the capsid protein C) and seven Non-Structural (NS) proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5). Whereas the cleavages at the junctions C-prM, prM-E, E-NS1, NS4A-NS4B, and likely also NS1-NS2A, are performed by the host signal peptidase located within the lumen of the ER, the remaining peptide bonds are cleaved by the virus encoded NS3 protease. Flaviviruses replication requires the viral protein NS5, which is an RNA-dependent RNA polymerase. An "antisense" negative strand RNA is produced by this enzyme, which then serves as a template for the synthesis of many new copies of the infectious positive strand RNA genome. The author acknowledge CDC to obtain this image.

4. Host Reservoirs

WNV is survive in nature in a cycle between mosquitoes and animal hosts. Some species like birds show symptoms of disease, and may die, while others become only infected do not show symptoms and act as carriers [28,29,30]. Although house sparrows and crows are play minor role in transmission. The American robin play important role in transmission of WNV in the United States [31,32]. Humans are considered as "dead-end" hosts for WNV, as the low level of viremia in mammals is usually not sufficient to be transmitted to mosquitoes, thereby ending the transmission cycle. The ability of mammals to serves as hosts *Aedes* mosquitoes, feed primarily on humans, become primary transmission vectors for WNV [33,34] **[Figure 3]**.

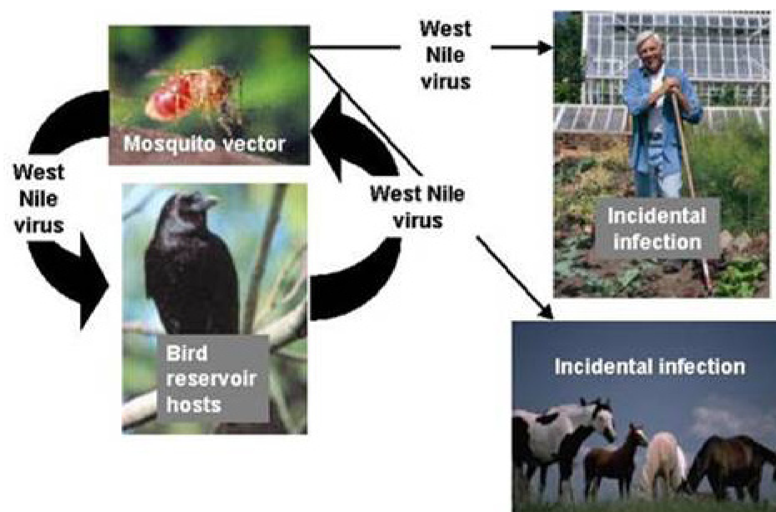

Figure 3: Transmission cycle of West Nile Virus The author also thankful to CDC to get above image

5. **Vector Response to Infection**

There have been many recent discoveries purposed at clarifying the transcriptomic and proteomic response to flavivirus infection in the mosquito vector. WNV demonstrate a consistent infection in mosquito cells *in vitro* and in live mosquitoes, there is growing evidence that the mosquito does show some immune responses against virus infection. Reports about the insect immune system come from experiments with *Drosophila melanogaster*, though recent examination of the mosquito immune responses is starting to expose related proteins and pathways [35,36,37]. The mosquito antiviral response is include two pathways: the innate immune pathway and the RNA interference (RNAi) pathway. The innate immune response is included of three signaling pathways: Toll, JAK-STAT, and IMD. The Toll and IMD pathways both ends in NFkB-mediated expression of antimicrobial peptides (AMPs) and IMD signaling has been shown to prevent RNA virus infection in *Drosophila* [38,39,40]. Not much is known about the role of mosquito AMPs in antiviral immunity. Their expression is induced by viral infection. Both Toll signaling and the JAK-STAT pathways have been show significant role in the inhibition of DENV infection in *Aedes aegypti* [52,53] and may also be significant at the time of infection of *Culex* with WNV. The RNAi pathway in mosquitoes is both activated by viral ds RNA and has been shown to be crucial for preventing alphavirus infection in *Aedes* and *Anopheles* [41,42,43]. The RNAi pathway is induced at the time of WNV infection in *Culex pipiens* [44]. Infection with DENV was also found to actively control mosquito immune responses *in vitro* [45]. Evidence for a transcriptomic approach of flavivirus infection was found at the time of a comprehensive study of *Aedes aegypti* infected with WNV, DENV, and YFV [46].

Genes play significant role in transcription and ion binding. There is upregulation and down regulation of genes takes place here and genes coding for proteases and cuticle proteins were to be up regulated at the time of infection with all three viruses. Serine proteases play significant role during viral propagation and blood digestion, although there have been several studies related their effect on flaviviral infection in the mosquito [47,48]. Another report of flaviviral infection in *Drosophila* recognized several insect host factors relevant at the time of dengue virus infection in the mosquito. In regarding to WNV infection, a recent transcriptomic analysis of *Culex quinquefasciatus* exposed that several genes takes part in metabolism and transport are upregulated at the time of infection [49,50]. The virus infect a variety of cell types and organs in the mosquito vector, and other host factors which play significant role in WNV infection of the mosquito that have yet to be reported.

West Nile (WNV) virus is mosquito-transmitted flavivirus that cause significant morbidity and mortality worldwide. Disease severity and pathogenesis of WNV infection in humans depend on many factors, including pre-existing immunity, strain virulence, host genetics and virus–host interactions [51,52,53]. Among the flavivirus-host interactions, viral evasion of type

I interferon (IFN)-mediated innate immunity has a critical role in modulating pathogenesis. DENV and WNV have evolved effective mechanisms to evade immune surveillance pathways that lead to IFN induction and to block signaling downstream of the IFN-α/β receptor. Molecular mechanisms of DENV and WNV antagonize the type I IFN response in human cells [54,55].

6. Molecular Classification

Classifications of WNV were depends on cross-neutralization reactions and showed that WNV is a family member of the Japanese encephalitis virus serocomplex. Recent studies on molecular phylogeny suggest this antigenic classification and expose the presence of up to eight different genetic lineages of WNV. Lineage 1 is further divided into three clades. Clade 1a consists of African, European and American isolates; clade 1b groups the Australian Kunjin Virus (KUNV), which has been reveal a subtype of WNV and clade 1c clusters isolates from India. Clade 1a reveal close genetic relationships between globally different areas which are suggested to be the result of WNV transmission *via* migratory birds WNV inside clade 1a can be further classified into various clusters. The only one endemic genotype has been identified in India (1c) and one in Australia (1b), suggests that WNV was spread into these areas only once, as well as it occurred in the American continent, where WNV was endemic in 1999 in the East Cost of the US [56].

The first North American WNV isolate was show relevancy to a strain isolated from a dead goose in Israel (lineage 1) North American WNV was originated from this epidemic in the year 1998 outbreak. However, recent studies suggest that the 1998 Israel epidemic was not the directly relevant of North American epidemics, but rather that both epidemics derived from the same (unknown) areas . Lineage 2 initially consists of WNV strains only identified in Africa and Madagascar, which have been speculated to be less neuroinvasive than those consists in lineage 1. Recent outbreaks occurs in Europe (Austria, Hungary and Greece) have been related to lineage 2 strains. Other lineages of WNV of unidentified human pathogenicity due to lineage 3 (Rabensburg isolate 97-103), isolated from *Culex pipiens* mosquitoes in the Czech Republic in 1997 and Lineage 4 (LEIVKrnd88-190), isolated from *Dermacentor marginatus* ticks in 1998 in Russia. It has been suggested that WNV Indian isolates that were classified as linage 1c forms a new cluster termed lineage 5. A new lineage of WNV (strain HU2925/06) that includes evolutionary branch with lineage 4 has been recently reported in Spain [57] **[Figure 4]**.

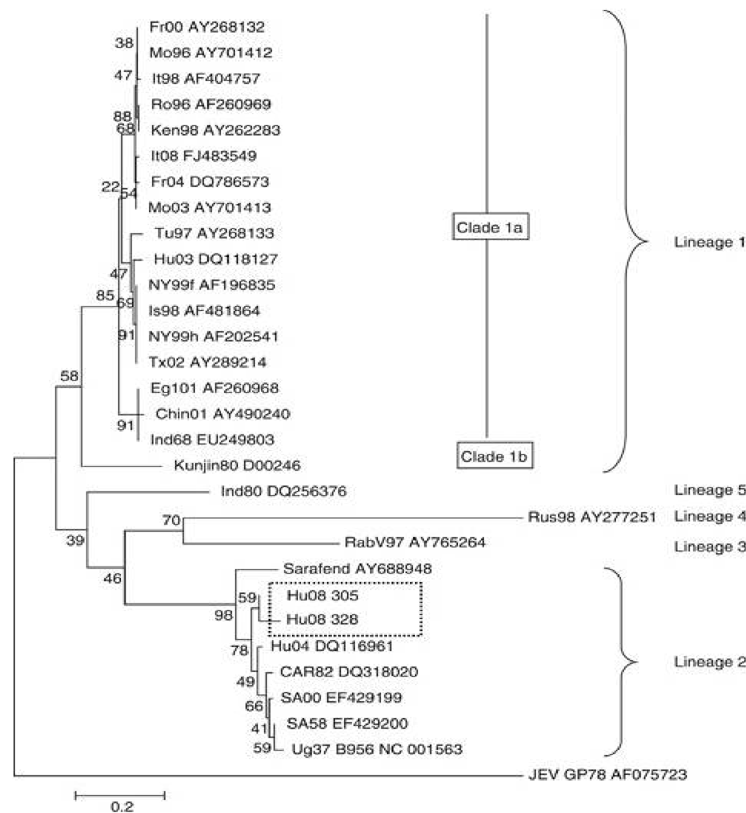

Figure 4: Phylogenetic tree of West Nile virus strains based on a 282nt fragment of the Envelope gene. The tree was constructed with the program MEGA (Molecular Evolutionary Genetic Analysis) by neighbor-joining. Bootstrap confidence level (1,000 replicates) and a confidence probability value based on the standard error test were calculated by MEGA. WNV strains are named according to the following rules: a set of letters corresponding to the place where the strain was isolated (Fr, France; Mo, Morocco; It, Italy; Ro, Romania; Ken, Kenya; Tu, Tunisia; Hu, Hungary; NY, New York; Is, Israel; Tx, Texas; Eg, Egypt; Chin, China; Ind, India; Rus, Russia; Rab, Rabensburg; CAR, Central African Republic; SA, South Africa, Ug, Uganda), 2 numbers for the isolation year (ex: 00 = 2000, 96 = 1996), and GenBank accession number. Sequences obtained from the 2 horse samples in Hungary 2008 are highlighted (rectangle). JEV, a close flavivirus, was used to root the phylogenetic tree. The author thanks to research gate for this image.

7. Molecular Epidemiology

The WNV outbreak occurred in North America in the late summer of 1999 in New York City due to this outbreak of mosquito borne encephalitis death occurred of humans, birds, and horses [58]. WNV transport throughout the United States and into Canada, Mexico, and the Caribbean **[Figure 5]**. From 1999 to 2016, 46,086 cases were reported to the CDC, with 2,017 (4%) deaths, and 21,574 reported cases of neuroinvasive disease, with 1,888 (9%) deaths [Figure 6]. As in most cases the virus is spread by the *Culex* mosquito vector. WNV transmission may occur through blood transfusion, organ transplantation, and laboratory-acquired infection has also been reported. The first reported Acute Encephalitis Syndrome (AES) outbreak in Kerala, India, occurred in Kuttanad region between January and February 1996, causing 105 cases and 31 deaths. In India, presence of West Nile antibodies in humans was first reported from Bombay (now Mumbai) by Banker in 1952. Smithburn et al confirmed the report by detecting the WNV neutralizing antibodies. During a post sero-epidemiological study, detected WNV neutralizing antibodies among humans at South Arcot district of Tamil Nadu. WNV has been isolated from sporadic cases of encephalitis and mosquitoes. Work postulated a hypothesis of a zoogeographical interface of Japanese encephalitis and West Nile virus. The hypothesis

proposed the intermingling distribution of JEV and WNV at the south Indian peninsular region [59].

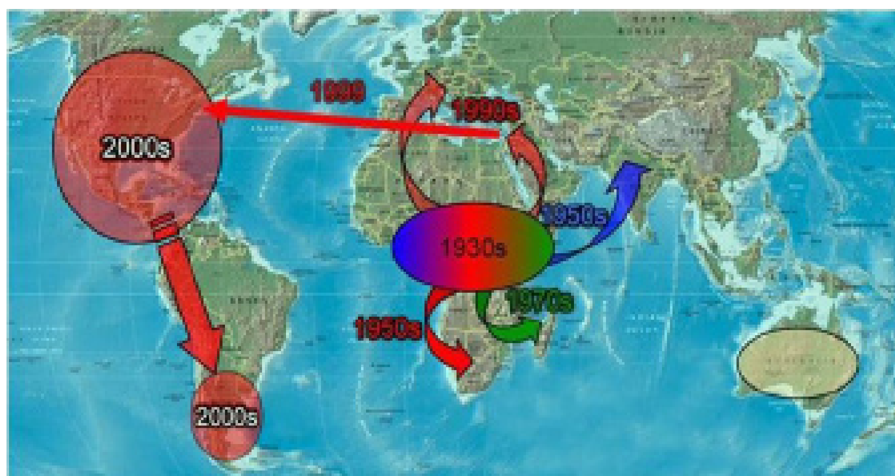

Figure 5: Worldwide outbreaks of West Nile virus. The authors also thankfully acknowledge the ILRI Clippings for this image.

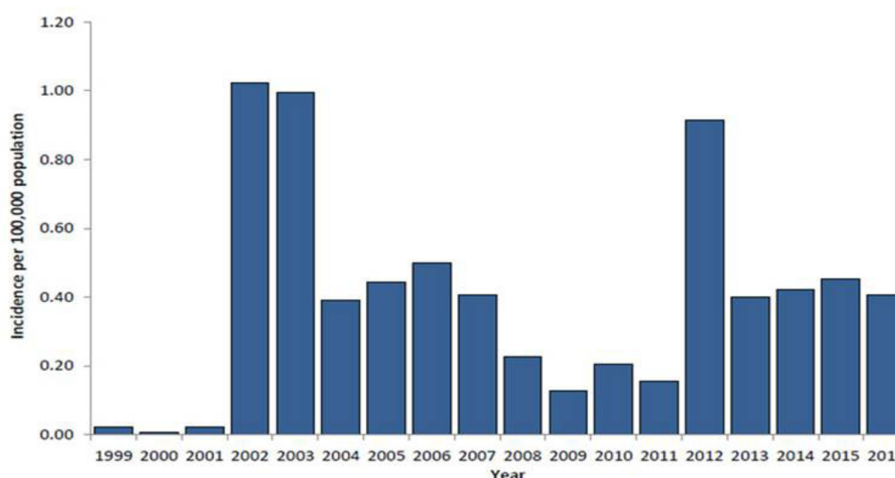

Figure 6: West Nile virus neuroinvasive disease incidence reported to CDC by year, 1999-2016. The author acknowledge the CDC to get above mentioned information

8. Clinical Features

Infections in humans are mainly subclinical, but reported infection demonstration may range from fever and CNS affected in encephalitis causes death. Encephalitis occurs only in a small subset of patients; concatenation to chronic neurological condition may induce acute paralysis after encephalitis. Individuals with neurological involvement that may result in death and may have high risk of mortality after acute illness. In about 75% of infections people have few or no symptoms. About 20% of people develop a fever, headache, vomiting, or a rash. In less than 1% of people, encephalitis or meningitis occurs, with associated neck stiffness, confusion, or seizures. Recovery may take weeks to months. The risk of death among those in whom the nervous system is affected is about 10%. Among people over 70 years of age, the case-severity rate ranges from 15% to 29% [60-62]. Higher mortality is also seen in infected infants and in immunocompromised patients. Risk factors for encephalitis are cardiovascular disease or chronic renal disease, hepatitis C virus infection, and immunosuppression. In some cases convalescent patients may have persistent or severe infection identified through molecular

based assays like PCR of the urine, which suggested ongoing viral replication in kidney tissues [63,64].

9. Pathogenesis

WNV is a neuroinvasive disease involve in progression of meningitis or encephalitis to poliomyelitis- like condition with acute flaccid paralysis that can also cause respiratory troubles. It is estimated that 10% cases are fatal [65,66]. Older age individuals are at high risk of infection but neuroinvasive diseases have been also reported in young people and children. Hypertension and diabetes are considered as potential risk factor for severe WND, Immunocompromised people easily infected by this disease. A fully functional immune (innate and adaptive) response (humoral and cellular) has been shown to be necessary to compete with WNV infection in animal models. It is well familiar that overall, humoral immune response has capable of control viral load while T-cell mediated response is necessary for clearance of the virus from the Central Nervous System (CNS). WNV is able to infect neurons of the CNS, brain, stem and spinal cord [67,68].

The pathogenesis of WNV infection is similar to that of other Flaviviruses. After primary inoculation, WNV is replicate in skin Langerhans dendritic cells before it travels to the lymph nodes and blood stream from where it transfers to the spleen and kidneys and, finally reaches to the CNS resulting in inflammation of the medulla, brain stem and spinal cord. The Viral entry mechanism in CNS is yet to be fully elucidated. The main mechanisms include: *via* leukocytes, direct entry across the brain barrier [69,70].

As several viruses, WNV has developed different mechanisms to block the action of immune system like it inhibit Interferon gamma (IFN) and, thus, to escape the host antiviral activity of IFN-stimulating genes. Different reports suggest that nonstructural proteins NS1, NS2A, NS4B and NS5 play important role to control IFN α/β signaling by several pathways [71,72].

10. Laboratory Diagnostics

Routine laboratory diagnosis of WNV infection is primarily based on serodiagnosis, followed by virus isolation and identification. Serologically, WNV infection can be inferred by immunoglobulin M (IgM) and immunoglobulin G (IgG) capture ELISA. Recently several investigators have reported PCR-based detection systems for the rapid diagnosis of WNV infection in clinical specimens that are negative for virus isolation, suggesting that nucleic acid-based assays hold great promise for the diagnosis of WNV infection. In addition, other PCR-based methods, like Reverse transcription loop-mediated isothermal gene amplification (RT-LAMP) assay, have been developed for the diagnosis of WNV RNA [73].

10.1. Immunological diagnosis

The detection of WNV infection is mainly based on clinical criteria and testing for antibody responses. The incubation period for WNV infection is about 2 to 14 days. The anti-WNV IgM mainly from Cerebrospinal Fluid (CSF), is used for detection. Cross-reactivity with related flaviviruses (Japanese encephalitis virus, St. Louis encephalitis virus, YFV, and DENV), if suspected, can be accessed through plaque neutralization assays (PRNT). Replication of WNV has been reported in human monocytes *in vitro* and with higher efficiency in polymorphonuclear leukocytes; this could cause transmission via blood transfusion [74,75]. Thus many rapid diagnostics have been developed for blood donor testing using Nucleic Acid Testing (NAT), an amplification-based transcription technique, which detects WNV-infected individuals before they become symptomatic [76,77]. RNA mostly became undetectable after 13.2 days, although it was rarely found to persist for 40 days. IgM and IgA antibodies decreases significantly, while the IgG level left elevated for 1 year after detection of viremia. Antibody to WNV NS5 persists *in vivo*, and thus NS5 antibody cannot be used to differentiate recent from old WNV infection [78,79].

10.2. Molecular diagnosis

Several methods for identification of viral RNA have been used for WNV surveillance. Generally Reverse Transcription Polymerase Chain Reaction (RT-PCR) technique, quantitative real-time RT-PCR and Isothermal gene amplification techniques like LAMP that is one-step, single tube, cost effective real-time reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay was developed for identifying the West Nile (WN) virus [80]. The RT-LAMP assay is a novel technique of nucleic acid amplification that is highly specific, sensitive, rapid and cost effective under isothermal conditions with a set of six specially designed primers that recognize eight distinct regions of the target. The complete process is very simple and rapid, and amplification can be occur in less than 1 h by incubating all of the reagents in a single tube with reverse transcriptase and *Bst* DNA polymerase at 63°C. *Bst* DNA polymerase has strand displacing activity. There are several formats for end point detection of gene amplification like agarose gel electrophoresis, real-time monitoring in an inexpensive turbidimeter, in practice, generally the visual inspection for amplification is performed through observation of color change following addition of SYBR green I dye, (a fluorescence ds intercalating dye) When the sensitivity of the RT-LAMP assay was compared to that of conventional RT-PCR, it was found that the RT-LAMP assay demonstrated 10-fold higher sensitivity compared to RT-PCR. All these assays have been extensively used in mosquito pools, animal and human samples [81,82].

11. Therapeutics and Vaccines

Advance therapeutic alternatives against WNV are mainly supportive; there are no FDA-approved vaccines or treatments available. RNA interference (RNAi) is a major pathway of antiviral defense in plants and insects. The pathway involves the processing of Double-Stranded RNA (dsRNA) into short 21–22 bp effector RNA molecules by the RNAse III domain-containing Dicer 2 enzyme. The fidelity of Dicer cleavage and downstream strand selection in the RNAi process is maintained by Dicer-associated proteins (TRBP and PACT in mammalian cells) These dsRNA serve as targeting moieties when loaded into a RISC complex to selectively downregulate mRNA targets. Argonaute proteins (e.g. Ago2) play a major role in the knock down of gene expression by the RISC complex, mediating selective endonucleolytic cleavage of target RNAs. The successful avoidance or downregulation of the RNAi machinery is vital for arboviruses to productively infect their arthropod vectors. There are several reports to identify individual susceptibility markers, recombinant antibodies, peptides, RNA interference, and small molecules with the ability to directly or indirectly neutralize WNV. Till now effective therapy is still lacking [83].

A recent approach to search for new antiviral agent candidates is the assessment of long-used drugs commonly administered by clinicians to treat human disorders, as part of drug repositioning (finding of new applications to licensed drugs). Among some of the drugs already tested as antivirals are lithium, statins, or valproic acid [84,85]. Since there is evidence supporting that WNV infection shares common points with Parkinson's disease, study was undertaken whether drugs used for the treatment of Parkinson's disease could provide novel tools for antiviral intervention. In this way, assessed the effect of four antiparkinsonian drugs (L-dopa, Selegiline, Isatin, and Amantadine) in WNV multiplication in cultured cells from different origin. L-dopa, Isatin, and Amantadine treatments significantly reduced the production of infectious virus in all cell types tested, but only Amantadine reduced viral RNA levels. This results indicates that Amantadine, as possible therapeutic candidates for the development of antiviral strategies against WNV infection.

There are recently four USDA-licensed vaccines available for equines in which two are attenuated whole WNV one is a non-replicating live canary pox recombinant vector vaccine, and another is an attenuated flavivirus chimeric vaccine. Passive immunization has been used in a some cases; Sometimes it also causes allergic reactions. A case study of two WNV encephalitis patients treated with alpha interferon, the standard of care for infection with the related flavivirus hepatitis C virus, showed significant melioration and an improved recovery course. Several strategies are being used for the advancement of a vaccine in humans that may significant for use. Reports include live inactivated vaccines, recombinant subunit vaccines, vectorized vaccines, DNA vaccines with constructs that express the WNVE protein, live recombinant vaccines, and an inactivated strain based on nonglycosylated E and mutant

NS1 protein [86]. A neutralizing WNV-specific monoclonal antibody E16 (MGAWN1), which enters in the CNS in animal models, generates neutralizing antibodies in phase I trials. Very effective results were seen with a chimeric vaccine based on the WNV prM and E proteins inserted into the yellow fever 17D vaccine moiety (ChimeriVax-WN02). It was shown to be effective, safe and immunogenic in phase II clinical trials, with high seroconversion rates, yet it is no longer available [87,88].

12. Preventive measures and control

Prevention and control of WND require strategy that includes vaccination, mosquito control and clinical management. Preventive tools are necessary for inhibiting WNV infections other than drugs and vaccines can also aid to armed the transport of the infection by avoiding mosquito bites [89]. These simple measures can be summarized under the title ‘fight the bite!’ and consists of the use of insect repellents, the removal of standing water where mosquitoes lay eggs, the reduces the outdoor activities cooccurence with the maximum activity of mosquitoes, reporting dead birds to local authorities and helping mosquito control programs [90].

13. Future Directions

WNV has now persisted and causes endemics in North America. Transmission of the mosquito vectors harboring WNV to include *Aedes albopictus*, a mammal-biting mosquito. It is believed that the increase in our knowledge of WNV with the mosquito vector will lead to new boulevard for therapeutics and preventive measures. Mosquito responses at the levels of protein and gene expression [91]. Many strategies to novel targets to concentrate our efforts to prevent or block WNV infection. For example, a single-chain human monoclonal antibody developed through phage display against the envelope protein reveled both protection and therapeutic efficacy when detect in the murine model. Current advancement in nanoparticle technology have also been used in vaccination studies of murine WNV infection and show significant efficacy of TLR9-targeted biodegradable nanoparticles, which produce a large number of circulating effector T cells and antigen-specific lymphocytes . Potency to relevant viral susceptibility mechanisms, processing host antagonism of chemokine responses as has been noted in infection with the related flavivirus hepatitis C virus.may show infectious mechanisms used by WNV. The rate of vector discovery, virus, and host molecules of pathogenesis provide critical insights for the controls and therapeutics for WNV [92].

14. Conclusion

The recent amanation and transport of WNV in America and increase in number and severity of outbreaks in Europe shown one of the major zoonotic treats in years. Although our information about WNV infection has increased in recent years, some panorama of WNV activity still essential to be further addressed: the ways by which WNV colonizes new habitats

and the role that climatic (temperature, humidity, *etc.*) factors play; the differences in WNV disease demonstration between the US and other parts of the world, mainly Central and South America; a better knowledge about WNV immunity, pathogenicity, and the factors which causes virulence; the long-term manifestations of WNV infection and the results of persistent infections; the progression of national and international surveillance programs to monitor WNV outbreaks and to take appropriate strategy to control it. The search for more efficient, rapid, and specific and sensitive diagnostic assays that can be easily adopted in all over the world; and the search for cheap human vaccines for high risk targeted populations and for new antiviral targets for therapeutic usage. Advancement on our current knowledge on WNV infection will greatly help to fight not only future transmission of WNV to habitats around the world, but also of other Flaviviruses.

15. Acknowledgment

The authors thank Dr. D. K. Dubey, Director, Defence Research and Development Establishment (DRDE), Ministry of Defence, Govt. of India for his support, constant inspiration and providing the necessary facilities for this study. This manuscript is assigned DRDE accession no. DRDE/VIRO/009/2018.

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Chapter 4

Vectorial Control Based on the General Characteristics of Phlebotomine Sand Flies

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Abstract

Sand flies (Diptera: Psychodidae: Phlebotominae) are vectors insects of zoonotic diseases, caused by protozoa such as *Leishmania* spp. Leishmaniases are diseases of great global epidemiological importance, endemic in tropical and subtropical areas of the Americas, where they constitute a significant public health problem. In this chapter we will discuss the vector control measures based on the general characteristics of phlebotomine sand flies.

Keywords: sand fly; leishmaniasis; vector control.

1. Introduction

Sand flies (Diptera: Psychodidae: Phlebotominae) are vectors insects of zoonotic diseases, or even anthropogenic diseases (in the Old World), caused by protozoa such as *Leishmania* spp. [1], bacteria, for example *Bartonella bacilliformis* or viruses belonging to three different genera: (i) the Phlebovirus [2] including sandfly fever Sicilian virus, sandfly fever Naples virus, Toscana virus and Punta Toro virus; (ii) the vesiculovirus including Chandipura virus and (iii) the orbivirus including Changuinola virus.

The Phlebovirus virus belonged to the family Phenuiviridae, order Bunyavirales and

transmitted by sand flies are widely distributed in the Mediterranean region, Africa, Indian subcontinent, Middle East and Central Asia. Being that the major viruses that are pathogenic to human health, the Sicilian Fever Virus (SFSV) and the Naples Fever Virus (SFNV) are the causative agents of transient febrile illness in humans [4], while the Toscana Virus (TOSV) can cause central nervous system infections, as occurred during the hot season in Italy, then in other Mediterranean countries (Spain, France, Portugal, Greece, Turkey, Tunisia), thus presenting an emerging health problem [5].

Pathogenesis has been reported since the early 20th century and likely new cases will continue to be observed within the local populations where phleboviruses are known. In addition, the increasing movement of humans, animals and their belongings may introduce this virus by displacing its vector, so all regions where sand flies are present should be considered as a potential risk for phlebovirus [4].

Estimates indicate the existence of 988 phlebotomine species and subspecies on all continents, except Antarctica. Among them, two genera are important carriers of human leishmaniasis, the genus Phlebotomus, found in Europe, Africa and Asia, and Lutzomyia in South and Central America [6].

Leishmaniases are endemic in tropical and subtropical areas of the Americas, where it represents a significant public health problem [7]. These parasitoses present zoonotic character and affect men and diverse species of wild and domestic animals, that can harbor several species of parasites [8].

Leishmaniasis manifests itself in three forms: visceral (kala-azar), cutaneous and mucocutaneous. Approximately one million new cases of these diseases are reported and 30,000 deaths occur annually [9].

Globally every year, 50 000 to 90 000 new cases of visceral leishmaniasis are recorded. In 2015, 90% of cases reported to WHO (World Health Organization) were observed in seven countries: Brazil, Ethiopia, India, Kenya, Somalia, South Sudan, and Sudan. However, cutaneous leishmaniasis (CL) is the most common form of leishmaniasis, causing dermatopathies. About 95% of CL cases are found in the Americas, the Mediterranean basin, the Middle East and Central Asia [6].

Thus, in this chapter vector control measures based on the general characteristics of phlebotomine sand flies will be approached.

2. Sand Flies

2.1. General Features

Sand flies are small insects, measuring from two to three millimeters, that have in their body intense pilosity and long and thin legs [10]. They are also known for their painful bites that may cause allergic reactions [11].

The period of activity of these insects begins at dusk and during the day they remain in their shelter. Their attraction to hosts is related to temperature and body odor, being eclectic in relation to their food preference [10].

2.2. Biological Cycle

Sand flies are holometabolous insects, with the phases from egg to adult varying with the food supply and climatic conditions. In most species, in the first in star larvae, a single pair of caudal bristles can be noticed, being that the others exhibit two pairs [10]. Immature stages develop in relatively humid places, with low light and are protected from severe climatic changes by layers of decaying organic materials [12].

The development time of the larvae of some species is 18 days, but this period may be prolonged for months depending on the environmental conditions [13].

After the adult emerges from the pupa, in 24 hours, the external genitalia of the males undergoes a rotation of 180º and from this point, they will be ready for copulation [10]. Adults of both sexes need carbohydrates (sugars) as a source of energy for mating, posture and infectivity of *Leishmania* spp. in their digestive tract [11]. In addition to sugars, females need blood from vertebrates for the maturation of their eggs. Some species feed only once between the postures, while others need several repasts for one oviposition cycle [10], presenting gonotrophic discordance and increasing the chance of protozoal transmission.

The longevity of adult insects in the natural environment is unknown, however, laboratory studies have shown that sand flies can survive between 20 to 30 days [10]. Regarding their dispersion distances under natural conditions, some species can reach from 243m in urban areas [14] up to 700m in a rural community [15], such is the case of *Lutzomya longipalpis.*

2.3. Habitat

The natural habitat of sand flies is characterized by a small variation of temperature and humidity, which favors their presence, since they are sensitive to desiccation. The minimum modification of these factors in microhabitats is enough to alter the population dynamics of these insects [16].

Some species reproduce in peridomestic situations and shelter in human habitation, such as the *Lutzomyia* genus that is seen in and around households and reproduces in organic waste, including manure, animal feces (chickens, rodents), garbage, rodent holes, cracks and fissures in walls with high temperature and humidity [17].

Phlebotomine larvae have already been found in samples of soil and organic matter in different microhabitats, including bases of trees, ground of open forest, soil from under fallen logs, soil of roots and bases of palm trees [18], animal burrows and shelters of domestic animals [19].

2.4. Vector Competence

The vectorial competence of a phlebotomine sand fly is observed by its capacity to become infected with the protozoan of the species of *Leishmania* spp. and transmit it to a susceptible host. This proof is an evidence that a particular insect is a vector of a specific species of *Leishmania*. This parameter is one of the criteria to be evaluated in vector capacity studies, in which the interaction between vectors-parasite-host that are involved in the eco epidemiology of leishmaniasis is searched [20].

Ecological associations and transmission dynamics are also related to the success or failure of transmission of *Leishmania* spp. [21]. So for the characterization of the vectorial competence, its essential to identify the location of the different forms of the protozoan in specific parts of the bowel of the sand flies by microscopy [19], mainly demonstrate the presence of infectious metacyclic forms of the parasite in the anterior midgut of the vector and the experimental transmission [6].

2.5. Control

2.5.1. Mechanical and Chemical Control

Some of the control strategies directed to sand flies are the internal residual spraying (IRS), the treatment of mosquito nets with insecticides, synthetic sex pheromones and environmental management.

The application of insecticides to the walls and roofs of households (IRS) [23] and in animal shelters (eg poultry houses and stockyard) is considered effective in reducing the population of sand flies [24]. Environmental concerns and the risk to human health surrounding the use of organochlorides and other chemical groups have gradually led to their replacement with synthetic pyrethroids (α-cypermethrin, cypermethrin, deltamethrin and λ-cyhalothrin), which are currently used by heads of public health agencies in several countries [25]. On the other hand, in the north of Morocco, cutaneous leishmaniasis has been reduced with house spraying with α-cypermethrin [26].

However, the IRS can be useful in particular situations when a high density of these insects is found near or in human habitations, as well as in poultry, stockyard and dog shelters that may represent a "natural attraction" to the vectors [27-28].

Mosquito nets impregnated with slow release insecticide are also used in the intervention against leishmaniasis, and their use provides a significant reduction in the incidence of the disease in endemic areas [29]. Through intervention with mosquito nets in Bangladesh, a decrease of 70-80% of the density of *Phlebotomus argentipes* has been noted [30].

In studies in the Indian subcontinent, using mosquito net treated with insecticides, a reduction of 25% in the sand fly density in the internal area has been verified [31].

Another control strategy is the use of synthetic pheromone ((S) -9-methylgermacrene-B) of *Lu. longipalpis* species to improve the effectiveness of sand fly control programs, when used with an effective insecticide. The combination of pheromone and insecticide has as a mechanism the action the attraction and death of insects of both sexes, preventing the females from looking for the hosts in the transmission of leishmaniasis and the males in establishing the mating in other places [32].

The feasibility of using synthetic pheromone attraction to attract *Lu. longipalpis* for a long time has been proven and therefore can be used in the control of sand flies and in the prevention of visceral leishmaniasis [33].

It should also be recommended the management of the environment followed by application of insecticide of the group of pyrethroids [34] used so far, or another that is more effective. This management of the environment is fundamental in urban areas to reduce the density of vectors that proliferate very close to the population, in their wooded backyards.

2.5.2. Microbial Control

Strategies are being made through paratrangenesis, which consists of the use of genetically modified symbiotic bacteria that secrete effector molecules that kill infectious agents. This process has been viable for controlling the transmission of pathogens by arthropod vectors [35].In underdeveloped countries, new vector insect control programs are being deployed in an interesting way, as is the case of sand flies [36].

However, the knowledge of the symbiosis of insects can show new ways to control insects vectors of important diseases, through the directed manipulation of the symbionts or host-symbiont associations [37].

In the two main known genera of phlebotomines, *Lutzomyia* represented 57% of the bacteria belonging to *Proteobacteria phylum* (Gram-negative bacteria), while *Phlebotomus*

expressed (47%) *Proteobacteria* and (40%) *Firmicutes*. Such a difference in the composition of the intestinal microbiota can be justified by several factors, including the diversity between the evolution of the two subgenera [38].

For the development of a paratransgenic platform to control the transmission of leishmaniasis, a non-pathogenic strain of *Bacillus* (*Bacillus subtilis*) isolated from phlebotomine *Phlebotomus papatasi* was used as a strong candidate for paratransgenic. Even though this bacterium has advantages such as being of genetic manipulation and easy to culture, not pathogenic, its use for the paratransgenic control of *Leishmania* may be challenging because of its ability to establish colonies in the intestines of several species of phlebotomine. For these reasons, it will be of great epidemiological importance to expand a regional strategy for each endemic area with different bacterial isolates [36].

2.6. Sand Flies Saliva, Leishmania Infectivity, and Vaccination

Phlebotomines infected with *Leishmania* spp. inoculate the parasites when making the blood replast in a vertebrate host. In this process, the vector saliva is inserted along with the protozoa into the skin of the host. This saliva is composed of molecules that trigger haemostatic, inflammatory and immunological responses of the host [39].Some of these molecules are immunogenic and develop strong immune responses in animals, including humans [40]. The humoral response against saliva from sand flies has been suggested as a possible epidemiological marker for exposure to vectors in endemic areas of leishmaniasis [41].

Continuous exposure to uninfected phlebotomine bites or immunization with salivary proteins are known to induce cellular and humoral immune responses [42-43].

A study with positive children in the delayed hypersensitivity test (DHT) showed that they were protected from infection because of their ability to expand the effective immune response against *Leishmania* spp. antigens. Although this group offers high concentration of anti-saliva antibodies to *Lutzomyia longipalpis*, its role in protecting against infection formation is not yet clear. Probably, the antibodies are capable of damaging the action of the salivary products, decreasing their functioning on the macrophages, however, favoring a greater activities of the antigens and the amplification of the cellular immunity. Therefore, it is not yet known whether the reduction of parasitic load may induce immune response [44].

Recently, a salivary protein of phlebotomine of the species *Lu. longipalpis*, LJM11, was identified as an immunogenic molecule for humans, dogs and mice exposed to bites of this vector [45].This protein belongs to the family of "yellow" proteins that are present in the salivary gland of phlebotomines of the genera *Lutzomyia* and *Phlebotomus*. Its function was instituted as a high affinity binders of proinflammatory biogenic amines [46].It has already been observed that this molecule is competent to cause a cellular immune response in verte-

brates and an additional protection against *Leishmania*. It is important to emphasize, that the immunization with the LJM11 protein led to a protection against *Leishmania* major infection transmitted by Lu. longipalpis, thus highlighting the coverage capacity of this salivary molecule [47].

3. General Considerations

The main concern of this chapter is the vector control measures based on the general characteristics of the sand flies, which were described above and showing that one of the main strategies for control of sand flies in the world are spray and mosquito nets treated with insecticide, but these insects are showing resistance to dichlorodiphenyltrichloroethane (DDT) and deltamethrin [32-33], highlighting the importance of new insect control techniques.

The text also describes a new artifice of the use of synthetic pheromone as an insecticide, that its action is positively proven in the control of sand flies [30], being of great interest its viability and commercialization as a product for the use of vector control.

The role of health education in implementing vector control programs for leishmaniasis should be recognized. The outcome of an effective program can be compromised unless the people involved understand the needs of an intervention and are proactive in maintaining vigilance for the prevention of this zoonosis.

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Chapter 5

Climatic Variables and Malaria Transmission

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Abstract

Malaria is one of the major vector-borne diseases caused by plasmodium spp. and transmitted to humans by anopheles mosquitoes. Malaria transmission is highly dependent on climatic variables such as temperature, rainfall and relative humidity. Malaria parasite *Plasmodium* completes its life cycle in two main hosts i.e., mosquito, humans and also in South-East Asian macaques (a natural host of *P. knowlesi*). It completes its sporogony in anopheles mosquitoes which are poikilothermic and requires ambient temperature to complete their life cycle. The duration of sporogony of the parasite inside the mosquito is highly influenced by the temperature. Moreover, malaria transmission is more in rainy season as the female anopheles mosquito lays eggs in water collection; therefore rainfall provides breeding places for mosquitoes which further increases the transmission intensity. Relative humidity has indirect effect on parasite development and survival of mosquito. The life cycles of parasite and the mosquito are completely dependent on temperature and relative humidity. The current chapter deals with the complex relation between the climatic variables and malaria transmission.

Keywords: Climatic Variables, Temperature, Rainfall, Relative Humidity, Plasmodium Parasite, Anopheles Mosquito

1. Introduction

Climate is defined as the long time pattern of weather components such as temperature, humidity, wind, and precipitation etc in a particular area over a period of time. These climatic components are also referred as meteorological variables. Change in climate can influence the human health either directly by extreme weather events such as heat waves, floods, cyclones or indirectly via changes in biological and ecological processes that influence the transmission of vector-borne diseases [1].

In vector-borne diseases, the abundance of vectors is affected by various physical factors such as temperature, rainfall, humidity etc. Moreover, circadian rhythm affects the feeding, resting, and oviposition which are restricted to optimum times, regardless of ambient temperature. Other factors such as forest ecosystem, temporary ground water pools with direct sunlight, vegetation near human settlement, agricultural practices, and human behaviour of the vector can also contribute to the disease burden. In addition, migration, urbanization, poor health infrastructure and other socio-economic contribute [2].

Of various vector-borne diseases, malaria is one of the major diseases and the link between climate and malaria distribution has long been established. Sustained transmission of malaria depends on favorable climatic factors for both mosquito and plasmodium parasite. Temperature, rainfall, and humidity are important, as well as the wind and the duration of daylight. The circadian rhythm affects other behaviors of the vector, such as feeding, resting, and oviposition which are restricted to optimum times, regardless of ambient temperature. Every single element that influences the climate and with it the entire ecosystem, is strongly altered by humans and their activities [3].

2. Malaria Statistics

Malaria continues to be the world's most widespread and serious vector-borne disease. In 2016, an estimated 216 million cases and 445000 deaths were occurred globally [4]. Most of these cases were from WHO regions of Africa (90%), followed by South-East Asia (7%) and the rest from Eastern Mediterranean region (2%) [4]. It is caused by *Plasmodium* parasite and transmitted by *Anopheles* mosquitoes.

3. Plasmodium Parasite

In order to understand the relation between malaria transmission and climatic variables, it is necessary to have brief knowledge of causative agent and its transmitter. As we know that malaria is caused by protozoan *Plasmodium* spp. and transmitted by female *Anopheles* mosquitoes. Of 250 species of plasmodia, five species i.e., *P. vivax, P. falciparum, P. malariae, P. ovale and P. knowlesi* infect humans [5]. *P. vivax* and *P. falciparum* are considered to be most widespread parasites. *P. falciparum* is most prevalent in African region (with 99% malaria cases), whereas *P. vivax* is predominant in Americas (64% cases) and 30% above cases, in South- East Asia and 40% in Eastern Mediterranean region [4]. Moreover, *P. knowlesi,* malaria of macaques (*Macaca fascicularis, M. nemestrina*) recognized as causative agent of human malaria from South East-Asian countries [6].

3.1. Life cycle of Plasmodium

Malaria parasite *Plasmodium* completes its life cycle in two hosts i.e., mosquito and

humans **(Figure 1)**. Infection starts when female anopheles mosquito picks up plasmodium parasites in a blood meal taken from an infectious person and inoculates gametocytes into the healthy human host during another blood meal [7]. In the gut of mosquito, gametocytes develop into sporozoites and this process takes 7-20 days. Then the sporozoites moves to salivary glands of mosquito and inoculated into another human when it bite and suck another blood. Sporozoites, moves along bloodstream and infect liver cells, where they mature into shizonts and release merozoites upon rupture [In *P. vivax* and *P. ovale*, the merozoites remain dormant (hypnozoites) in the liver cells and can become active and releases into bloodstream causes relapse even after weeks or years]. The initial replication in the liver is also known as exo-erythrocytic shizogony [8]. The released merozoites in the blood infect red blood cells and multiplies into trophozoite (ring stage), mature into schizont, which rupture releasing merozoites. The cycle of merozoites to schizonts and back to merozoites is referred as erythrocytic schizogony [9]. In Red Blood Cell (RBC), some merozoites develop into male (micro) and female (macro) gametocytes. These micro and macro gametocytes are ingested by anopheles mosquito, during blood meal and multiplies inside the mosquito is known as sporogony (sexual cycle) [10]. Inside the mosquito stomach male and female gametocytes fuses and generate zygote which is subsequently develop into motile ookinetes which invade the midgut wall of the mosquito to develop as oocysts. The oocysts grow, divide and release sporozoites, which make their way to the salivary glands of the mosquito. When the mosquito loaded with sporozoites take another blood meal, inoculation of sporozoites into a new person's bloodstream, causing malaria infection in the human host [5,11].

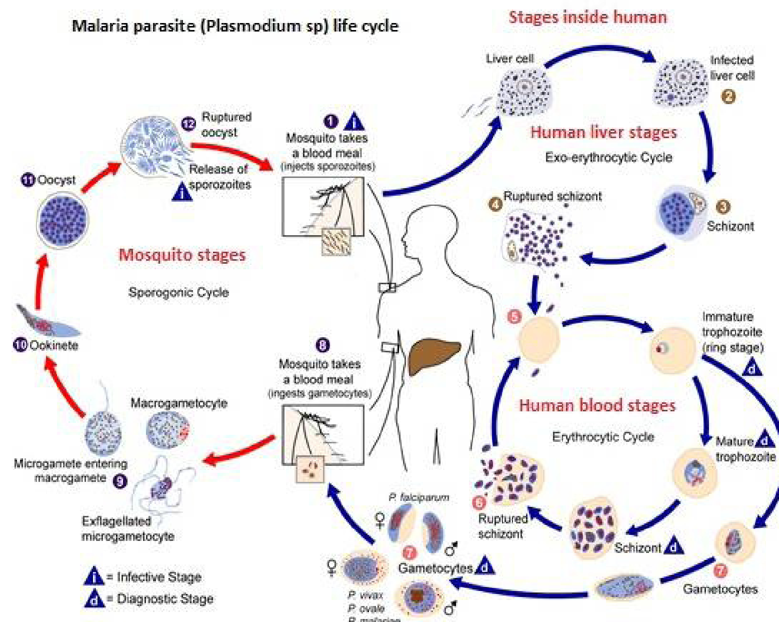

Figure 1: Life cycle of plasmodium parasite in two hosts i.e., humans and anopheles mosquito (Courtesy: Ref. 12)

Table 1: Identified dominant malaria vectors of different regions (Courtesy: Ref. 13-15)

Dominant malaria vectors in different regions			
Americas	Europe and Middle-east	Africa	Asia
An. freeborni *An. pseudopunctipennis* *An. quadrimaculatus* *An. albimanus* *An. albitarsis* *An. aquasalis* *An. darlingi* *An. marajoara* *An. nuneztovari*	*An. atroparvus* *An. labranchiae* *An. messeae* *An. sacharovi* *An. sergentii* *An. superpictus*	*An. arabiensis* *An. funestus* *An. gambiae* *An. melas* *An. merus* *An. moucheti* *An. nili*	*An. barbirostris, An. lesteri* *An. sinensis, An. aconitus* *An. annularis, An. balabacensis* *An. culicifacies, An. dirus* *An. farauti, An. flavirostris* *An. fluviatilis, An. koliensis* *An. leucosphyrus, An. maculatus group* *An. minimus, An. punctulatus* *An. stephensi, An. subpictus* *An. sundaicus, An. dthali,* *An. superpictus, An. sacharovi* *An. maculipennis, An. pulcherimus*
9	6	7	24
Grand total = 46			

4.1. Life Cycle of Anopheles

Anopheles mosquitoes are poikilothermic (change their body temperature according to the surroundings) and requires ambient temperature to complete their life cycle. Anopheles mosquitoes exploit different habitats for breeding. The female anopheles mosquito lays eggs in water collection; therefore breeding increases in the rainy season when water collects in bottles, tyres, broken water pipes, open tins/cans, open tanks, temporary water ponds formed due to rainfall, agricultural places, etc [16-18]. Female anopheles mosquito undergoes four stages in the life cycle: egg, larva, pupa, and adult **(Figure 2).** The first three stages are aquatic and adult female lays eggs directly on water and the eggs take two days to 3 weeks (in colder climates) to hatch. Larvae of mosquito have well developed head, thorax and segmented abdomen. They spend most of their time on feeding on algae, bacteria etc in the surface of micro layer. In pupa stage, head and thorax merged into cephalothorax with abdomen curving around underneath. After few days as pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges. This complete cycle from egg to adult stages are strongly influenced by ambient temperature. The life cycle completes in five days in temperate conditions and 10-14 days in tropical conditions. The adult males feed on nectar or other sugar sources, whereas females on sugars for energy and blood meal for egg development. After taking blood meal, female mosquito rest for few days for digestion and egg development. This process depends on the

temperature and takes 2-3 days in tropical conditions. Female lays fully developed eggs and resumes seeking host. The cycle repeats until the female dies. The life span of female mosquito is up to one month (or little longer) but in general mosquitoes do not live longer than 1-2 weeks where as males can survive about a week in nature [19,20].

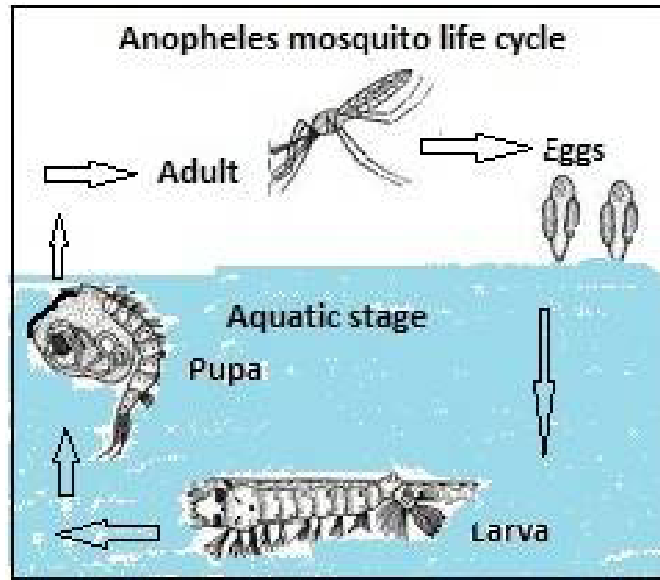

Figure 2: Life cycle of Anopheles mosquitoes

5. Parasite Inside the Vector

After ingested by the mosquito, the parasite undergoes development within the anopheles mosquito before infectious to humans. The time required for the development inside the mosquito is referred as the extrinsic incubation period (EIP/sporogony) which takes 10-21 days. This extrinsic incubation period is completely depends on the parasite species and temperature. Moreover, if the mosquito dies before the completion of extrinsic incubation, then it will not transmit plasmodia to humans [EIP is often longer than mosquito life expectancy] [19,20].

6. Transmission Intensity

The intensity of malaria disease depends on various factors such as vectorial capacity, entomological, entomological inoculation rate, urbanization, population migration change in the land use, agricultural practices, construction of dams/irrigation canals, resistance to insecticides etc [2,21,22,23]. Malaria transmission in human depends on vectorial capacity, longevity of the vector, the duration of the sporogony, interaction between the human and infected mosquito etc. Entomological parameters such as vector distribution rates, feeding behavior, biting rate also contribute in the disease transmission [24]. Moreover, malaria risk will be determined by the vectorial capacity which is further dependant on climatic variables [25].

7. Relation between Climatic Variables and Malaria Transmission

Apart from the above factors, environmental changes, climatic factors affect the biology and ecology of anopheles vectors, and their disease transmission. The complex relations between climatic factors and malaria transmission **(Figure 3)** have been widely reported across the world [26-28]. There are three main climatic factors that affect malaria transmission are temperature, rainfall (precipitation) and relative humidity [29]. The plasmodium parasite and their vector (anopheles mosquito)) are influenced by these factors. Temperature influences the life cycle of the parasite and mosquito; whereas rainfall provides breeding places for mosquitoes. The third climatic factor relative humidity has indirect effect on parasite development and on the survival capacity of anopheles mosquitoes [30]. Moreover, these climatic factors not only influence the malaria incidence but also constitute driving forces of malaria epidemics [31-33]. The affect of main climatic variables is described below.

7.1. Temperature

Temperature plays a fundamental role in parasite multiplication inside the mosquito. The temperature range for malaria transmission is between 15 to 40 °C and the number of days required for a mosquito to complete its life cycle depends on the temperature ranges and humidity [34]. Temperature directly influences the mosquito life cycle at different stages including biting rate, gonotrophic cycle (a physiological process of digestion of blood meal and development of ovaries) and survival probability. As the temperature increases, the rate of blood meal digestion also increases, which in turn accelerates ovaries development, egg laying and reduction of gonotrophic cycle. This will make the mosquito to feed more frequently on humans, thereby increasing the disease transmission intensity. The life cycle of mosquito from egg to adult takes 10 days at an optimum temperature of 28˚C. The duration gets prolonged at lower temperatures and reduced at high temperatures. The daily survival of the mosquito is dependent on the temperature, 90% of mosquitoes survives at temperatures 16˚C-36˚C [35]. Moreover the duration of parasite extrinsic incubation period (sporogony) also depends on temperature. Increased temperature leads to reduction in the duration gonotrophic and sporogony cycles which enhances the rate of transmission [36,37]. The average duration of EIP of plasmodium spp. is provided in the table **(Table. 2)**. Both gonotrophic and sporogony cycles are highly sensitive to temperature changes [38]. The minimum temperature required for main malaria parasites, *P. vivax* is 14.5–16.5˚C and *P. falciparum* is 16.5–19˚C for their development inside mosquito [27]. The optimum temperature for parasite development is about 20-30˚C [39]. The parasite completes its sporogony in five days temperature exceeds 30˚C [40]. However, the survival rate of mosquito decreases at 40˚C [41], *Anopheles culicifacies* a rural vector of India cannot survive more than 24 hrs [42].

An iconic study on degree day model was developed by Detinova et al., (1962) to

define the EIP of *P. falciparum* inside the mosquito has been applied in numerous studies over the years. Moreover, numerous researchers across the world have been working on the relation between temperature and development of parasite inside the mosquito or mosquito population dynamics [31,44-46]. In all these models, variations are observed in EIP period and temperature and it could be depend upon mosquito species, mosquito condition, parasite strain, environmental fluctuations and specific vector-parasite combinations etc [47]. Small fluctuations in temperature can either lead to increased malaria incidence and at very high temperature can kill the mosquito, parasite. Moreover, the intensity of malaria transmission will vary spatially and temporally depending on environmental fluctuations and specific vector-parasite combinations.

Table 2: Number of days required for sporogony at different temperatures (Adapted from ref. 2 & 23)

Parasite	Duration of sporogony at 20˚C	Duration of sporogony at 25˚C	Duration of sporogony at 28˚C
P. falciparum	22-23 days	12-14 days	9-10 days
P. vivax	16-17 days	9-10 days	8-10 days
P. malariae	30-35 days	23-24 days	14 days
P. ovale	_	15-16days	12-14 days
P. knowlesi	_	_	_

7.2. Rainfall

Rainfall is considered as a predominant factor for malaria transmission in arid and semi arid regions [48]. It is not only providing breeding places for mosquitoes to lay their eggs, but also modifies the effect of temperature which results in the increasing in the relative humidity which improves the mosquito survival rates [49]. But the relation between rainfall and mosquito abundance is best studied when temperature is not limiting factor. The first three stages of mosquito life cycle is aquatic phase which requires water for laying eggs, larval development and this water is provided through rainfall. Rainfall provides new breeding places and new water to existing ones [50,51]. The persistence of larval habitats and their development is highly depending on the frequency, duration and intensity of rainfall. Heavy rainfall during wet/monsoon season may flush away the vector breeding places [52]. Rainfall based malaria transmission is seasonal in arid, semi arid or high land regions. Several studies have reported increased risk of malaria following heavy rainfall [48, 53, 54]. Moreover, heavy rainfall in the highlands, arid and semi arid regions led to malaria outbreaks [55]. But the amount of rainfall and lag period (time between rainfall and malaria) varies from place to place. The lag period between *P. vivax* and *P. falciparum* was different based on the significant association between the climatic variables [31,32,48]. On contrary to this, there was no association between rainfall and malaria transmission observed in north eastern India [56,57].

7.3. Relative humidity

It is well known that relative humidity has indirect effect on parasite development and survival of mosquito [27]. There is a positive association was observed between rainfall and relative humidity. As the rainfall increases, relative humidity also increases. On the other hand, temperature has negative effect on humidity, as temperature increases humidity decreases. Relative humidity plays important role in arid, semi arid or dry zones. Relative humidity more than 60% along with the temperature between 20 to 30˚C favours plasmodium parasite development inside the anopheles mosquito [27]. Humidity levels between 55 to 80% are same for *P. vivax* and *P. falciparam* parasites. At the same humidity levels, *P. vivax* completes its sporogony in 15 to 20 days when the temperature ranges from 15 to 20˚C; the number of days decreases to 6 to 10 days when the temperature fluctuated between 25 to 30˚C. *P. falciparum* completes sporogony 20 to 30 days when the temperature ranges between 20 to 25˚C, 8 to 12 days between 30 to 35˚C temperature [34]. Though humidity is not main climatic factor, significant association between relative humidity and malaria incidence was reported [58]. A study from China, reported the significant association between the relative humidity and malaria cases [59]. Studies from India have reported the positive association between malaria incidence and relative humidity [48,60].

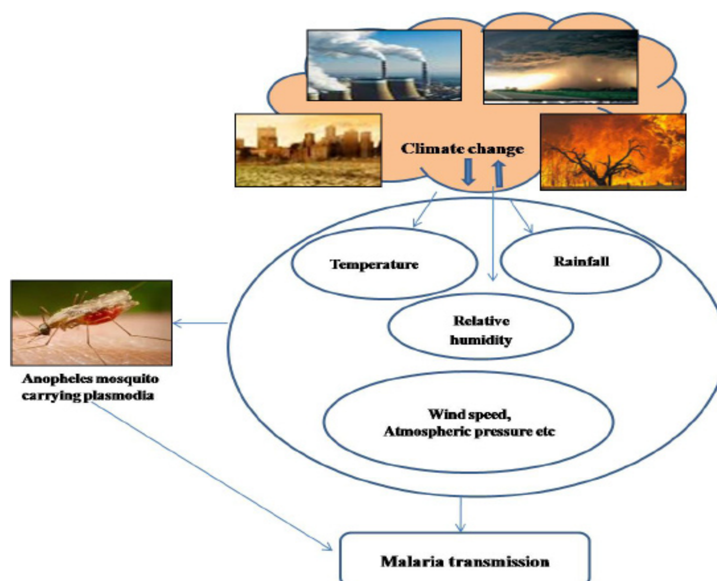

Figure 3: Climatic model of malaria transmission

8. Conclusion

Despite of widespread transmission, it is still difficult to predict future malaria intensity, particularly in the face of climate change. Because the parasites that cause malaria are so strongly tied to mosquitoes for transmission, malaria incidence will change as the climate changes. Though there are numerous studies and mathematical models based on temperature, rainfall and relative humidity to predict malaria transmission/epidemics, it is still unclear and debatable matter how the changes in transmission will occur [25,26,31]. To control the malaria transmission, strong vector control strategy, malaria control interventions, improved health

care system and development of rainfall cutoff based prediction models for dry lands/semi arid regions will help to control the malaria disease in future.

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Citation of this book chapter: Lingala MA, Climatic variables and malaria transmission, Chapter 5, in Vector-borne diseases and treatment. 2018; Vol 2: 1-12.

Chapter 6

Japanese Encephalitis Virus: Displacing of Virus Genotype and Efficacy of Vaccination

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Abbreviations: AES: Acute Encephalitis Syndrome; Arbo: Athropod Borne; C6/36: *Aedesalbopictus*C6/36 clone; E: Envelope; G: Genotype; Genotype I: GI; Genotype II: GII; Genotype III: GIII; Genotype IV: GIV; Genotype V: GV. JEV: Japanese Encephalitis Virus; PS: PS: Pig Kidney Epithelial; RD: Human Rhabdomyosarcoma

1. Introduction

Japanese Encephalitis Virus (JEV) is a mosquito-borne virus which causes Acute Encephalitis Syndrome (AES). After more than 60 years since JEV was discovered in Japan in 1935, the virus spread to many Asian countries, Western Pacific countries and North Australia, with approximately over 3 billion individuals lived in epidemic areas in 2011 [2,3,34,36]. JEV is a leading cause of AES for children in Asian countries with a high morbidity and mortality. It was claimed that if there had been no vaccination to prevent human from the disease, its consequence would have been more severe than any other disease. JEV has only one serotype but five genotypes (GI-V). The emergence and distribution of genotypes were varied between different geographical regions and periods. At the time of vaccine development, most of JEV strains isolated in humans belonged to JEV GIII, thus vaccine for humans was developed from GIII strains [1,14,24] **(Table 1).** At the beginning of 21st century, it was predicted that JEV GIII and GII would emerge in Australia and some northern Asian countries, respectively [30]. However, the emergence of genotypes from mosquitoes, pigs, and humans from 1935 to 2016 in Asia, Western Pacific region, and Australia showed that this prediction did not come true. Moreover, the JEV genotype isolated from humans shifted from GIII to GI in the recent decade in most the Asian countries [17,22,25]. Besides this, the appearance of the JEV GV in several Asian countries was also reported in 2000s after long time of first detection in 1952 in

Malaysia [16,28,31] **(Table 1)**. It raises a question about the efficacy of Japanese encephalitis vaccine with the emerging JEV genotypes.

2. Japanese Encephalitis Virus

2.1. Origin, vector, and amplifying hosts

Japanese encephalitis virus was firstly isolated from a human in Tokyo, Japan in 1935. Comparison of *JEV* with other Flaviviruses suggested that jev might evolved from an African ancestral virus, but recent phylogenetic analysis showed results of the origin of JEV might be from its ancestral in the Indonesia-Malaysia region [7,29,30].

JEV belonged to Arbovirus which shared the ability of transmission by Arthropod vectors. JEV is maintained in nature in a cycle between vertebrate host and mosquitoes which primarily from the *Culex* genre. *Culex spp* acts not only as a vector but also as sub-amplifying hosts since mosquitoes can transmit JEV to the next generation through eggs. The vertical transmission JEV in mosquitoes makes virus control in nature become very difficult. Moreover, this species feeds on birds, creates the natural cycle between mosquitoes and avian species. Particularly, *Culex spp* feeds on mammals and transmits JEV to humans and animals [15,34,36]. The role of animals is amplifying factors and subsequent source infection to mosquitoes. Additionally, pigs are considered the most efficient amplifying host of JEV, but other livestock species also act as amplifying hosts such as horses and cattle [21,32,39].

2.2. Morphology

The mature virus particles are 45-50nm in diameter and possess a spherical symmetry consists of the inner core or an icosahedral nucleocapsid protein surrounding the genomic RNA, a lipid bi-layered membrane, and an envelope. The envelope proteins are reported to carry Hemagglutinating (HA) activity and immunogenicity relating to neutralization. The virion is about 69-70 kilodaltons (kDa) in size and 200S of deposition value [8,39,42].

2.3. Molecular characteristics

JEV genome is a single positive-strand RNA genome, approximately 11 kb in length, encodes 10 proteins consisting of three structural proteins and seven non-structural proteins. Structural proteins are C protein (core protein), M protein (membrane), and E protein (envelope) [14,18,42] **(Figure 1)**.

JEV has only a single serotype but is divided into five genotypes (GI-GV) based on the E gene or the complete genome. The genotype distribution is different spartio-temporally [28]. In Vietnam, a long-term phylogenetic study showed that from 1964 to 1988, Vietnamese isolates were classified into one genotype and evolved slowly with evolution rate of ≤3.2%

[11] **(Table 1).**

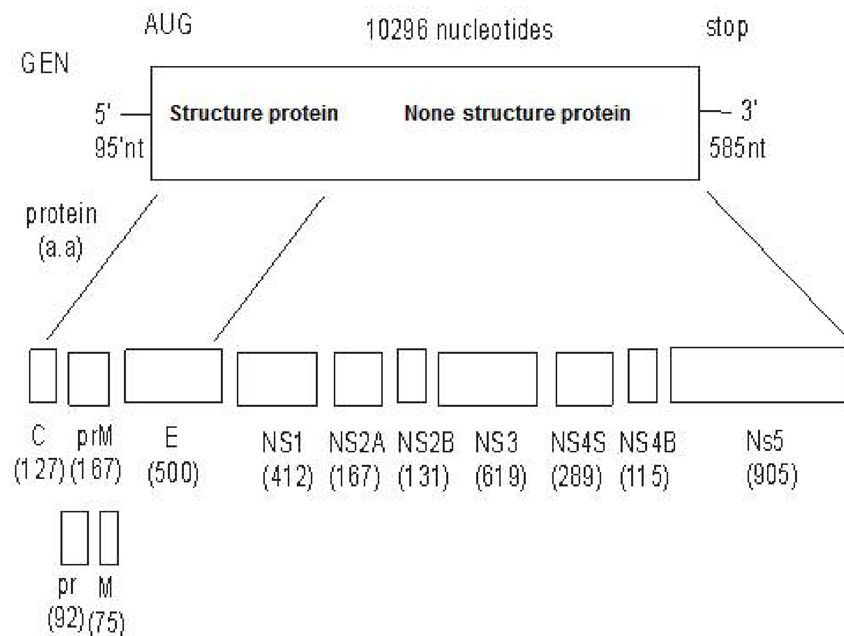

Figure 1: JEV genome schematic and protein translation.

2.4. Virulence

The virulence of the five genotypes differ between them. In 1960s-1970s, JEV detected in humans in Asian countries mainly belonged to GIII. Recently, JEV detected in humans in Malaysia, Indonesia, and Northern Australia belonged to GII. The situation in Thailand, Vietnam, and China was different, since all isolated JEV belonged to GI. The etiological role of GIV and GV in humans had been still unknown, although virus belonging to GIV was detected in mosquitoes in Indonesia [8,9,27]. Moreover, experimental studies showed that GIV virus was less virulence than GIII virus but the GIV has been not detected from humans up to now. Besides that, several GIII strains became less virulence after number of passages, such as SA 14-17-2 strain [16,29,31].

3. Appearance and Replacement of JEV Genotypes

The first JEV strain detected from brain tissue of Japanese patient in 1935 belonged to GIII. After this first detection, GIII was reported as epidemic strains in many Asian countries, such as China, Korea, Vietnam, Thailand, Malaysia, Indonesia, and India **(Table 1).** In 1994, the GI was firstly reported in Japan from mosquitoes. However, some molecular epidemiology studies after that when analyzing strains collected before 1994 showed the much earlier appearance of GI in Northern Asian countries and Vietnam: China in 1979 in mosquitoes, South Korea in 1983 in mosquitoes, Vietnam in 1990 in humans, Australia in 2000, and India 2009 **(Table 1).** After that, GI strains became predominant in these countries where GIII used to be the predominance. It is noted that the first detections of GI were mainly in mosquitoes and swine. This phenomenon suggested that GI might adapt better in mosquitoes and swine than in human. However, recent studies in Vietnam, Japan, China and India showed that GI

was also the agent causing encephalitis in humans [6,12,18]. In Vietnam, although GI was detected in 1990s in humans it suggested that GI might circulate in mosquito's population much earlier **(Table 1).**

Table 1: Emerging of JEV Genotypes in the Asian Pacific Region and Northern Australia in Recent Decades

Country	Year of JEV genotypes emerged or notified				
	GI	GII	GIII	GIV	GV
Japan	1994	Undetected	1935	Undetected	Undetected
China	1979	Undetected	1948	Undetected	2009
Korea	1983	Undetected	1987	Undetected	2010
Vietnam	1990	Undetected	1964	Undetected	2018
Thailand	1963	1983	1964	Undetected	Undetected
Australia	2000	1995	Undetected	Undetected	Undetected
Malaysia	Undetected	1970	1965	1965	1952
Indonesia	Undetected	1981	1979	1981	Undetected
India	2009	Undetected	1956	Undetected	Undetected

JEV GV was detected in the 1950s in Malaysia and Singapore in mosquitoes and birds, respectively. Over 60 years, it was not detected until recently, the new emerging of GV was noticed from mosquitoes in several Asian countries, such as China in 2009, Korea in 2010 [16,31,33], and Vietnam in 2018 (Unpublished data) **(Table 1).**

The prediction of emerging new type or new genotype is also interesting topic of recent decades. For examples, Schuh *et al*. have performed bioinformatics analysis and calculated that time of the most recent common ancestor of GI strains in Vietnam was 1953 [29]. This was the source of widely spread to neighboring countries such as China, Japan, South Korea, Thailand and became the most predominant genotype in these countries [29]. These results showed strong evidence completely different with the previous prediction of the emerging and predominance of GII in Northern Asia and the emerging of GIII in Australia [30].

In the recent decades, the trend of JEV genotype replacement occurred in many countries. Before the 1990s, JEV from humans mainly belonged to GIII. GI was detected only in Thailand in five patients. However, after the 1990s, the emerging of GI was reported as increasing worldwide [11,22,23].

Table 2: The Last Time GII and GIII Strains in Asian Pacific Countries and Australia

Countries	The last time of GII and GIII strains	
	GII	GIII
Japan	Undetected	1994
China	Undetected	2007
South Korea	Undetected	1994
Vietnam	Undetected	2004
Thailand	1992	1992
Australia	2000	Undetected

The surveillance data showed that when GI appeared and spread throughout Asian countries, GI and GIII co-circulated just for a short period of time before GIII disappeared. GI became the single genotype found in Japan, South Korea, Vietnam, and Thailand **(Table 2)**. Similarity, GII appeared in Thailand and Australia in 1983 and 1995 and but disappeared in 1992 and 2000, respectively, while GI appeared and spread **(Table 2)** [4,26,35]. The *in-vitro* studies using different cell-lines originated from humans, mosquitoes and swine could provide the experimental illustration of this phenomenon. JEVs were amplified and maintained by C6/36 cells (originated from mosquitoes) after 10 passages whereas that by RD (originated from humans) and PS (originated from swine) only limited within 8 and 6 passages, respectively. This result showed that GI strain amplified and maintained more efficiently on C6/36 and PS but not RD, whereas GIII strain amplified and maintained more efficiently on RD [5].

One example of the genotype replacement was in Vietnam. A phylogenetic analysis of JEVs in Vietnam from 1964 to 2011 based on 1,500 nucleotide sequences of E gene showed that, the first GIII strain was detected in humans in Vietnam in 1964, and in mosquitoes in 1979, whereas GI strains were first detected in humans and mosquitoes in 1990 and 1994, respectively **(Figure 2)**. After 2004, GI was the only genotype detected in Vietnam, demonstrating that the GIII strains had been displaced by GI strains [4,19,22]. All the Vietnamese GI strains belonged to the GI-b clade defined by Schuh *et al*. including the major GI strains circulating widely in temperate climates, such as China, Japan, Korea, and Thailand. Whereas sub-genotype GI-acontains strains circulating only in the tropical climates, such as Thailand and Australia [28].

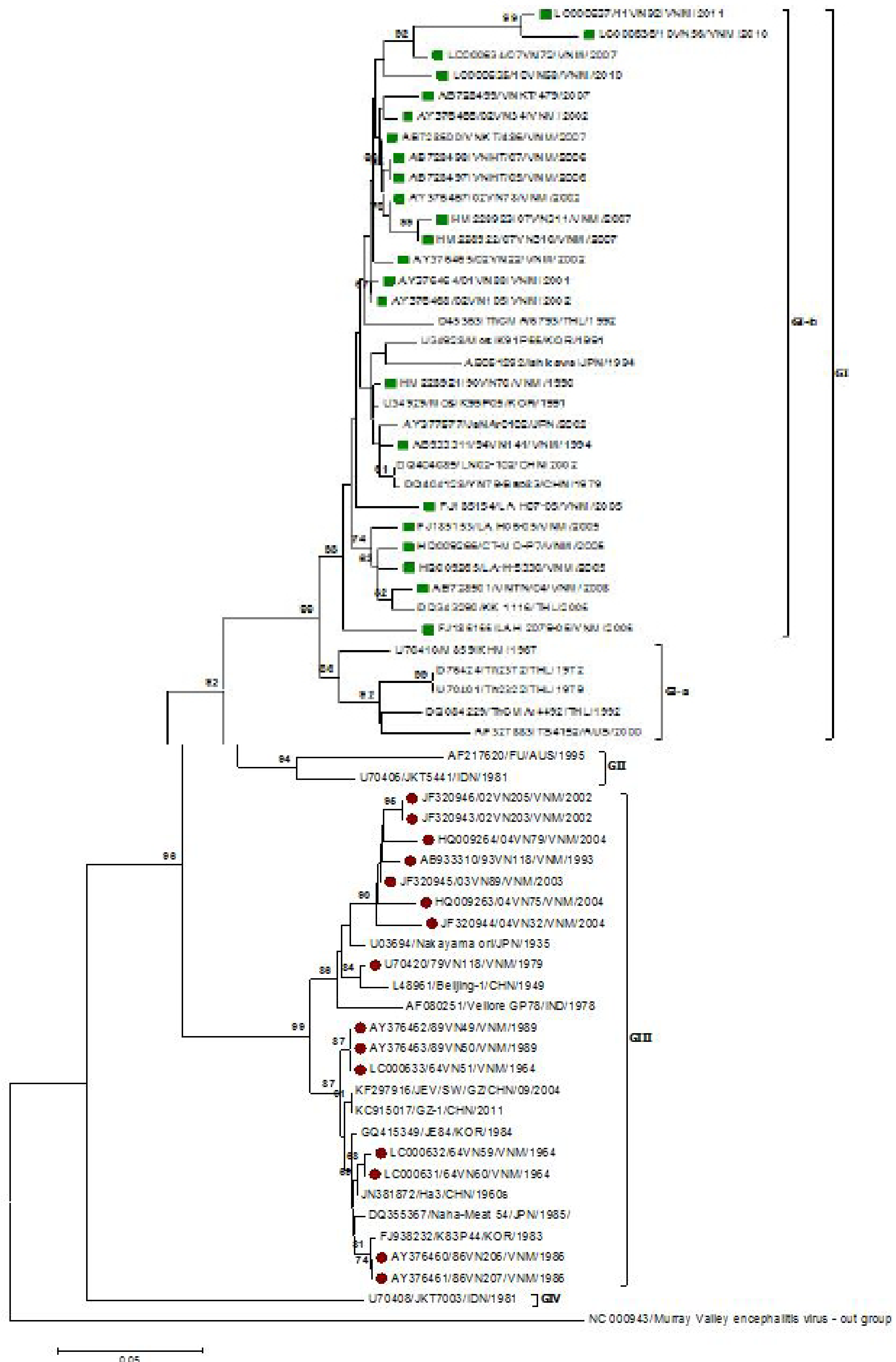

Figure 2: The phylogenetic tree of E gene of GI and GIII strains in Vietnam, 1964–2011. Marked red circle: GI strains; Marked green square: GIII strains [4].

4. Japanese Encephalitis Vaccines

Japanese Encephalitis vaccine was researched and developed by Mitamura *et al*. since 1936. This first licensed vaccine was a mouse brain-derived using Nakayama strain developed in Japan in 1954. In 1965, this vaccine used a combined physics-chemistry method to produce a purified, safe and high effectiveness vaccine [13]. Another mouse brain-derived vaccine using Beijing-1 strains was also used worldwide, especially in Asia, Europe and the USA [40]. Owing the vaccine strains protective efficacy, they have been used worldwide in the last 30 years with the efficacy of 80-91% after the second dose of JE vaccine. Not only produced in Japan, the mouse brain-derived vaccine technology has been transferred to several Asian countries, such as China, India, Taiwan, Vietnam, and Thailand to develop domestic products. However, the antibody declined over time leading to booster doses every 3-4 years till 15 years old. In addition, the cost of a purified process is quite expensive. That is the reason why only some developed countries, such as Japan, Korea had introduced this vaccine to control the disease nationwide [10,20,41]. Another first generation JEV vaccines is cell culture inactivated JEV vaccine. The first cell culture inactivated vaccine cultivated on Primary Hamster Kidney cells (PHK) using Beijing-3, P-3 strain with the efficacy of about 76-95% was used in Chinese nationwide campaigns until the mid-2000s [40]. Lived attenuated SA14-14-2 virus vaccine was replaced the PHK inactivated JE vaccine after that and has been used in China, Nepal, India, Sri Lanka and South Korea [3,40].

After the first generation of JEV vaccine, the development of second generation JEV vaccine has continued. Chimeric live-attenuated JEV vaccine is a recombinant vaccine based on a chimeric of JEV (SA14-14-2 strains) and Yellow Fever Virus (YFV17D) and was approved in Australia and Thailand [40]. It is a two doses therapy, whole-life protection and already commercial but its high cost is a consideration issue for the developing countries in Asia [20]. Vero cell lived-attenuated JEV vaccine using SA14-14-2 strain is licensed in the USA, Europe, Canada, Switzerland and Australia [40]. Inactivated Vero cell- derived vaccine was licensed in China and two other vaccines were licensed in 2009 and 2011 in Japan, respectively [40]. Another one developed by Novartis company, which has been approved and licensed in 2009 by the US Food and Drug Administration (FDA). These new generation vaccines are safe and produce a good sero-conversion rate of more than 83% [13,24,38,40]. In Vietnam, cell-derived JE vaccine has been developed in order to replace the mouse brain-derived vaccine [41].

Nowadays, JE vaccines for human are abundant and available. However, to achieve the target of control Japanese Encephalitis in Asia, it is necessary to have a safe vaccine, ready to produce large number of doses, high immunogenicity, two doses in the life therapy, acceptable price for the impoverish rural in Asia. It is also raised a question about new vaccines for specific populations such as infants or children infected with HIV.

5. Japanese Encephalitis under the Impact of the Vaccine

If the vaccine had been not available for prevent human from the disease, JE would have been one of the most serious health problems in Asia. The surveillance of JE pre- and post-vaccine era in Japan, Korea provided the evidences of this judgment. In Japan, before the introduction of JE vaccine (1949–1958), the incidences of JE were very high with 2,882 cases annually. After JE vaccine was introduced to susceptible population (1986–2000), the incidences of JE were dropped down to only 12.6 cases annually with a 99.6% reduction comparing to previous period. In Korea, in the period when JE vaccine was not used (1949–1958), the incidences of JE were 1,669 cases annually. After JE vaccine was introduced national-wide (1986–2000), the annual incidence reduced 99.9% and there were only 1.1 cases annually. In China, the first big outbreak of JE was recorded in 1966 with 150,000 cases, the second one occurred 5 years later with 180,000 cases were reported. After that, due to the enhancement of introducing JE vaccine, number of JE case was declined significantly with annual incidence of 20,000-40,000. However, in China, another issue needed to be considered is the available of stock vaccine which would be required huge number of doses in the control JE period in the future [10,37,39]. Similarity, in Vietnam, JE vaccine has been introduced in Expanded Program of Immunization (EPI) since 1997 for children at 1 to 5 years of age in high risk areas. The program started with 11 districts of 11 high risk provinces in the North meaning only 1 district in each province used JE vaccine. The coverage rate of JE vaccine by district in Vietnam was 1.63% (11/676 districts) in 1997. Every year, the enhancement of JE vaccination was consolidated. Till 2011, the vaccine coverage rate in district was 75.44% (510/676 districts). As the results of this enhancement, the incidence of suspected viral acute encephalitis syndrome in Vietnam had been declined to 1,000 cases/year, meaning the rate of suspected viral AES reduced from 4.2-4.8/100,000 population before 1997 to 1.2-1.8/100,000 population in the recent years [41].

Hitherto, the Japanese encephalitis vaccine has been produced using genotype III of JEV strain protecting human from the other genotypes of JEV.

6. Concluding Remarks

JEV is a mosquito born virus, the leading etiology cause AES for children with high morbidity, mortality and sequela. JEV has five genotypes, the emerging and circulating of each genotype change by periods and geographical regions. Before 1990, most of human isolates were JEV GIII. Thus, selected JEV GIII strain was used to develop vaccine for human till now. But in recent decades, JEV GI emerged in a lot of Asian countries causing AES in human. The shift genotype was took place when JEV GI appeared and spread in Asia, yielding the quiescent of JEV GIII or GII in areas where those genotypes were circulating before.

To prevent human from JE disease, three generations of JE vaccine were licensed in the world in order to supply purify, safer and high effectiveness vaccines. Several North Asian countries had controlled JE disease thanks to national wide vaccination such as Japan, Korea. And other countries have also controlled JE disease when increasing JE vaccination for children such as Vietnam, China yielding JE incidence rate dramatically reduce. It showed the efficacy of JE vaccine producing from JEV genotype III to protect human from the other genotypes of JEV.

7. Acknowledgments

The authors thank the Vietnam National Foundation for Science and Technology Development support under grant number: 106.16-2011.68.

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