

# Vector-Borne Diseases & Treatment

## Chapter 1

# Chikungunya Fever: Biology and Epidemiological Aspects

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## Abbreviations

CHIKV: Chikungunya; ECSA: East/Central/South African; MCC: Maximum clade credibility; IOL: Indian Ocean Lineage; Ae.: Aedes; PHB: Prohibitin; PtdSer: Phosphatidylserine; PVEERs: mediated virus entry-enhancing receptors; GAGs: Glycosaminoglycans; PAHO: Pan American Health Organization; ZIKV: Zika virus; DENV: Dengue; RT-qPCR: Polymerase Chain Reaction using Reverse Transcriptase Reaction in Real Time; RT-PCR: Polymerase chain reaction; IgM: Immunoglobulin M; IgG: Immunoglobulin G; RT-LAMP: Real-time Accelerated ; ELISA: Enzyme Linked Immuno Sorbent Assay; IVM: Integrated Vector Management; WHO: World Health Organization; SIT: Sterile insect technique; RIDL: Release of Insects carrying a Lethal gene; IIT: Incompatible Insect Technique; CI: Cytoplasmic Incompatibility; Bti: *Bacillus thuringiensis israelensis*; IRS: Indoor residual spraying; Medea: Maternal effect dominant embryonic arrest.

## 1. Introduction

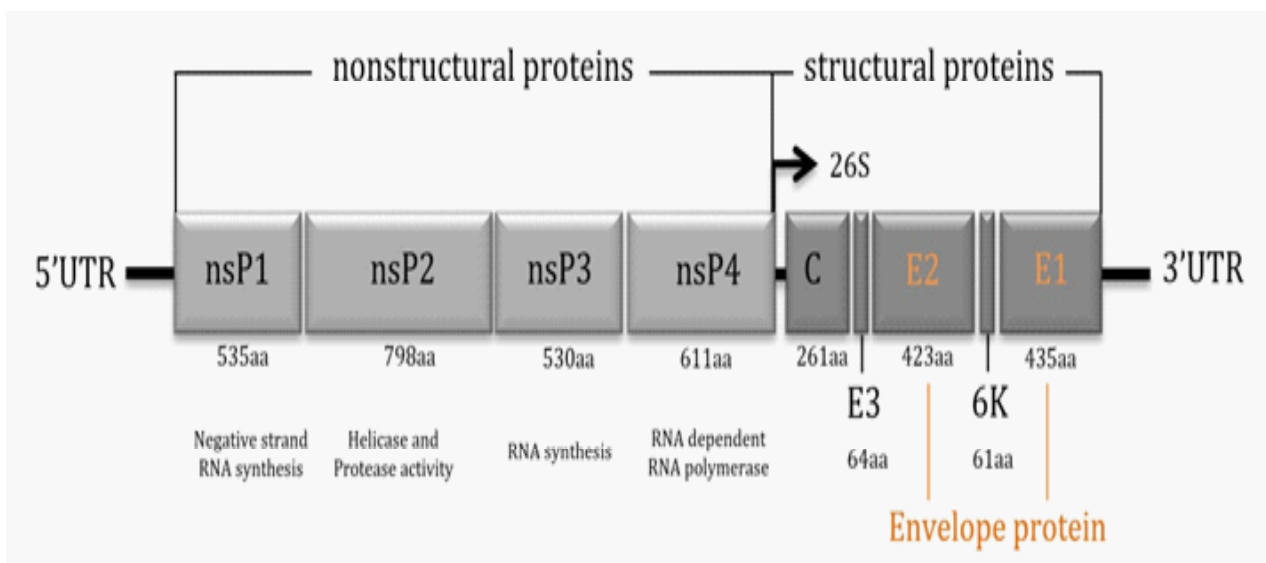
Vector-borne diseases transmitted by mosquitoes like *Aedes aegypti*, in particular Chikungunya fever, is quite alarming [1]. Chikungunya fever was first described during an outbreak in Tanzania in 1952. In the last 50 years, Chikungunya virus (CHIKV) has spread beyond

the African lands and has caused explosive outbreaks, which include millions of cases in the Indian Ocean, Asia and, most recently, in Europe and the Americas [2]. The name Chikungunya comes from the Bantu language spoken by the Makonde tribe-an ethnic group in southeast Tanzania and northern Mozambique - as a descriptive term, which can be translated as the disease that bends up the joints due to arthralgia that can last for months [2,3]. The etiological agent of Chikungunya fever is CHIKV, an *Alphavirus* belonging to the *Togaviridae* family [5].

## 2. Chikungunya Virus Structures

CHIKV is an enveloped virus with icosahedral symmetry, belongs to the Semliki Forest antigenic group of the genus *Alphaviridae*, which includes other arthritogenic viruses such as o'nyong-nyong, Ross River, Barmah Forest and Mayaro [5,6]. The genome, which consists of a single 11.8Kbp positive sense RNA strand, is closely related to the o'nyong-nyong virus and encodes a 2,472 amino acid non-structural polyprotein and a 1,244 amino acid structural polyprotein [7]. The polyproteins give rise to 4 non-structural proteins (nsP1, nsP2, nsP3 and nsP4) that make up the viral replication machine and 5 structural proteins (C, E3, E2, 6K and E1) [8,9].

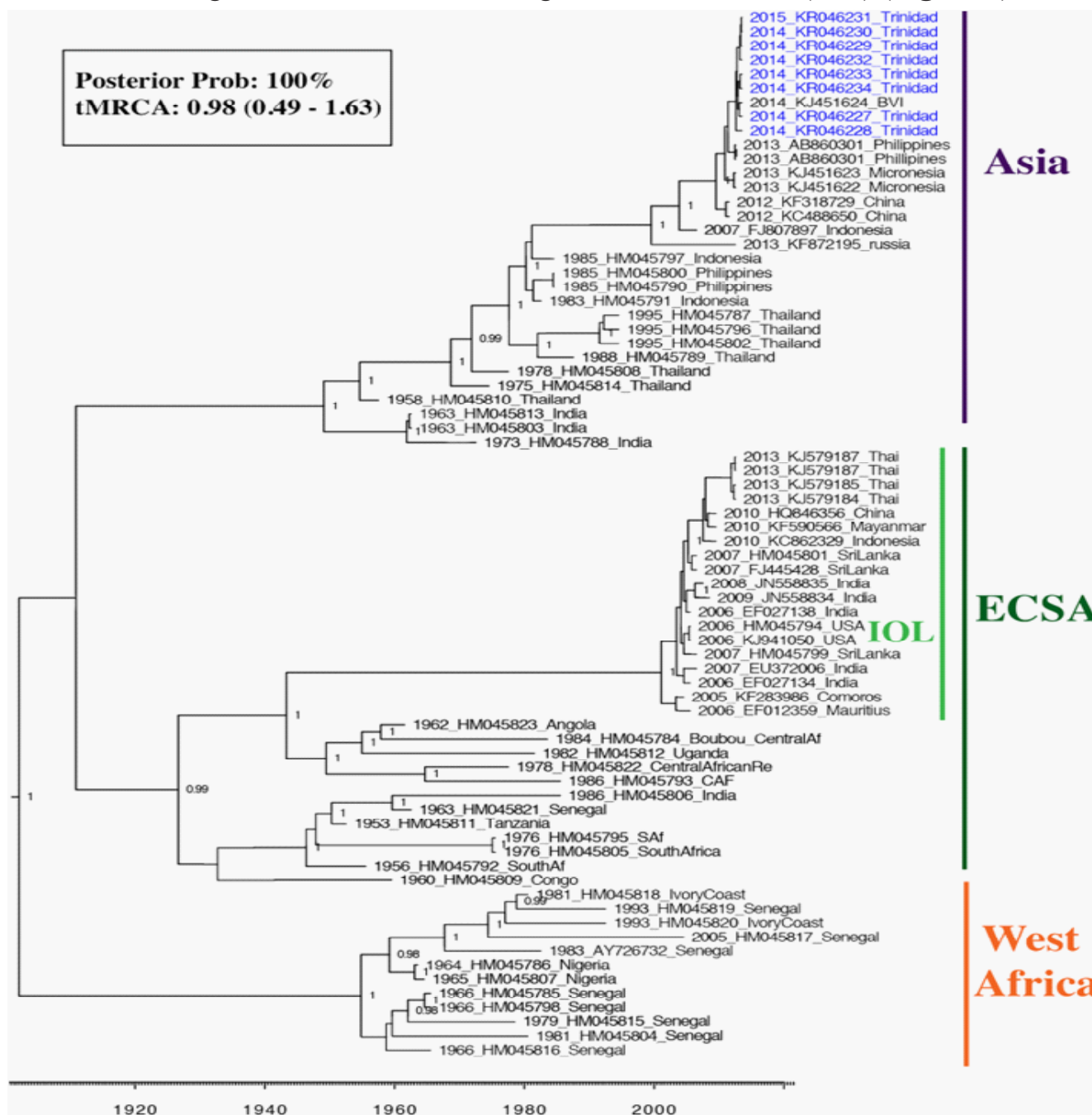
The virion is 70 nm in diameter and consists of repeating units of the E1 and E2 transmembrane glycoproteins (240 heterodimers of E2/E1 arranged as trimeric spikes on its surface), the capsid (C), a host-derived lipid bilayer, and a single molecule of genome RNA. The genome encodes the nonstructural proteins (nsPs) at the 5' end and the structural proteins at the 3' end. The 5' end of the genome has a 7- methylguanosine cap, while the 3' end is polyadenylated [10]. The nsPs are translated from genomic RNA and the structural proteins from a subgenomic RNA (**Figure 1**) [11].



**Figure 1:** Schematic representation of the chikungunya virus genome. Organization of nonstructural and structural proteins throughout the genome as well as the non-translatable regions at the 5' and 3' ends. The function and size of amino acids is shown for each protein. C, capsid; AA, amino acid.

Until 2004, there were three different genotypes of CHIKV revealed by phylogenetic analysis that evolved independently in different geographic regions. The West African genotype is derived of isolates from Senegal and Nigeria. The East/Central/South African (ECSA) genotype is an enzootic genotype in Africa, and it has been related to epidemics in the Indian Ocean. The Asian genotype, including isolates from Asian countries such as Singapore, Malaysia, and Thailand has been associated with spread in the Pacific Region [12–15].

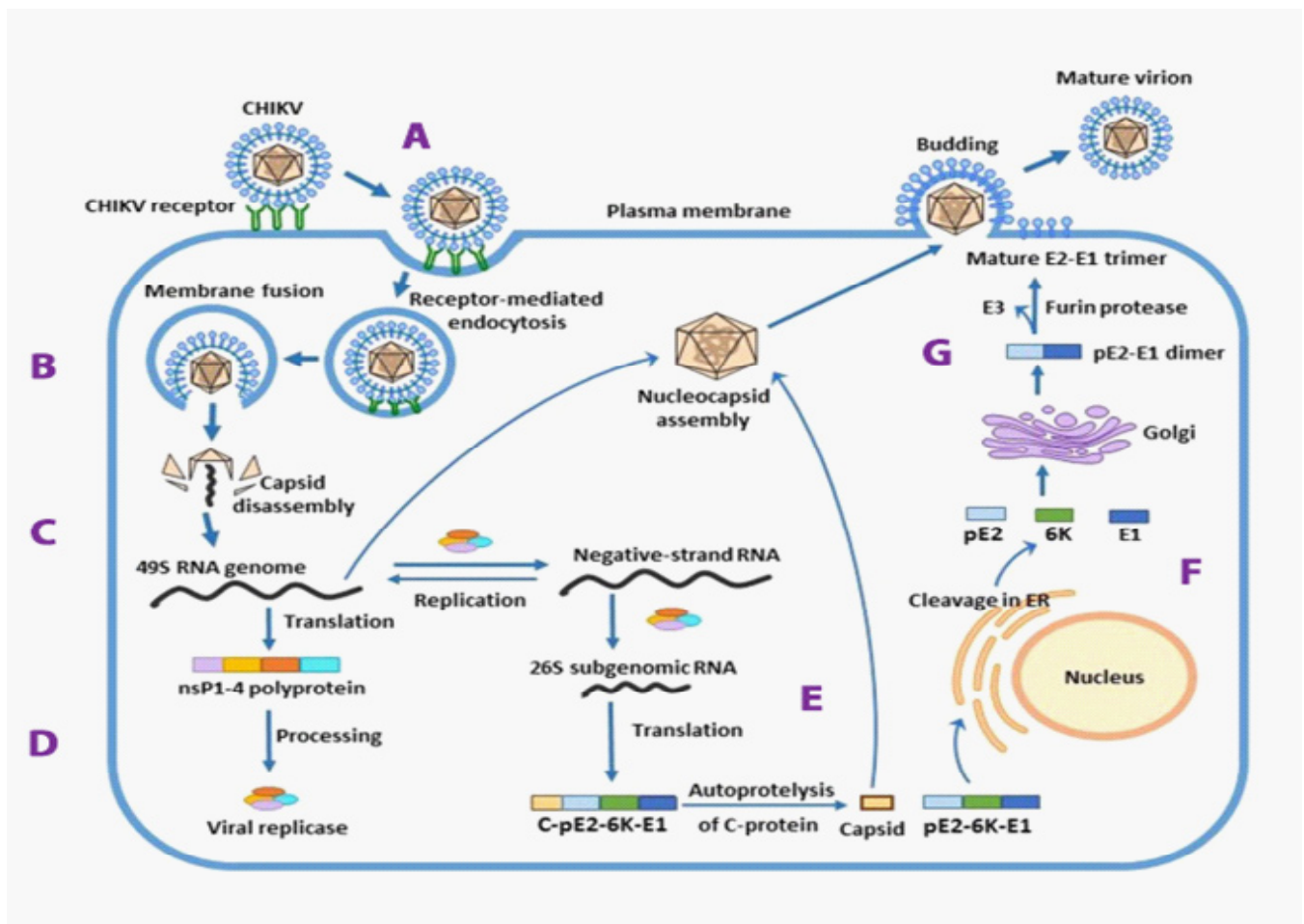
The origin of the 2005 epidemic in the Indian Ocean was initially attributed to the ECSA lineage. When the epidemic began in Kenya in 2004, the first CHIKV isolates of the La Réunion Island exhibited an alanine residue at position 226 in the E1 envelope protein, but subsequent isolates showed a replacement of alanine for valine residues (A226V). This and other substitutions gave rise to the fourth lineage, the Indian Ocean (IOL) (Figure 2).



**Figure 2:** Maximum clade credibility (MCC) phylogeny based on the complete coding region of 74 chikungunya virus sequences. In detail, the presence of IOL representing a novel ECSA [16].

This mutation, may have contributed to the adaptation of the virus to the *Aedes albopictus* mosquito, leading to the resurgence of CHIKV in 2004. In addition, another adaptive mutation was identified and analyzed, the E2-L210Q point mutation that was identified in the E2-glycoprotein coding region. This mutation contributed to the increase in CHIKV infection in both mosquitoes and cells, and it acted mainly on epithelial cell infection [17,18]. These two mutations have been shown to cause a dramatic increase in CHIKV infectivity with spreading to Europe and the Americas due to the widespread distribution of the *Ae. aegypti* and *Ae. albopictus* vectors [16,19,20].

Like all alphaviruses, CHIKV enters the target cell by endocytosis facilitated by the interaction of the E2 envelope glycoprotein with receptors on the surface of the target cells, which may be fibroblasts, macrophages, monocytes or endothelial cells [21,22]. Some receptors (DC-SIGN, L-SIGN, heparin sulphate, laminin and integrins) are implicated in the process, but their roles are still unclear [9]. Prohibitin (PHB), phosphatidylserine (PtdSer)-mediated virus entry-enhancing receptors (PVEERs), and glycosaminoglycans (GAGs) have also been suggested as CHIKV receptor proteins in mammalian cells [23–25] and ATPsynthase  $\beta$  subunit in mosquito cells [26,27]. It appears that these proteins facilitate the initial interaction of CHIKV with the cell surface instead of virus absorption; even CHIKV can proceed in the absence of such proteins.



**Figure 3:** Schematic representation of the replication cycle of chikungunya virus. The figure was adapted from Abdelnabiet al., 2015 [28]

CHIKV enters the cell by endocytosis following the binding of the E2 protein to specific receptor(s) on the cell surface (A). Within the endosome, conformational changes occur in viral envelope glycoproteins due to the low pH environment, allowing for fusion between the E1 envelope glycoprotein and the endosomal membrane (B) [29]. There is release of the viral nucleocapsid in the cytoplasm where it is disassembled to release the viral RNA genome (C).

There by, the viral genome is translated by the host cell machinery to generate a non-structural polyprotein that is cleaved to produce the nsP123 precursor and free nsP4 protein. The nsP123 precursor interacts with nsP4 and host cell proteins to form an initial replication complex (viral replicase) that synthesizes the negative strand RNA (D) [21]. The negative strand RNA is then used as a template to synthesize positive-stranded genomic RNA and sub-genomic RNA (26S RNA) that drives the expression of the polyprotein precursor (C-pE2-6K-E1) (E) [29,30].

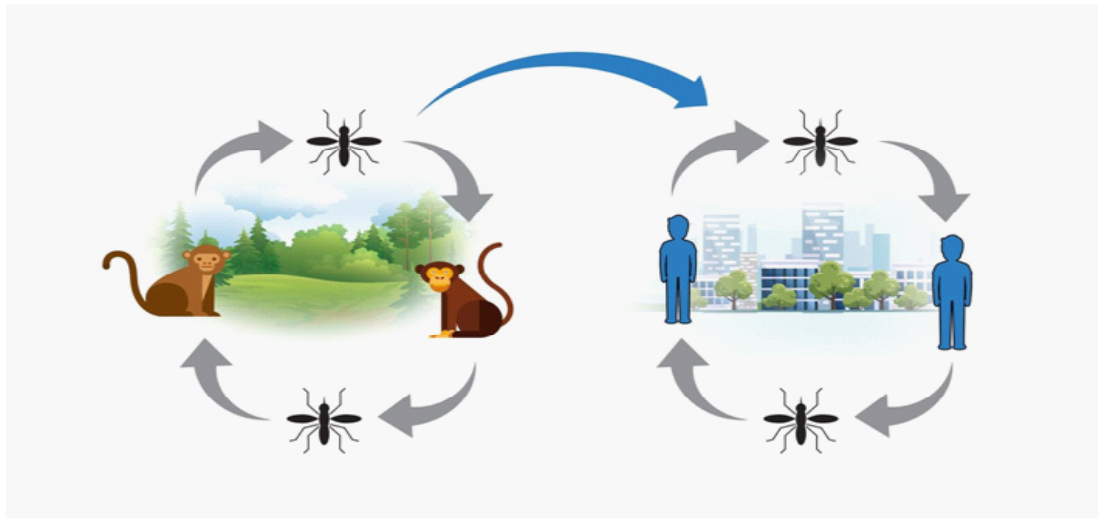
The capsid protein (C) is then released from this polyprotein by its autoprotease activity, while the remaining polypeptide pE2-6K-E1 is processed in the endoplasmic reticulum (ER) (F). Glycoproteins pE2 and E1 form heterodimeric complexes that migrate to the cell membrane through the Golgi complex. During this migration to the cell surface, pE2 is cleaved by a cell furin or furin-like proteinases to form mature E2 and E3. Finally, nucleocapsid complexes collect in the cytoplasm and sprout through the cell membrane by acquiring a lipid bilayer envelope containing virus-encoded E1-E2 glycoproteins (G) (**Figure 3**) [29].

### 3. Transmission Cycle

There are two distinct CHIKV transmission cycles that have been characterized: enzootic/sylvatic and urban. The enzootic cycle occurs in Africa, where arboreal mosquitoes, mostly *Aedes* and *Culex* species, such as *Ae. furcifer*, *Ae. vittatus*, *Ae. fulgens*, *Ae. luteocephalus*, *Ae. dalzieli*, *Ae. campaxhynchites*, *Culex annulirostris*, and *Mansonia uniformis*. Nonhuman primates would be the main reservoirs and hosts of amplification with high seroprevalence rates and levels of viremia in response to experimental infection [31]. The main CHIKV vectors of urban cycle are *Ae. aegypti* and *Ae. albopictus*. The enzootic transmission cycle can spread to infect people who live near enzootic mosquito vectors that may be involved in an interhuman transmission during small outbreaks (**Figure 4**). CHIKV was introduced in urban areas through the anthropophilic vectors present at the site, initiating human-mosquito-human transmission leading to epidemics in Africa [32].

CHIKV can initiate a sustained, urban transmission cycle that only relies on *Ae. aegypti* and/or *Ae. albopictus* and human amplification hosts. This endemic/epidemic cycle results in high levels of human exposure to mosquito transmission, particularly because these vectors live in close proximity to people [32]. *Ae. aegypti* and *Ae. albopictus* are species that have some significant differences, such as the high degree of anthropophily and the preference for

more anthropic sites as well as lower vegetation cover by *Ae. aegypti*, which is the opposite of what occurs with *Ae. albopictus* [33,34]. Both species share similarities in their life cycle and mode of reproduction [22,35].



**Figure 4:** Life cycle of the Chikungunya virus in Africa showing the interconnection between the sylvatic cycle on the left and urban cycle on the right. The virus is maintained in a sylvatic cycle consisting of non-human primates and different species of forest-dwelling mosquitoes including Aedini mosquitoes (*Ae. Africanus*, *Ae. furcifer-taylori*, *Ae. dalzieli*, etc.). The urban cycle virus is maintained between the mosquitoes *Ae. aegypti* and *Ae. albopictus* and humans.

The biology of *Ae. aegypti* is ideal for the development of epidemic events because this species is extremely urban and human-related. Adult females mainly feed on humans, and they are known for gonotrophic discordance (females often have several partial blood meals during a single gonotrophic cycle). This increase significantly affects the chances for virus transmission. Females tend to deposit and spread their eggs in artificial containers as preferred locations for their larvae, and they rest inside houses with ready access to human hosts [31].

The main form of dissemination of CHIKV is through the bite of infected female mosquitoes, although there are cases in which maternal-fetal transmission occurs [36] and there is the risk of transmission through blood transfusion as with other arboviruses. However, this event involving CHIKV has not yet been confirmed [37].

*Ae. albopictus* is zoophilic and anthropophilic, aggressive, silent, active throughout the day, and has a longer lifespan than other mosquitoes (up to 8 weeks). In recent decades, it has spread to several areas that are known to be *Aedes* free [38]. It appears that most of the new introductions of *Ae. albopictus* were caused by diapause eggs contained in timber and tires exported from Asia around the world. *Ae. albopictus* eggs can persist in diapause during low winter temperatures that are unfavorable to adult survival.

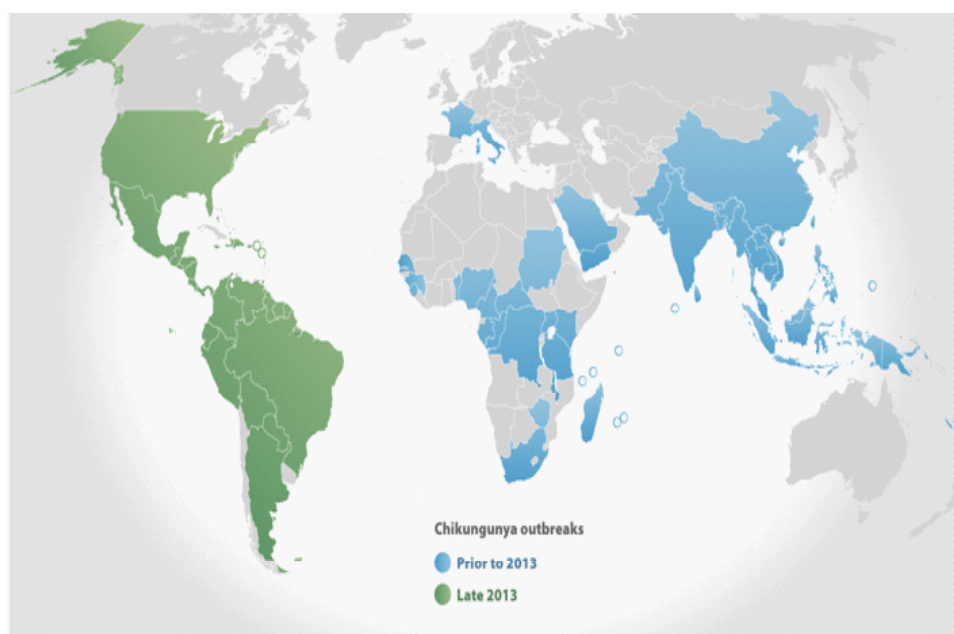
Although the infectivity of different CHIKV strains varies widely for both *Ae. aegypti* and *Ae. albopictus*, humans develop high-titer viremias that generally persist during the first 4 days after the onset of symptoms, with peaks estimated on the first day of symptoms at approximately 9 viral RNA copies/mL and infectious titers sometimes exceeding 7 PFU/mL. These titers generally exceed the oral infectious dose 50% levels for both epidemic vector species,

allowing efficient transmission among humans by mosquitoes [7]. Humans serve as a reservoir for CHIKV during urban epidemic periods, and other vertebrate hosts, such as monkeys, rodents, and birds, serve as a reservoir for the virus to keep circulating in the environment [39].

### 3. Global Expansion and Epidemiology

According to Diallo and colleagues (2016), some ecological factors can potentially contribute to dissemination of CHIKV, such as the temperature, which impacts human migration and the mosquito presence area range; rainfall and vegetation; the availability of breeding sites and new vector species; and human demographic changes linked to population movements due to migration, tourism, and global trade [35].

The number of countries reporting Chikungunya fever cases has increased and reflects the growing number of reported cases and people affected by this disease (**Figure 5**) [40]. The described scenario has roots in factors related to the selection of insecticide-resistant populations, climate changes, globalization and the human population's disregard for eliminating potential mosquito breeding sites. Other factors include the absence of a specific vaccine and medication and the low efficiency of conventional methods for mosquito population control, which is caused by the precariousness of the offered services [2–5].

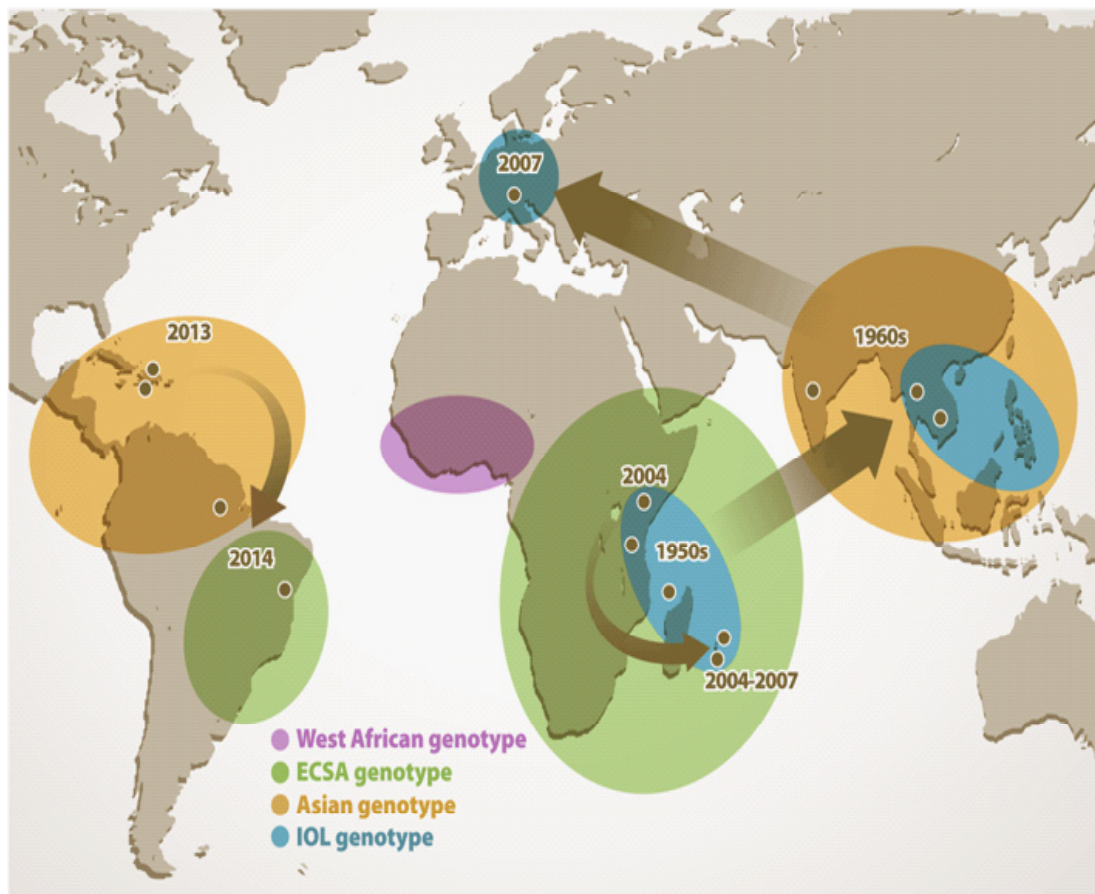


**Figure 5:** Distribution of chikungunya virus all over the globe, concentrating mainly on tropical and subtropical regions according to PAHO/WHO [42]

CHIKV was first isolated in 1952 after an epidemic in Tanzania [4,43,44]. Later on, other countries also started to report Chikungunya cases outside the African continent, especially in Asian countries (1958–1973) [45].

The major outbreak period was between 2004 and 2007; according to the World Health Organization (WHO), more than 272,000 people were infected during an outbreak of Chikungunya in the Indian Ocean islands, including La Reunion and Mauritius, Organization (WHO), more than 272,000 people were infected during an outbreak of Chikungunya in the Indian Oc

ean islands, including La Reunion and Mauritius, where *Ae. albopictus* was the presumed vector. This outbreak wave might have started in Kenya (after disease re-emergence), and it was later spread to the Indian Ocean Islands. A mutated CHIKV may be responsible for this rapid spread; once it improves the vector competence of *Ae. albopictus* makes this mosquito more relevant in the transmission process and not only *Ae. Aegypti* [2,19,45,46].



**Figure 6:** Global expansion of CHIKV between the continents of Africa, Asia and Europe between the years of 1950 and 2015. It originated in Africa, radiated to Asia, Europe and later to America.

The inclusion of a second species transmitting the virus allowed it to reach a wider range of areas, such as Europe during the Italian outbreak in 2007 [47]. The possible reason for the Italian outbreak was the migration of people infected with the virus, who introduced the infection in a coastal village in Italy. This outbreak (197 cases) confirmed that mosquito-borne outbreaks by *Ae. albopictus* are plausible in Europe [2]; later on, cases of Chikungunya were also reported in Croatia [48].

The Union of Comoros had, in 2005, 63% of their main island population infected with CHIKV; the entomological surveillance also detected virus circulation in the *Ae. aegypti* population [49]. In 2006, an outbreak in India had more than 1.5 million cases of Chikungunya with *Ae. aegypti* implicated as the vector [2].

The first reported/confirmed case in the Americas occurred in 2013 on Saint Martin Island [28,50,51]. However, dengue-like infections that were previously reported could have been Chikungunya instead of Dengue, which could be related to an unclear diagnostic protocol or crossed infections [44]. In 2015, the PAHO mentioned more than 690,000 suspected cases



and more than 37,000 confirmed cases of Chikungunya. From the total number of suspected cases, Colombia was responsible for around 350,000 of them with fewer than 150,000 laboratory confirmed cases. Also, Brazil contributed 265,000 suspected cases. However, in 2014, more than 1 million suspected cases were reported in the same region (**Figure 6**) [2].

#### **4. Symptoms, Diagnostic and Treatment**

CHIKV has an incubation period that usually takes between 3 and 7 days, but it may range from 1 to 12 days. The viremia period is approximately 10 days, starting 2 days after the beginning of symptoms. Approximately 70% of the infected individuals have symptoms, but they have a very low mortality rate [52].

Chikungunya fever presents in three distinct phases, the acute, subacute and chronic phases. The first is an acute or febrile phase in which the symptoms are characterized by high fever with a sudden onset and polyarthralgia, which occurs in approximately 90% of the cases and is accompanied by back pain, headaches and fatigue. Retro-ocular pain, chills and conjunctivitis may occur at this stage as well. The fever usually lasts until the fourth day [53].

The subacute phase occurs soon after. At this stage, a fever is not usually present. Severe arthralgia occurs, which can lead to immobilization of the patient; myalgia; pain affecting the head, throat and muscles; red spots; skin eruptions; constipation and conjunctivitis. During diagnosis, there are descriptions of additional symptoms, such as generalized pruritus; maculopapular rash; and the onset of purpuric, vesicular and bullous lesions. In rare cases, peripheral vascular disease and fatigue may develop [54,55].

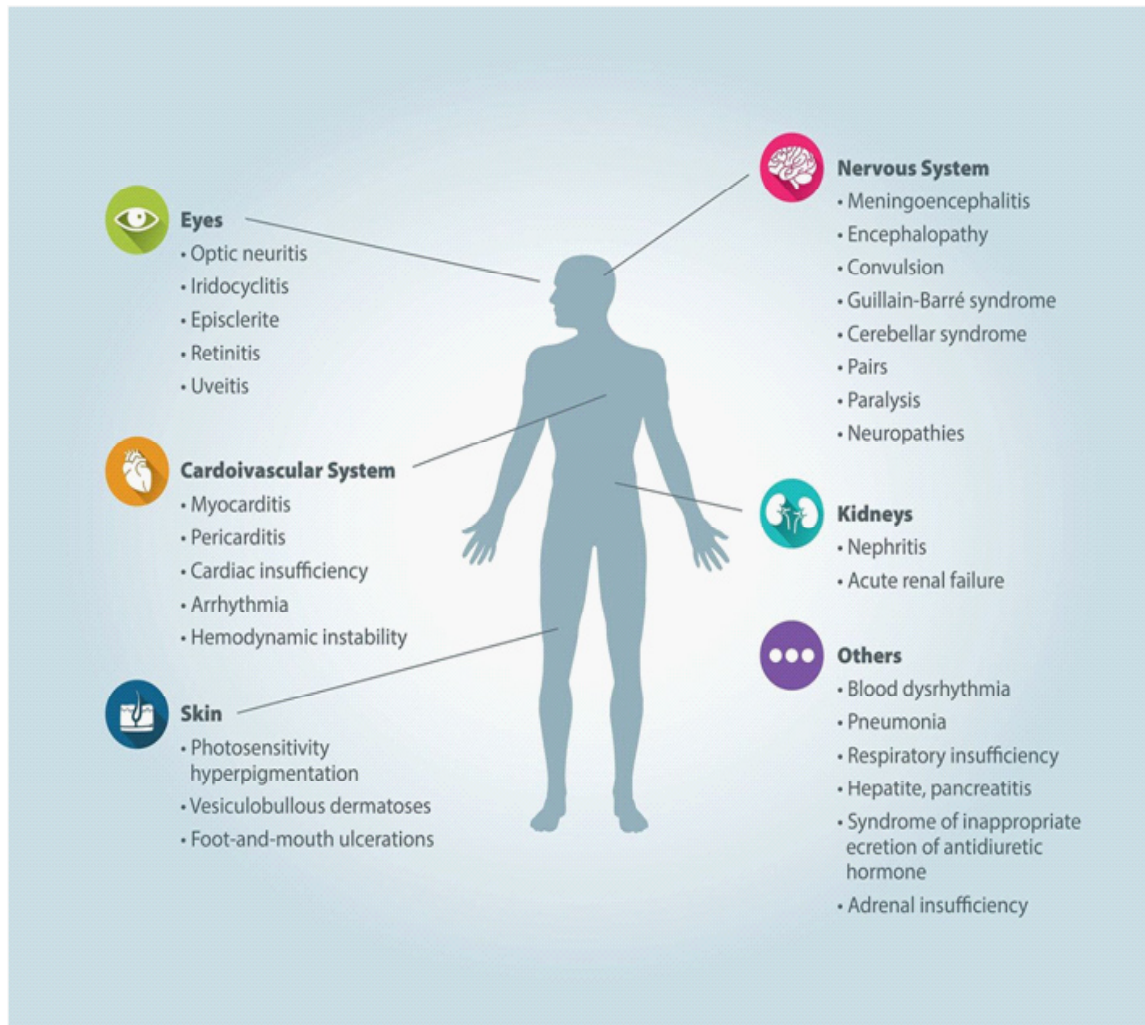
The chronic phase corresponds to the period after 3 months from the initial symptoms, but in some cases can evolve to the chronic phase. At this stage, some symptoms may vary according to the sex and age of the infected individual and may manifest mainly as inflammatory polyarthritis and tenosynovitis [56].

Most patients remain symptom-free for approximately 4 months, but it is common to last for 20 months. In less frequent cases, the symptoms may remain for years, as in a case of a patient who had chronic rheumatism at 24 months. In addition, the presence of CHIKV-specific IgM antibodies has also been reported [57,58].

In their research, Sissoko [57] observed that those over 45 years of age are more susceptible to developing persistent arthralgia. Other factors, such as underlying disorders and severity of pain during infection, may also increase the duration of symptoms [58,59]. Other manifestations were documented during an epidemic, such as neurological, ocular and hemorrhagic complications [60].

Atypical and severe manifestations may occur when CHIKV-infected patients are also

infected by immunological viruses or during drug toxicity. In those cases, the patient may not have fever or joint pain (**Figure 7**) [61–64].



**Figure 7:** Atypical manifestations in CHIKV-infected patients, showing the system and organs affected as well as the resulting manifestations.

CHIKV infection is usually more severe when it occurs in newborns, children, people over 65 years old, and people with pathological comorbidities such as febrile convulsion, diabetes, asthma, heart failure, alcoholism, rheumatic diseases, sickle cell anemia, thalassemia and hypertension. CHIKV infection may also develop to a severe stage in patients using aspirin, anti-inflammatory medications and high doses of paracetamol [64].

Vertical transmission of infected pregnant women is 48%. In the first trimester of pregnancy, CHIKV infection is usually severe and may lead to fetal death [65]. Vertical transmission in pregnant women has also been documented in the last trimester of gestation. In newborns, the symptoms can appear on the fourth day after birth and may develop as a fever, difficulty feeding, cutaneous manifestations, pain and distal edema [65,66].

In severe cases, neurological complications including cerebral edema, intracranial hemorrhage, seizures and encephalopathies may occur in addition to hemorrhagic complications and myocardial involvement [67]. Children usually have the same symptoms as adults. They may have neurological manifestations, such as seizures, altered level of consciousness, blind-

ness, and acute flaccid paralysis [58,68].

It is not possible to detect CHIKV only by analyzing the clinical picture of the patients because it is easily mistaken for other arboviruses such as Dengue and Zika. Cases of double simultaneous infection of more than one arbovirus have also been reported. The Zika virus (ZIKV), Chikungunya (CHIKV) and Dengue (DENV) co-circulate in much of the tropical Western Hemisphere. Three cases of patients in Ecuador who had co infection with ZIKV-CHIKV and three cases of CHIKV were reported. The cases were diagnosed through the Polymerase Chain Reaction using the Reverse Transcriptase Reaction in Real Time (RT-qPCR) [69]. There was evidence of coinfection among DENV-CHIKV in several countries, like Angola, Gabon, India, Madagascar, Malaysia, Myanmar, Nigeria, Saint Martin, Singapore, Sri Lanka, Tanzania, Thailand and Yemen (**Figure 8**) [70,71].



**Figure 8:** Countries with past or current autochthonous transmission of CHIKV, DENV and ZIKV.

Seventeen samples of CHIKV-positive sera were collected during the dengue outbreak in Delhi in 2006, and six of the 17 samples were positive for both CHIKV and DENV [72]. Another study by Wagoner et al. analyzed serum samples from 346 patients with suspected arboviral disease during the acute phase. A multiplex RT-qPCR was performed for ZIKV, CHIKV and DENV, and the detected viremia for each virus was quantified. Two hundred sixty-three patients were positive for a virus, 192 were positive for a single virus (monoinfections), and 71 were positive for 2 or all 3 viruses (coinfections). For each virus, the mean viremia was lower in coinfections than in monoinfections [69,73].

Laboratory tests are the fastest and most efficient method for diagnosing CHIKV infection. The reverse transcriptase reaction followed by polymerase chain reaction (RT-PCR) is a molecular method that can identify the genetic material (RNA) of the virus in the blood. Another method is real-time accelerated reverse-transcription-loop-mediated isothermal amplification (RT-LAMP); this method is similar to RT-PCR, but the cost of analyzing the samples is lower [74–76]. The sensitivity of the LAMP method is 2.7 copies/reaction for CHIKV, and -

no sophisticated instruments are required, which makes this method adaptive to field diagnosis and small-scale hospitals [77].

The rapid diagnostic test of CHIKV is a tool for faster virus detection. The serology test detects the presence of antibodies in the patient's blood. In this method, serodiagnostics are used for detecting immunoglobulin M (IgM) and immunoglobulin G (IgG) against CHIKV in blood and serum samples [78]. Two days after infection, it is already possible to detect the presence of IgM and IgG antibodies through an ELISA immunofluorescence assay. However, only IgG antibody remains in the serum for a longer period of time [79,80].

Virus isolation in cell culture is also used. This method can identify small levels of virus. The samples collected during the viremia period are inoculated into mosquito culture cells or in mammalian cell culture. However, because this approach requires more time, from 7 to 12 days, it is not commonly used [64].

To date, there are no vaccines or antivirals for the preservation and treatment of CHIKV. The therapy used is palliative and focuses on relieving the symptoms alone; it is used in combination with hydration and rest care. Symptomatic treatment consists of the use of analgesics, such as paracetamol; non-steroidal anti-inflammatory drugs (ibuprofen, naproxen, diclofenac, nimesulide, and acetylsalicylic acid); antipyretics and saline solution [58,64]. Aspirin is contraindicated in the acute phase because there is a risk of developing Reye's syndrome and bleeding [64].

Several studies have been performed to better understand the genomic structure, replication cycle and functions of viral loci and proteins in different alphaviruses. These studies showed that the vertebrate immune system eliminates viral infections through apoptosis in a few days, which is a very short time interval compared to invertebrates. However, CHIKV infection in humans is mainly characterized by the persistence of symptoms for a long period of time. According to recent studies, CHIKV has tropism for muscle satellite cells, which serve as a reservoir for the virus [5,71,81].

## **5. Vector Control and CHIKV**

There is no current vaccine for CHIKV. Therefore, preventive measures to reduce transmission are a primary focus for decreasing human-vector contact and vector control [82]. Hence, the vector control associated with entomological surveillance is used to reduce and maintain the vector density below the levels of epidemic transmission [35].

To perform vector control, a methodology known as Integrated Vector Management (IVM) is used, a strategic control approach that is promoted by the WHO. According to the WHO [83], the IVM is defined as “a rational decision-making process for the optimal use of

resources for vector control,” and it considers the following five elements: advocacy, social mobilization and legislation; collaboration within the health sector and with other sectors; an integrated approach to disease control; evidence-based decision-making; and capacity-building. In this sense, the IVM proposal is that control is not only a health sector, but it is a network of collaboration between the public, private and community sectors, the latter of which is a key factor to guarantee the sustainability of actions [84].

The IVM consists of several interventions with proven effectiveness, which are used separately or together, aiming at viable economic control and reducing dependence on any single intervention. This strategy also helps reduce the selective pressure of resistance to insecticides, increasing the useful life of insecticides and/or drugs [84].

IVM, as the main *Aedes* control measure, seeks to eliminate the vector in all life stages through either eliminating breeding sites, which permit the larval development, or eliminating the adult mosquitoes using insecticides [85]. The first type of control is achieved by making it impossible for mosquitoes to access these containers or by constantly emptying and cleaning them, eliminating the aquatic stages of mosquitoes using insecticides or biological control agents. Adult control involves chemical control methods that can be applied with residual surface treatments or space treatments [83]. According to the WHO [86], the control methods, which are categorized into environmental, biological and chemical, can be used separately or in combination.

### **5.1. Environmental methods**

This strategy aims to change the environment to prevent or minimize the spread of vector and human contact with the vector-pathogen. It is based on removing, destroying, recycling or preventing the possibility of containers that accumulate water (temporary), such as bottles, disposable cups, gutters, and other potential breeding grounds. Also, permanent containers (i.e., water tanks and barrels) are covered. Additional approaches are adequate disposal of tires, installation of screens in the windows of homes and the improvement of services of adequate water distribution to the population. These actions could be the pillar of dengue vector control [86].

### **5.2. Biological methods**

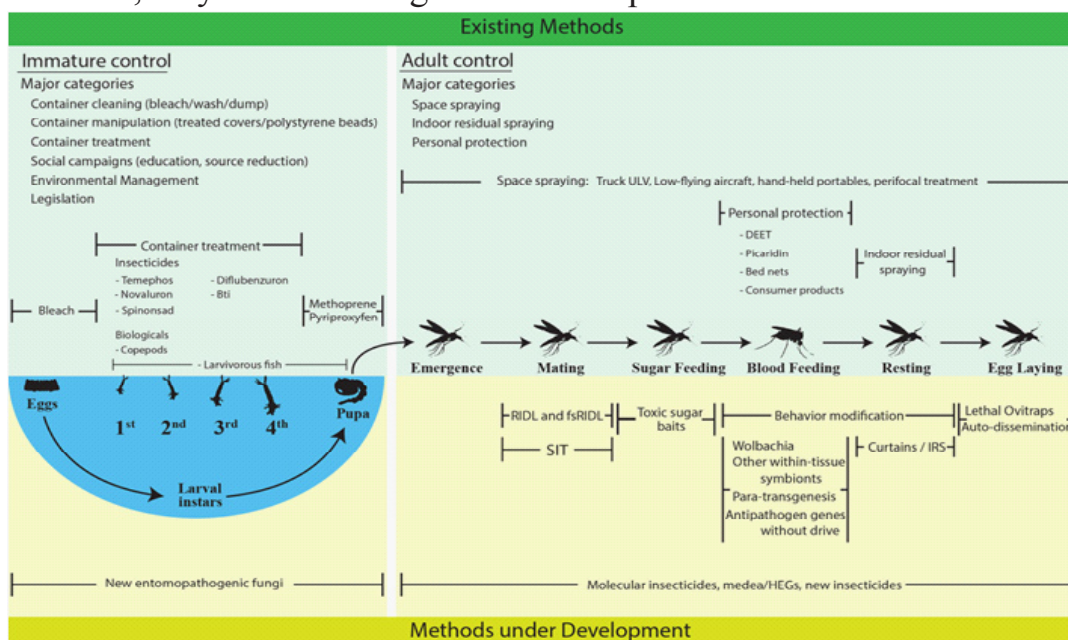
This approach is based on the use of predatory and parasite organisms of mosquitoes or competing species that reduce their populations. Some larvivorous fish and predatory copepods (small freshwater crustaceans) are examples of organisms that can be used against the immature larval stage vector. These organisms should be appropriately raised and distributed to potential mosquito breeding sites [86].

### 5.3. Chemical methods

This type of method can be used to target larval (larvicidal) and adult (adulticide) stages of the mosquito. Chemicals used as larvicides are restricted to containers that cannot be handled according to environmental control, except in emergency situations. Containers for water storage (i.e., water box) should be of low toxicity to other species and should not change the color, odor or taste of water (the WHO has specific guidelines on the use of chemicals in drinking water). Regarding adulticides, their use is intended to impact some parameters of mosquitoes linked to disease transmission, such as population density and longevity. Adulticides may be applied to either ‘residual surface treatments’ or ‘space treatments’. The first one is indicated for specific routine use in real estate and concentrates many potential breeder containers. The space treatments seek to rapidly and largely destroy the vector population, and it is only recommended for control in emergencies to reduce or prevent an epidemic [86,87].

The implementation of the current vector control methodologies faces difficulties that compromise its effectiveness. These difficulties are mainly related to urban infrastructure problems, such as disordered urban growth, inefficient and deficient garbage collection, irregular occupation of areas, and an irregular water supply [88,89]. Other difficulties are the selection of resistant populations to larvicides/insecticides or the inefficiency of the insecticide in reaching the target vector during its application [87,90,91]. Faced with the challenges encountered by the control methods (traditional methods), new tools are being developed and evaluated to complement the traditional control [89,92,93].

These strategies are being called "innovative" and involve several different areas with mechanisms of action ranging from social measures, selective monitoring, different dispersion of insecticides, and new chemical/biological control compounds to molecular procedures [89,94]. The main concepts of some of these innovative approaches will be described below (Figure 9). Of note, they are still being evaluated in pilot tests.



**Figure 9:** Examples of existing and developing *Aedes* control methods. *Bacillus thuringiensis israelensis* (Bti), Female-specific flightless OX36404C (fsRIDL), Indoor residual spraying (IRS), Maternal effect dominant embryonic arrest (Medea), Homing endonuclease genes (HEGs). *The figure was adapted from Achee et al., 2015 [90].*

#### 5.4. Eco-bio-social control

This approach is conducted by various sectors of the community and includes health and environmental education to avoid pesticides. Therefore, the activities focus mainly on eliminating water reservoirs and covering potential breeding sites (working not only to remove the mosquito but also correcting or minimizing the social and environmental deficiencies that favor proliferation). It also involves installing screens on windows and doors [89]. This type of strategy allows those agents, formerly mere receivers of information, to also be agents of vector control [95–97].

#### 5.5. Autodissemination

This approach uses the mosquito itself to spread larvicide in the potential breeding sites. In this strategy, *Aedes* females are attracted to "dissemination stations" (breeding impregnated with the larvicide pyriproxyfen powder), which are distributed throughout the city. When these containers are used as breeding grounds by *Aedes* females, the larvicide will attach to the female's body. Then, when this same female visits another breeding site, the larvicide present in this female will contaminate the water there, making the container incompatible with the survival of the immature mosquito forms [89,98].

#### 5.6. Autocidal control

In 1950, a species-specific technique for insects was developed for birth control of its own population [99]. SIT can control agricultural pests, and several species have developed their own programs. There are numerous success cases [100]. The approach involves the mass rearing of target species, sterilization (via an ionizing source or chemosterilizer) and subsequent release of sterile males. After mating this sterile male, the generation of offspring will be made unfeasible, reducing the reproductive potential of the wild population over time [99,101,102].

Other SIT-based technologies have been developed, such as the "Release of Insects carrying a Lethal gene" (RIDL) [103], and the Incompatible Insect Technique combined with SIT (IIT/SIT). The RIDL technology uses a transgenic *Ae. aegypti* strain carrying a gene responsible for conditioned mosquito lethality, which is transmitted to the offspring after copulation of the transgenic male with the wild-type female [103–105].

Regarding IIT/SIT, the technique is based on the use of a symbiont that is naturally found in populations of several species of insects, the *Wolbachia* bacteria. Males infected with

this bacterium, when copulating with uninfected females, have their offspring affected by Cytoplasmic Incompatibility (CI) that occurs at the time of embryo fertilization [106,107]. The Wolbachia, as a control tool, can be used in another approach, as well as with infected mosquito release, with the purpose of replacing the natural population of *Aedes* by a population infected with bacteria. Such an approach is based on the generated CI, which leads to infeasible offspring that considerably reduces the lifetime of an adult mosquito. Wolbachia's capacity can reduce or eliminate arbovirus transmission, including CHIKV [89,94,108,109].

The innovative approaches, such as autocidal control, will likely become viable tools soon. However, until evaluations are finalized, the focus should remain on improving existing measures [93].

According to Diallo *et al.* [35], to reduce CHIKV's chances of being a threat to public health, it is necessary that control measures are effective and sustainable. However, this goal will only be achieved if perseverance and quality work are linked to a multidisciplinary scientific network (i.e., entomology, virology, and epidemiology). Even so, the success of vector control programs requires an educated and committed community.

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