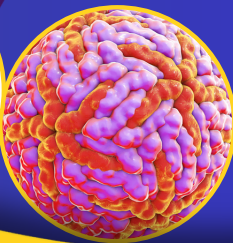
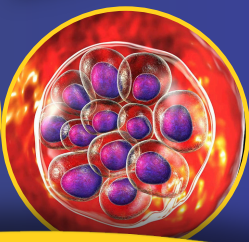
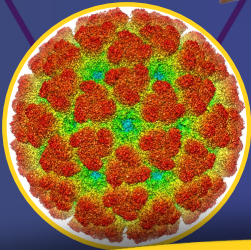


Vector Borne Diseases in India:

Public Health Challenge



DR. M. RAJA

DR. P. K. SRIVASTAVA

DR. S. SELVAKUMAR

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Authours

Dr. M. Raja

Dr. P.K. Srivastava

Dr. S. Selvakumar

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PG and Research Department of Advanced Zoology and Biotechnology

Loyola College (Autonomous),

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Phone :+91-44-28178200



Email: cimserloyola@gmail.com

Website: www.loyolacollege.edu

A Tribute to Legendary Personality in Entomology: Late Prof. T. N. Ananthakrishnan



Prof. T. N. Ananthakrishnan (1925–2017) was a distinguished Indian entomologist and an eminent scientist known for his pioneering contributions to the fields of insect taxonomy, ecology, and physiology. He specialized in the study of thrips (Thysanoptera), and his extensive research significantly advanced our understanding of their taxonomy and biology. Below are highlights of his life and career:

Education and Career

- Prof. Ananthakrishnan earned his degrees from prestigious institutions in India and began his academic career at Loyola College, Chennai.
- He later joined the Entomology Research Institute in Chennai, where he served as its founder-director.
- His leadership played a vital role in establishing this institute as a center of excellence in insect science.

Contributions to Entomology

1. Taxonomy and Systematics

Authored over 200 research papers and several books, many focusing on the taxonomy of thrips, a challenging group of insects. Described numerous new species of thrips, helping to build a foundational understanding of their diversity.

2. Insect Ecology and Physiology:

- Conducted groundbreaking research on insect-plant interactions.
- His studies highlighted the ecological roles of thrips, including their role as pollinators and pests.

3. Academic Contributions

- Mentored a generation of entomologists and inspired students across India and abroad.
- His research legacy continues to influence studies in entomology and agriculture.

Recognitions and Awards

- Prof. Ananthakrishnan was widely recognized for his scholarly contributions. He was a fellow of several scientific academies, including the Indian National Science Academy (INSA).
- He received numerous accolades for his work, reflecting his status as a luminary in his field.

Publications

- Authored books such as "*Bionomics of Thrips*" and "*Dynamics of Insect-Plant Interactions*", which are considered landmark contributions to entomology.

Legacy

Prof. T.N. Ananthakrishnan's work laid a robust foundation for future research in insect biology, particularly in India. His focus on thrips remains invaluable to both theoretical entomology and applied agricultural sciences, as these insects play critical roles in ecosystems and crop health.

A TRIBUTE TO (LATE) PADMA SHRI DR. PYLORE KRISHNAIER RAJAGOPALAN



Pylore Krishnaier Rajagopalan (PKR)

27.10.1930 - Mukteshwar, Uttarakhand

29.04.2024 - Chennai, Tamil Nadu

Pylore Krishnaier Rajagopalan was a distinguished public health expert who made significant breakthroughs in the research of Kyasanur forest disease (KFD), a prevalent tick-borne viral ailment and Japanese Encephalitis (JE) a mosquito borne viral zoonotic disease in South India. PKR, as he was fondly referred, was born in Mukteshwar, Uttarakhand in the year 1930. He did his M.Sc in zoology with special paper of entomology in 1951 from Banaras Hindu University (BHU). He studied dengue, Japanese encephalitis, ticks and mites, Western Equine encephalitis, bird migration, and yellow fever; in Bangkok, Japan, USA, and Germany. In addition, he spent time at the British Museum to understand mosquito taxonomy. He was the first Director of ICMR-Vector Control Research Centre (VCRC) at Puducherry and continued for 15 years until his retirement in 1990. His five-year project on integrated control of lymphatic filariasis (LF) provided ample opportunities to develop the LF elimination programme in the country. His significant contributions to Kyasanur Forest Disease, Japanese encephalitis, lymphatic filariasis, and malaria, will be referred by scientists for many years to come. He initiated M.Sc (Medical Entomology) at VCRC.

Rajagopalan conducted an extensive investigation of KFD's epidemiology and transmission dynamics, establishing a critical foundation for understanding tick-borne viruses and zoonotic infections. His work has advanced scientific knowledge and informed public health strategies for disease control. His response to KFD discovery was an accidental one. In one interview he said "that When an episode of human and monkey deaths was reported from the forests near Sagar, Shimoga district, it was thought that yellow fever (YF), which was eluding India, had broken out. On investigations, it was found to be a tick-transmitted hemorrhagic disease, closely related to Omsk hemorrhagic fever, belonging to the Russian Spring Summer Encephalitis group of viruses. The first isolation was from the tissues of a dead monkey collected from Kyasanur forest, near Barige village, in Sagar taluk, and hence named KFD".

He was awarded the Padma Shri by the Government of India in 1990 and has been felicitated with various other awards such as P.N. Raju oration award of the Indian Council of Medical Research in 1980, Om Prakash Bhasin Award for Science and Technology in 1985 and Charles University, Prague, Gold Medal in 1988. He is an elected Fellow of the Royal Society of Biology and the Royal Society of Tropical Medicine.

My association with him is since 1988 when he was expert in my selection board for Assistant Director in UPSC. I have been visiting his house at Chennai and meeting with him in different conferences. He used to call me "PK".

Dr Rajagopalan lost his wife Leela Rajagopalan in December 2023. After a gap of 4 months, he left all of us on April 29, 2024, at his residence in Chennai, India, after a brief illness. PKR's departure was a great shock to all scientific community engaged in vector control. He is survived by his two sons and a daughter.

As a tribute to this great personality and the doyen of medical entomology, we request all young scientists working in the field of medical entomology and vector control who did not have the opportunity to meet and associate with this great man, to read his articles especially Review Article - at "Ebola, KFD and Bats", originally published in Journal of Communicable Diseases, Volume 51, Issue 4 - 2019, Pg. No. 1-4.

P K Srivastava & Sridharan Subramanian
ABSOLUTE HUMAN CARE FOUNDATION
(CIN: U85320DL2017NPL311121)



FOREWORD

The book on “Vector-Borne Diseases in India: Public health challenge” will provide a value-added opportunity for knowledge sharing among the academicians, research scholars, students and persons engaged in vector control operation and update recent trends in vector biology and control. The book covers various vector borne diseases, its pathogen, vectors and control measures.

The newer technology like artificial intelligence and its use has also been highlighted. The challenges posed in implementation of traditional strategies in the light of infrastructure development, and climate change are discussed which will be of great use to younger generation.

The goal of bringing out the book is to promote scientific information interchange between researchers, developers, students and practitioners working worldwide. Department of Advanced Zoology and Biotechnology is organizing the International Conference in order to articulate research talents and to motivate the research aptitude. The release of this book during the conference will not only popularise about emerging threat and challenges due to various vector borne diseases but also boost the younger generation to have the latest reference on the subject.

I am sure that this book will enrich the knowledge and develop interest in entomology and vector control.

(Dr. M. Raja)



PREFACE

The Third International Conference was conceptualized following discussions with Dr. M. Raja of Loyola College and Dr. Selvakumar, CVCO of Chennai Corporation who readily accepted to organize it jointly by the Centre for Insect Molecular Studies and Environmental Research (CIMSER), PG & Research Department of Advanced Zoology and Biotechnology, Loyola College, Chennai-34, and Absolute Human Care Foundation (AHCF)-A Non-Profit Organization, New Delhi. We are very fortunate to have a platform of a very reputed college which is celebrating its Centenary in 2025. The overwhelming response of academia, Scientists, researchers, public health officials, vector control experts, industry professionals in the field of Entomology and Vector-borne diseases, pest control through social media groups prompted us to gear up for organizing the conference. Dr Raja's proposal of bringing out the book entitled "**Vector Borne Diseases in India: Public Health Challenge**" was appreciated by different subject experts who promptly responded and shared their manuscripts.

The book contains 17 chapters on various vector-borne diseases and I feel that it will serve in updating the knowledge and skill of all those involved in dealing with such vector borne diseases.

I thank to all those who have contributed the chapters of the book without whom, bringing out the book would not have been possible.

The special thanks are due to Dr M. Raja, Assistant Prof, Department of Zoology, Loyola College, Dr S. Selvakumar, CVCO of Chennai Corporation, Dr Sushma Srivastava Editor-in-Chief, AHC Foundation, Dr Anju Viswan K, Scientist C of ICMR-VCRC Puducherry-Koraput Field station and Dr Jayalakshmi Krishnan, Assistant Prof, Department of Biotechnology, Central University of Tamil Nadu, Thiruvavur, India for their support in compilation and editing the book.

P.K. Srivastava

Dr P. K Srivastava
Director
AHC Foundation

**REGD OFFICE: 21-C, NAVYUG APPT. BODELLA PH-2, VIKAS PURI,
NEW DELHI 110018, INDIA**

Phone No.: +91-9910455099

EMAIL: absolutehumancarefoundation1@gmail.com

www.absolutehumancarefoundation.com

ACKNOWLEDGEMENTS

The book entitled “**Vector Borne Diseases in India: Public Health Challenge**” released on 9th December 2024 during the Third International Conference on “**Emerging Challenges in the Prevention and Control of Vector-Borne Diseases**” jointly organized by the Centre for Insect Molecular Studies and Environmental Research (CIMSER), PG & Research Department of Advanced Zoology and Biotechnology, Loyola College, Chennai-34, and Absolute Human Care Foundation (AHCF)-A Non-Profit Organization, New Delhi is an outcome of scientific thoughtful discussions focussing on challenges in prevention and control of vector-borne diseases.

Loyola College, a Catholic Minority Institution, was founded by the Society of Jesus (Jesuits) in 1925. Loyola College affiliated to University of Madras became autonomous in 1978. The department of Zoology has produced 256 PhDs. Till 2024. The Absolute Human Care Foundation (AHCF) is a non- profit organization established in the year 2017, to improve the lives of poor and weaker sections of society with its mission to contribute to the growth and development of health, education, agriculture, and skill development in India. The foundation has been involved in improving capacity building on various vector borne diseases and brought out a book on “Entomological Intelligence” which is a compilation of all presentations made by subject experts in technical sessions during covid-19 period.

The book could be brought out only due to contribution of subject experts of different vector-borne diseases which is highly acknowledged.

The thanks are due to Dr Sushma Srivastava Editor-in-Chief, AHC Foundation, Dr Anju Viswan K, Scientist C of ICMR-VCRC Puducherry-Koraput Field station and Dr Jayalakshmi Krishnan, Assistant Prof, Department of Biotechnology, Central University of Tamil Nadu, Thiruvavur, India, Ms Arokia Ahino Jessie A, Jisha Hannie Selson and Eljeeva Devakumari T CIMSER, Department of Advanced Zoology & Biotechnology, Loyola College, Chennai

Last but not the least, the thanks are also due to the publisher from Loyola College and Siga press without whose support, this book could not have been brought out in short time.

Editors

Dr P K Srivastava, Director AHCF, Dr M. Raja, Assistant Prof, Department of Zoology, Loyola College and Dr S. Selvakumar, CVCO of Chennai Corporation

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ARTHROPODS OF MEDICAL IMPORTANCE

M Raja

Associate Professor & Research Supervisor

Centre for Insect Molecular Studies and Environmental Research (CIMSER)

P.G. & Research Department of Advanced Zoology and Biotechnology

Loyola College (Autonomous), Chennai – 600 034

Mobile: 9962992520, Email: cimserloyola@gmail.com / loyo_raja2003@loyolacollege.edu

Arthropods, the largest phylum in the animal kingdom, encompass a vast array of species that hold significant medical importance for human health. These organisms can impact human health in two primary ways: directly, as parasites and venomous species, and indirectly, as vectors transmitting various pathogens.

The phylum Arthropoda contains a wide diversity of animals with hard exoskeletons and jointed appendages. Many familiar species belong to the phylum Arthropoda – insects, spiders, scorpions, centipedes, and millipedes on land; crabs, crayfish, shrimp, lobsters, and barnacles in water. Arthropods are considered the most successful animals on Earth. The phylum includes more species and more individuals than all other groups of animals combined. Over 85 percent of all known animal species are arthropods (Fig.1).

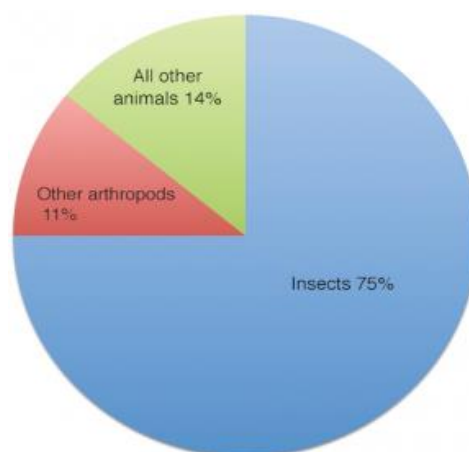


Figure 1. Proportion of arthropods versus all other animals in kingdom Animalia.

The burden of arthropod-borne diseases (ABDs) is particularly pronounced in developing countries, where healthcare systems may be ill-equipped to manage outbreaks. These diseases disproportionately affect vulnerable populations, exacerbating health disparities. The World Health Organization (WHO) estimates that vector-borne diseases account for more than 17% of all infectious diseases, causing over 700,000 deaths each year.

Arthropod-borne diseases are infections transmitted to humans through the bite of infected arthropods. The vectors not only facilitate the transmission of pathogens but also play a critical role in the life cycle of these organisms. Common vectors include mosquitoes, which are responsible for diseases such as malaria, dengue fever, Zika virus, and West Nile virus. Ticks transmit pathogens that cause Lyme disease, Rocky Mountain spotted fever, and tick-borne encephalitis. Sandflies are vectors for leishmaniasis, while triatomine bugs transmit Chagas disease.

The pathogens associated with arthropod-borne diseases can be classified into three main categories: viruses, bacteria, and parasites. Viruses include those causing dengue fever and Zika virus. Bacteria include those responsible for Lyme disease and tularemia. Parasites include Plasmodium species, which cause malaria, and Leishmania species, which cause leishmaniasis.

The spread of arthropod-borne diseases (ABDs) is influenced by various factors. Environmental conditions, such as climate change and urbanization, create favorable conditions for vector proliferation. Socioeconomic factors, including poor living conditions and inadequate access to healthcare, increase vulnerability to ABDs. Additionally, globalization, with increased travel and trade, facilitates the spread of both vectors and pathogens across borders. Controlling arthropod-borne diseases (ABDs) requires an

integrated approach that includes several vector control strategies. Chemical control involves the use of insecticides to reduce vector populations, although resistance to these chemicals is a growing concern. Biological control entails the introduction of natural predators or competitors to manage vector populations. Environmental management focuses on reducing standing water where mosquitoes breed through improved sanitation and urban planning.

Efforts to combat arthropod-borne diseases (ABDs) also involve public health education campaigns aimed at raising awareness about prevention methods such as using insect repellent, wearing protective clothing, and employing bed nets treated with insecticides. Ongoing research is crucial for developing effective vaccines and treatments. For example, vaccines for dengue fever have been developed but require further research for broader efficacy, and malaria vaccines are under continuous development with recent advancements showing promise in clinical trials.

Arthropod-borne diseases pose a significant threat to global health due to their widespread prevalence and impact on vulnerable populations. Arthropods play a dual role in human health as both direct threats through parasitism and venom and indirect threats through their role as vectors for numerous pathogens. The impact of these organisms on global health is profound, necessitating comprehensive control strategies that encompass integrated management approaches, public education, and ongoing research into innovative solutions. Addressing these challenges requires a multifaceted approach that combines effective vector control strategies, public health initiatives, community engagement, and ongoing research into vaccines and treatments. As environmental conditions continue to change, proactive measures will be essential in mitigating the risks associated with ABDs and protecting public health worldwide.

CLASSIFICATION OF MEDICALLY IMPORTANT ARTHROPODS

- **CLASS INSECTA (INSECTS)**

Order Diptera (Flies and Mosquitoes)

Mosquitoes (Family Culicidae)

Mosquitoes are perhaps the most significant arthropod vectors of human disease. Different genera are responsible for transmitting various pathogens:

1. *Anopheles* species: Primary vectors of malaria parasites
2. *Aedes* species: Vectors of:
 - Dengue virus
 - Yellow fever virus
 - Zika virus
 - Chikungunya virus
3. *Culex* species: Vectors of:
 - West Nile virus
 - Japanese encephalitis virus
 - Lymphatic filariasis

Flies

1. Sandflies (*Phlebotomus* and *Lutzomyia* species):
 - Vectors of leishmaniasis
 - Transmit bartonellosis
2. Tsetse flies (*Glossina* species):
 - Vectors of African trypanosomiasis (sleeping sickness)
3. Black flies (*Simulium* species):
 - Vectors of onchocerciasis (river blindness)

Order Siphonaptera (Fleas)

1. Rat fleas (*Xenopsylla cheopis*):
 - Primary vectors of plague (*Yersinia pestis*)
 - Transmit murine typhus
2. Cat and dog fleas (*Ctenocephalides* species):
 - Intermediate hosts for tapeworms
 - Cause flea allergy dermatitis

Order Phthiraptera (Lice)

1. Body lice (*Pediculus humanus humanus*):
 - Vectors of epidemic typhus
 - Transmit trench fever and relapsing fever
2. Head lice (*Pediculus humanus capitis*):
 - Cause pediculosis capitis
3. Pubic lice (*Phthirus pubis*):
 - Cause pediculosis pubis

• CLASS ARACHNIDA

Order Acari (Mites and Ticks)

Ticks

Hard Ticks (Ixodidae)

1. Deer ticks (*Ixodes scapularis*):
 - Vector Lyme disease
 - Transmit babesiosis and anaplasmosis
2. Dog tick (*Dermacentor*):
 - Vector Rocky Mountain spotted fever
 - Transmit tularemia

Soft Ticks (Argasidae)

- *Ornithodoros* species
- Transmit tick-borne relapsing fever

Mites

1. Scabies mites (*Sarcoptes scabiei*):
 - Cause scabies infestation
2. House dust mites (*Dermatophagoides* species):
 - Major cause of allergic reactions

Order Scorpiones (Scorpions)

- Medically important genera include:
 - *Centruroides* (North America)
 - *Tityus* (South America)
 - *Androctonus* and *Leiurus* (North Africa and Middle East)

Order Araneae (Spiders)

- Black widows (*Latrodectus* species)
- Brown recluse (*Loxosceles* species)
- Australian funnel-web spiders (*Atrax* and *Hadronyche* species)

Arthropods as vectors

Vector-borne diseases are those transmitted by vectors. A significant number of these diseases are zoonotic, meaning they can be passed directly or indirectly between animals and humans. Mosquitoes, fleas, sand flies, lice, ticks and mites serve as arthropod vectors. Arthropod vectors consist of mosquitoes, fleas, sand flies, lice, ticks, and mites. These creatures carry parasites by either injecting them directly into the host's bloodstream through their saliva or by introducing the parasites into a pool of blood that forms while they bite the skin.

Arthropods can act as vectors, meaning they significantly contribute to the spread of diseases. Examples of these vector species are mosquitoes, fleas, sand flies, lice, ticks, and mites, all of which are responsible for transmitting various illnesses. These vectors are known as hematophagous, as they feed on blood at certain stages or throughout their entire life cycle. In essence, these arthropods rely on blood for sustenance. Vectors can spread infectious diseases in two ways: actively and passively.

Biological vectors, like mosquitoes and ticks, have the ability to harbor pathogens that thrive inside them and pass them on to new hosts, typically through bites. On the other hand, Mechanical vectors, such as flies, can acquire infectious agents on their outer surface and transfer them via direct contact.



Figure 2. Mechanical vector and Biological Vector

Diseases can also spread through a mechanical or biological vector – usually an arthropod – an animal that transmits the disease from one host to another. In mechanical transmission, a mechanical vector carries the pathogen without being infected itself. For instance, a fly might land on waste and later transfer bacteria from that waste to the food it touches; a person consuming that food could then become infected with the bacteria resulting in diarrhea.

Biological transmission happens when a pathogen multiplies within a living organism, known as a vector, which then spreads the pathogen from one host to another. Arthropods are the primary vectors that facilitate this type of transmission. Most often, these arthropod vectors carry the pathogen by biting the host, leaving a wound that allows the pathogen entry. To aid in its transmission through the bite, the pathogen may partially reproduce in the arthropod's gut or salivary glands. For instance, hemipterans, known as "kissing bugs" or "assassin bugs," spread Chagas disease to humans when they bite and then defecate. This occurs when a person scrapes or rubs the contaminated feces into a mucous membrane or an open wound on the skin.

Biological insect vectors encompass mosquitoes that spread malaria and other illnesses, along with lice that transmit typhus. Additionally, other arthropods such as arachnids, especially ticks, can also serve as vectors by carrying Lyme disease and more diseases, while mites are responsible for transmitting scrub typhus and rickettsial pox. Since biological transmission requires the pathogen to survive and reproduce within the infected vector, it adds complexity to the biology of both the pathogen and its spread.

Arthropods depend entirely on their innate immune system to defend against infections. Research on the innate immunity of insects has significantly contributed to advancements in human immunology, including the identification of Toll-like receptors. While arthropod physiology is more straightforward than that of vertebrates, characterized by a single internal cavity (hemocoel) and circulating fluid (hemolymph), the key elements of innate immunity are similar in both groups. These consist of (i) physical and chemical defenses against infection at epithelial surfaces, (ii) circulating proteins that identify, attach to, or eliminate pathogens (humoral responses) through mechanisms like lysis, agglutination, and melanization, and (iii) circulating cells (hemocytes) that fight pathogens through phagocytosis and encapsulation.

PUBLIC HEALTH IMPACT

• DISEASE BURDEN

Arthropod-borne diseases (ABDs) pose a major global health threat, leading to significant illness and death. These diseases are primarily spread by vectors like mosquitoes and ticks, impacting millions of people around the world.

ABDs include a variety of illnesses such as malaria, dengue fever, leishmaniasis, and Chagas disease. Malaria alone accounts for over 200 million cases annually, with a high mortality rate, especially among young children. Dengue fever affects 50-100 million people each year, causing severe flu-like symptoms and sometimes leading to fatal complications. Leishmaniasis, transmitted by sandflies, results in 700,000 to 1 million new cases annually, with its visceral form being particularly deadly if untreated. Chagas disease, spread by triatomine bugs, infects 6-7 million people, often leading to chronic heart and digestive system disorders.

The prevalence of these diseases is influenced by factors such as climate change, urbanization, and global travel, which facilitate the spread of vectors. Efforts to combat ABDs include vector control programs, public health education, and improved access to healthcare. Addressing the socioeconomic conditions that contribute to the spread of these diseases is crucial for effective prevention and control.

MOSQUITO VECTORS AND THE DISEASES:

The *Anopheles* mosquito is a known carrier of diseases like malaria, filariasis, and arboviruses (arthropod-borne viruses). When it feeds on a host, it punctures the skin with its mouthparts and transfers parasites from its salivary glands directly into the bloodstream. Along with *Anopheles*, other insects like sand flies and black flies also act as pool feeders. Sand flies, which transmit Leishmaniasis, spread parasites through their saliva, while black flies, responsible for Onchocerciasis, release parasites into the bloodstream from their heads. Additionally, Tsetse flies carry the parasite that causes human African trypanosomiasis (or African sleeping sickness). *Aedes* mosquitoes also transmit diseases like avian malaria, dengue fever, and yellow fever, with their bloodsucking bites spreading at least six potentially fatal diseases.

Malaria

Malaria remains one of the most severe ABDs, caused by the *Plasmodium* parasite and transmitted by *Anopheles* mosquitoes. The World Health Organization (WHO) estimates that there are approximately 249 million cases of malaria each year, leading to over 608,000 deaths annually, predominantly among children under five years old². The disease is endemic in many tropical and subtropical regions, where environmental factors such as climate and socioeconomic conditions significantly influence transmission rates.

Dengue

Dengue fever is another major ABD, transmitted by *Aedes* mosquitoes. It is estimated that 50 to 100 million infections occur each year globally. The WHO reports that around 96 million symptomatic cases are recorded annually, with approximately 40,000 deaths attributed to the disease². The incidence of dengue has increased dramatically in recent decades due to factors such as urbanization and climate change, which facilitate mosquito proliferation.

Zika Virus

It is often asymptomatic but can cause symptoms such as fever, rash, joint pain, and conjunctivitis. Pregnant women who are bitten by an infected mosquito risk complications like miscarriage, preterm birth, or birth defects like microcephaly. Besides mosquito transmission, Zika can also be spread sexually.

Yellow Fever

Carried primarily by *Aedes* and *Haemagogus* mosquitoes, yellow fever causes symptoms like fever, headache, and muscle pain. It can lead to jaundice, giving the skin and eyes a yellowish hue. Diagnosis can be challenging, as it may resemble other diseases like malaria. An effective vaccine is available, and the World Health Organization's EYE Strategy aims to protect one billion people by 2026 in Africa and South America, where the risk is highest.

Chikungunya

The Chikungunya virus, spread by *Aedes* mosquitoes (such as *Aedes albopictus* and *Aedes aegypti*), has expanded its range over the past 30 years. It causes symptoms like fever and severe joint pain. While no vaccines or antiviral treatments are currently available, research is ongoing.

FLEA VECTORS AND THE DISEASES

Fleas transmit germs that cause disease primarily through the processes of feeding on hosts or through fecal contamination, when infected flea feces (poop; also called “flea dirt”) are scratched into an open wound. Fleas prefer animal hosts but will resort to biting people when animals are unavailable. Adult fleas find hosts by detecting body heat, movement, the vibrations caused by movement, and breathing.

Tapeworms

Fleas are common carriers of tapeworms, which can infect both animals and humans. Humans and pets may ingest infected fleas, leading to tapeworm infections. Symptoms often include the passage of small segments of the worm in stool, and untreated infections can cause fatigue, weight loss, and diarrhea. Treatment typically involves medication to kill the tapeworm.

Bartonellosis (Cat Scratch Disease)

Bartonellosis, also known as cat scratch disease, is caused by the *Bartonella* bacteria. While it is most often transmitted through scratches from infected cats, fleas can also spread the bacteria to humans. Symptoms include fever, fatigue, and swollen lymph nodes. Antibiotics are typically used for treatment.

Murine Typhus

Murine typhus, or flea-borne typhus, occurs when fleas infected with *Rickettsia typhi* bacteria bite humans. It causes fever, chills, body aches, nausea, and a rash. Although rarer in the US, it is common in tropical climates. Antibiotics are used for treatment.

Tungiasis

Tungiasis is caused by a specific flea species, the Tunga flea, which burrows into the skin after biting. It typically affects the feet and lower legs and creates painful, itchy lesions. Treatment involves removing or killing the flea with anti-parasitic medications.

Bubonic Plague

The bubonic plague, responsible for the historical Black Death, is still present today. Fleas infected with the *Yersinia pestis* bacteria can transmit the disease to humans. Early symptoms include swollen lymph nodes, fever, and chills, and if left untreated, the bacteria can spread to the bloodstream (septicemic plague) or lungs (pneumonic plague). Antibiotics are effective for treatment.

HOUSEFLY VECTOR AND THE DISEASES

House flies can carry a variety of pathogens, including bacteria, fungi, viruses, and parasites. These pathogens can cause serious diseases in humans and animals. House flies can spread disease by carrying pathogens on their feet or mouth parts and contaminating food or water. They can also contaminate food and eating utensils because they live with humans. These pests may also transmit the eggs of parasitic

worms, which cause their own issues. Houseflies transmitted many of helminthic eggs as *E. vermicularis*, *S. stercoralis*, *T. trichiura* and *T. caracanis*, *Trichomonas*, *Diphyllbothrium*, *hymenolepis*, *taenia* and *Dipylidium* species, and protozoa cysts and trophozoites as *E. histolytica*, *Giardia lamblia*, and some bacteria as *E. coli*, *Shigella* species and *Salmonella*. In addition to viruses, fungi are also isolated. Control methods are used for suppression of the housefly population.

House flies are believed to transmit at least 65 diseases to humans, including typhoid fever, dysentery, cholera, poliomyelitis, yaws, anthrax, tularemia, leprosy, and tuberculosis. They spread disease by regurgitating and excreting wherever they land, thereby mechanically transferring pathogens. Other diseases carried by house flies include Anthrax, Cholera, Salmonella, Tuberculosis and Typhoid.

Typhoid fever

It is caused by the bacterium *Salmonella enterica* serotype Typhi. It spreads through the fecal-oral route, meaning it is transmitted when a person consumes food or water contaminated with the feces of an infected individual. This can happen in areas with poor sanitation or where food handling practices are inadequate.

Trachoma

It is a bacterial infection caused by *Chlamydia trachomatis*. It spreads through personal contact, such as touching the eyes or face of an infected person, and also through contact with flies that have come into contact with discharge from the eyes or nose of an infected individual.

Anthrax

It can spread through contact with infected animals or contaminated animal products. It can also occur through inhalation of anthrax spores, or by eating or drinking food or water contaminated with spores.

TICKS VECTOR AND THE DISEASE

Ticks are indeed significant vectors of disease, transmitting a wide range of pathogens that can affect both humans and animals. They belong to the order Ixodida, which includes hard ticks (family Ixodidae) and soft ticks (family Argasidae). These arachnids are second only to mosquitoes in terms of their global importance as disease vectors to humans, and they are the primary vectors for various diseases in domestic and wild animals.

MITES VECTOR AND THE DISEASE

Mites are closely related to ticks, but they feed on tissue juice instead of blood and don't transmit as many diseases. Mites are responsible for transmitting a range of zoonotic diseases in India, including scrub typhus, scabies, demodicosis, and other conditions such as trombiculosis and mite dust allergy. Scrub typhus, caused by the rickettsial pathogen *Orientia tsutsugamushi*, is transmitted by the mite *Leptotrombidium deliense* and primarily affects humans through bites from infected mite larvae. Rodents serve as the reservoirs for this pathogen, facilitating its spread. *Sarcoptes scabiei*, the itch mite, causes scabies, a common skin condition that leads to intense itching and rashes. Infections such as dermatitis, trombiculosis, and mite dust allergies are linked to mites in the Pyemotidae family. Demodicosis, caused by the mites *Demodex folliculorum* and *Demodex brevis*, is another condition prevalent in India. *Dermanyssus gallinae*, the fowl mite, can cause pruritis in poultry and can be transmitted to poultry workers through contact with infected birds. There has been a resurgence of mite-borne diseases, particularly scrub typhus, in many parts of India, highlighting the urgent need for effective control measures.

Table 1: Vector, Vector borne diseases and pathogens

Vector	Species Name	Pathogen	Diseases
Mosquito 	<i>Anopheles stephensi</i> (Asian Malaria Mosquito)	Plasmodium	Malaria, Dengue
		Virus	Arboviruses Filariasis, Zika
	<i>Aedes albopictus</i> (Asian tiger Mosquito)	Filarial nematodes	Arboviruses, Zika
	<i>Aedes aegypti</i> (Yellow fever Mosquito)	Yellow Fever Virus	Yellow Fever, Zika, Dengue
	<i>Culex quinquefasciatus</i> (Southern house mosquito)	West Nile Virus	Arboviruses Lymphatic Filariasis, west nile.
Tick 	<i>Oxides scapularis</i> (black legged tick)	Bacteria	Lyme disease, Ehrlichiosis, Anaplasmosis.
	<i>Amblyomma americanum</i> (Lone Star tick)	Bacteria	Human monocytotropic ehrlichiosis, Rickettsioses, Tularemia (rabbit fever), Alpha gel syndrome.
		Virus (Bourbon)	Virus disease (Bourbon)
	<i>Rhipicephalus appendiculatus</i>	Protozoan (Theileria)	Theileriosis (in cattle)
	<i>Dermacentor variabilis</i> (American dog tick)	Bacteria	Tularemia (rabbit fever)
	<i>Dermacentor andersoni</i>	Bacteria	Rickettsia (Rocky Mountain spotted fever), Babesiosis, Q- Fever, Tick paralysis
	<i>Haemaphysalis spinigera</i> & <i>Haemaphysalis turturis</i>	Virus	Kyasanur Forest Disease (KFD)
	<i>Hyalomma</i> spp	Virus	Crimean-Congo hemorrhagic fever (CCHF)
FLEAS 	<i>Ctenocephalides felis</i>	Bacteria	Bartonellosis (cat scratch disease or cat scratch fever)
	<i>Xenopsylla cheopis</i> (rat flea)	Bacteria, tapeworm	Murine Typhus (Rickettsia typhi)
	<i>Ctenocephalides felis</i> (cat flea)	Bacteria	
	<i>Leptopsyllia segnis</i> (mouse Flea)	Bacteria	
	<i>Xenopsylla cheopis</i> (rat flea)	Bacteria	Bubonic Plague

FLIES 	<i>Musca domestica</i>	Bacteria	Cholera, Typhoid, Salmonellosis, Cholera; Conjunctivitis; Dysentery; Gastroenteritis, tuberculosis, Anthrax
	<i>Culicoides paraensis</i> midge	Virus	Oropouche fever
	<i>Simulium yahense</i> (Blackfly)	Filarial worm	Onchocerciasis (river blindness)
	<i>Phlebotomus papatasi</i> (Sand fly)	Protozoa	Leishmaniasis (Kala -azar)
		Virus	Sandfly fever (phlebotomus fever)
	<i>Glossina austeni</i> (Tsetse flies)	Protozoa	Sleeping sickness (African trypanosomiasis)
	<i>Triatoma infestans</i> , <i>Triatoma dimidiata</i> and <i>Rhodnius prolixus</i> (Triatome bugs)	Protozoa	Chagas disease (American trypanosomiasis)
	<i>Pediculus humanus humanus</i> (Lice)	Bacteria	Typhus, Louse-borne relapsing fever(LBRF)

ECONOMIC IMPACT

The economic impact of arthropod-borne diseases (ABDs) is significant, encompassing direct medical costs, lost productivity, vector control expenses, and research and development investments. Direct medical costs arise from hospitalization, treatment, and preventive measures such as vaccinations and vector control interventions. Lost productivity occurs when infected individuals miss work due to illness, leading to economic losses; for example, dengue fever has resulted in millions in lost wages in endemic regions. Vector control programs incur substantial operational costs and require community engagement for effectiveness. Additionally, the development of vaccines and innovative control strategies necessitates considerable research funding. Addressing this economic burden requires coordinated efforts from governments and health organizations to enhance prevention and treatment strategies, ultimately improving public health and economic stability.

CONTROL STRATEGIES

Integrated Vector Management (IVM)

Integrated Vector Management (IVM) is a comprehensive approach to controlling arthropod-borne diseases by combining multiple strategies to reduce vector populations and disease transmission. It involves the use of chemical control, biological control, environmental management, personal protection measures, community engagement, surveillance and monitoring, and supportive policies and legislation. By integrating these components, IVM aims to achieve sustainable and effective control of vector-borne diseases, ultimately reducing their impact on public health.

1. Chemical Control

Chemical control involves the use of various substances to manage vector populations effectively. Insecticides are chemicals specifically designed to kill or repel insects, and their application can be targeted to minimize impact on non-target species. Repellents are substances that deter vectors from biting humans, thereby reducing the incidence of disease transmission. Growth regulators are chemicals that disrupt the development of insect larvae, preventing them from reaching maturity and reproducing.

2. Biological Control

Biological control utilizes natural enemies to suppress vector populations. Natural predators, such as certain insects or birds, feed on vectors and help control their numbers. Parasitic organisms live on or in vectors, ultimately harming or killing them. Pathogenic microorganisms, including bacteria, fungi, or viruses, can be introduced to infect and kill vectors, effectively reducing their populations.

3. Environmental Management

Environmental management focuses on modifying the environment to reduce vector habitats. Source reduction involves eliminating standing water where vectors breed, which is crucial for controlling mosquito populations. Habitat modification entails altering environments to make them less conducive to vector survival and reproduction, significantly impacting disease transmission rates. Improved sanitation ensures clean living conditions, helping to prevent vector breeding and reducing human exposure.

4. Genetic Control

Genetic control methods aim to alter vector populations through innovative techniques. The Sterile Insect Technique (SIT) involves releasing sterilized male insects into the wild to mate with females, resulting in no offspring and gradually reducing the population. Gene Drive Systems ensure that specific traits are passed on more rapidly through generations, potentially reducing vector populations or altering their ability to transmit pathogens. Modified Symbionts involve introducing beneficial microorganisms into vectors, which can affect their reproductive capabilities or susceptibility to pathogens.

Personal Protection Methods

Personal protection methods are essential for reducing individual risk of infection in addition to Integrated Vector Management (IVM) strategies. Insecticide-treated bed nets provide a barrier against biting insects while sleeping, significantly lowering malaria transmission rates. Wearing protective clothing, such as long sleeves and pants, can reduce skin exposure to vectors. Applying insect repellents containing DEET or other effective ingredients helps deter bites from mosquitoes and other vectors. Installing screens on windows and doors can prevent vectors from entering living spaces, further reducing the risk of disease transmission. By integrating these diverse strategies, IVM aims to create a sustainable and effective framework for managing arthropod-borne diseases, ultimately protecting public health while minimizing environmental impacts.

FUTURE CHALLENGES

Insecticide Resistance

Insecticide resistance poses a significant challenge in managing arthropod populations that transmit diseases. This resistance develops through several mechanisms, including metabolic resistance, where insects evolve to detoxify insecticides more efficiently, allowing them to survive exposure. Target-site resistance involves changes at the molecular level that prevent insecticides from binding effectively, reducing their efficacy. Penetration resistance occurs when alterations in the insect cuticle slow down the absorption of insecticides.

To manage resistance, strategies include Integrated Resistance Management (IRM), which employs a combination of different insecticides with varying modes of action to prevent the selection of resistant populations. Non-chemical control techniques incorporate biological control agents and cultural practices to reduce reliance on chemical insecticides. Monitoring and scouting involve regularly assessing pest populations to identify early signs of resistance and adjusting management practices accordingly. Alternative control methods, such as biopesticides and genetically modified organisms (GMOs), are also being explored to provide sustainable options against resistant vectors.

Climate Change Impact

Climate change significantly influences the dynamics of arthropod-borne diseases by altering vector behavior and disease transmission patterns. Warmer temperatures and changing precipitation patterns enable vectors like mosquitoes and ticks to expand into new geographic areas, potentially introducing diseases to previously unaffected regions. Variability in climate can affect the life cycles of vectors and pathogens, leading to altered transmission seasons and increased incidence rates in certain areas. Adaptation strategies are essential for mitigating these impacts. Implementing robust surveillance systems to track changes in vector populations and disease outbreaks associated with climate fluctuations is crucial. Additionally, developing public health preparedness plans that account for potential shifts in disease patterns due to climate change ensures healthcare systems can adapt quickly.

Emerging Diseases

The emergence of new diseases linked to changing pathogen-vector relationships is a growing concern. As ecosystems change, previously unknown interactions between pathogens and vectors may arise, leading to the emergence of new diseases or the re-emergence of known ones. To address these challenges, effective strategies include establishing enhanced surveillance systems that can detect emerging pathogens early, allowing for timely public health responses. Additionally, developing flexible response strategies, such as vaccination campaigns and vector control measures tailored to specific outbreaks, is crucial for managing new threats.

CONCLUSION

Understanding medically important arthropods is vital for public health due to their significant role in transmitting diseases. These arthropods, such as mosquitoes, ticks, and flies, are vectors for various pathogens that cause illnesses like malaria, dengue fever, Lyme disease, and leishmaniasis. The impact of these diseases is profound, leading to high morbidity and mortality rates globally.

Continued research is essential to uncover new insights into the biology and behavior of these vectors, which can inform the development of more effective control strategies. Surveillance systems are crucial for monitoring vector populations and disease outbreaks, enabling timely interventions to prevent the spread of diseases.

Developing novel control strategies is also critical. This includes advancements in chemical control, such as new insecticides and repellents, as well as biological control methods that utilize natural predators or pathogens to reduce vector populations. Environmental management practices, such as eliminating breeding sites and improving sanitation, play a significant role in reducing vector habitats.

Additionally, genetic control methods, like the Sterile Insect Technique (SIT) and gene drive systems, offer innovative approaches to managing vector populations. Personal protection measures, such as insecticide-treated bed nets and repellents, are also essential components of a comprehensive strategy to protect individuals from vector-borne diseases.

By integrating these diverse strategies, public health initiatives can effectively manage arthropod-borne diseases, ultimately reducing their impact on human health and improving overall well-being.

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MALARIA: AN OVERVIEW

P K Srivastava¹, Pradhan M M², Jayalakshmi K³, Anju Viswan K⁴ and Anupam V Sharma⁵

¹Absolute Human Care Foundation, New Delhi

²Directorate of Public Health (VBD), Boudh, Odisha

³Vector Biology Research Laboratory, Department of Biotechnology, Central University of Tamil Nadu, Thiruvavur, India

⁴VCRC Field station, Koraput, ICMR-VCRC, Puducherry

⁵Department of Zoology, Hindu College, University of Delhi
Email: pkmalaria@yahoo.co.in

Abstract

Malaria is a significant public health challenge affecting people from across the globe. The disease is caused by protozoan parasites of the genus *Plasmodium* of which 4 species were known to cause human malaria namely *Plasmodium (P) vivax*, *P. falciparum*, *P. malariae* and *P. ovale*. Recently *P. knowlesi* has also been recognised to transmit human malaria. These parasites are transmitted to humans through the bites of infected female *Anopheles* mosquitoes. In some cases, malaria can also be transmitted through blood transfusion, organ transplants, or from mother to child during childbirth. According to WHO, there were an estimated 249 million cases of malaria in 2022, with malaria deaths of 608,000. In 2022, the African Region was home to 94% and 95% of malaria cases and deaths, respectively. Children under 5 years of age are the most vulnerable group affected by malaria. Since Malaria is a hot topic to date, our chapter will cover insights on malaria transmission, the parasite life cycle, and its prevention and control.

Keywords: Malaria, plasmodium, lifecycle, vectors, mosquitoes

History

Malaria is an ancient febrile disease and consistently incomparable parasitic cause of human suffering across the world. The footprint of the human genome shows signs of malaria in the ancestors of human species (Cholera *et al.*, 2008). There are references to this disease in Chinese documents from around 2700 BCE, clay tablets from 2000 BCE, Egyptian papyri from 1570 BCE, and Hindu scriptures from the sixth century BCE. Besides, the early Greeks were fully aware of the traits of ill health, malarial fevers, and big spleens seen in people living in marshy areas, including Homer in approximately 850 BCE, Empedocles of Argenton in approximately 550 BCE, and Hippocrates around 400 BCE. For around 2500 years, malaria transmission was assumed to be due to the miasmas rising from swamps persisted (Cox, 2010), although this has been contested. It has been accepted that the word malaria has been derived from the Italian word malaria, meaning bad air (Tuteja, 2007). Hippocrates could distinguish between quotidian, tertian, and quartan fevers as well as the clinical signs of the illness by 500 BCE (Taylor *et al.*, 2012). The hunt for the malarial agent was stepped up after Louis Pasteur and Robert Koch developed the germ theory in 1878–1879 and Antonie van Leeuwenhoek identified bacteria in 1676 (Cox, 2010). Malaria scientific studies could begin only after the discovery of malaria parasite by Charles Louis Alphonse Laveran in 1880, Sir Ronald Ross first demonstrated in 1897 that malaria parasite transmitted to healthy humans from infected birds through mosquito bites (Sinden, 1985). Within 20 years of Laveran's discovery of a crescent-shaped sexual stage of *Plasmodium falciparum* all the prevalent concepts regarding malaria changed (Bruce-Chwatt, 1981) between 1898 and 1900. Italian researchers Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi, and Ettore Marchiafava implicated mosquitoes as the carriers of human malaria, conforming the Ronald Ross's earlier discovery (Cox, 2010) in 1897. They demonstrated that the parasite originated in the anopheline mosquito and was transferred from the *Anopheles* mosquito transmitting the parasite to the person. Both Ronald Ross and Laveran

were awarded the Nobel prizes in 1902 and 1907, respectively for their historical achievement in malaria transmission discovery (Cox, 2010).

Malaria is caused through man-mosquito-man transmission cycle. The female anopheline mosquito after being infected by a parasitic blood meal from an infected person develops and carries the tiny infective stage of the malaria parasite- plasmodium- and transmit to a healthy person while attempting for a blood meal.

Plasmodia - the Malaria parasites

Malaria is caused by an apicomplexan protozoan parasite which belongs to the *Plasmodium* genus. More than 200 *Plasmodium* species have been formally described and each infects a certain range of vertebrate hosts (Sato, 2021). Following five varieties of malaria parasites naturally infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*. The first four species are specific for humans while macaque monkey is the natural host for the *Plasmodium knowlesi* and thus it has zoonotic cycle, mostly found in South-East Asia (Sato, 2021). Recently in some forest fringe indigenous communities of Malaysia, simian malaria parasites have been detected like *P. cynomolgi*, *P. inui*, *P. coatneyi*, *P. inui-like* and *P. Simi ovale* (Yap *et al.* , 2021).

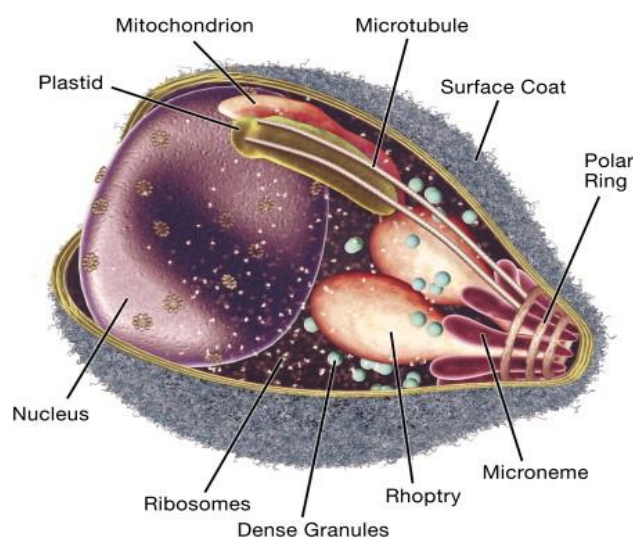


Figure 1. Structure of *Plasmodium*
(Source: Bannister *et al.*, 2000)

Malaria parasite life cycle

The life cycle of malaria parasite occurs in two types of hosts: human and anopheline mosquito. Though very complex, all malaria parasites infectious to human exhibit similar life cycles (Votýpka *et al.* , 2016). There are two discrete phases in the parasite life cycle: (a) asexual phase occurs in human and other primates and (b) sexual phase occurs inside the body of the female anopheles mosquito. The sporozoite stage is the infective stage of the parasite which after the bites of an infected female anopheline mosquito, enter to the body of the human host through its saliva , which is the beginning of the life cycle of the malaria parasite in the human host (Frischknecht & Matuschewski, 2017). Before infecting the erythrocytes, the sporozoites infect the liver cell (Shortt & Garnham, 1948). Sporozoites deposited in the dermis of the human host swiftly move to the liver which takes 30 to 60 minutes (Amino *et al.* , 2006) and multiply by thousands in the liver cells and this process is known as hepatic schizogony (Prudencio *et al.* , 2006). After infecting liver cells, sporozoites undergo different stages of development inside the human

body with some species-specific variances. During this phase, sporozoites multiply and evolve into merozoites. In case of *P. vivax* and *P. ovale* most of the times a dormant stage may persist in liver known as hypnozoites which cause relapses by invading blood streams even after many weeks and years (Ejigiri & Sinnis, 2009). After being released from liver, merozoites enter to red blood cells and multiply asexually manifold. In a red blood cell, one merozoite multiplies by the process of schizogony resulting in the production of 8-64 new merozoites. The number of merozoites depends upon the parasite species (Gerald *et al.*, 2011). These new merozoites are released back to the blood and the parasites repeat this intra-erythrocytic propagation cycle every 24 hours (*P. knowlesi*), 48 hours (*P. falciparum*, *P. ovale*, and *P. vivax*) or 72 hours (*P. malariae*). Some merozoites then differentiate into the next developmental stage; gametocyte which is responsible for the sexual reproduction (Josling & Llinás, 2015). The infected liver cells get necrosis and the liver merozoites get released to the circulation, thus the red blood cells get infected. This stage of the parasite in blood is known as erythrocytic schizogony. In this stage, the merozoites inside the red blood cells multiply, resulting in the destruction of millions of red blood cells. After the initial waves of asexual parasites, there occurs few intermittent cycles in which male and female gametocytes are produced during the blood stage. This process is called gametocytogenesis. Mature gametocytes are detected in the bloodstream at days 7 to 15 (Day *et al.*, 1998; Smith *et al.*, 1995).

Gametocytes are the sexual stage of the parasite that enter the mosquito body once the mosquito sucks gametocyte-containing human blood. The male and female gametocytes differentiate to create microgametes and macrogametes respectively, once they are activated by the unique environment of the mosquito midgut lumen (Bennink *et al.*, 2016). Gametocytogenesis or differentiation of gametocyte depends on the parasite species. *P. falciparum* needs several cycles of intra-erythrocytic propagation before it starts differentiation into gametocytes, whereas *P. vivax* continuously produces gametocytes even in its early intra-erythrocytic propagation cycles. Each gametocyte has a similar appearance during its early development. Gametocytes are differentiated into either male or female gametes which completes the life cycle of the malaria parasites inside the human body. In the mosquito body the parasite's zygote is the sole embryonic stage with a diploid genome and it is created when the microgamete fertilises the macrogamete (Sinden & Hartley, 1985). Recombination can occur in zygotes, according to genetic crossing experiments using gametocytes from two *P. falciparum* clones with various allelic variations (Walliker *et al.*, 1987).

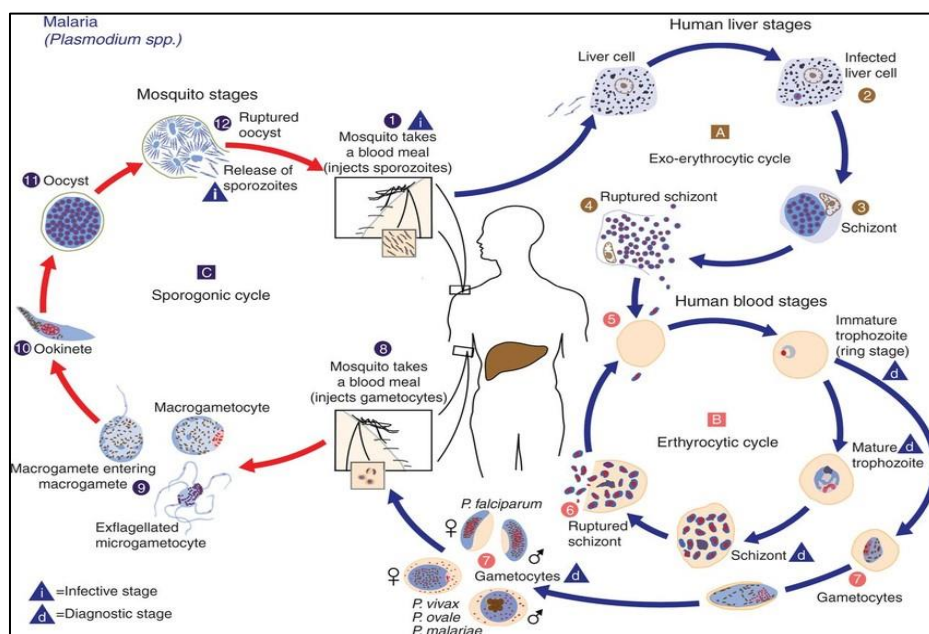


Figure 2. Life cycle of malaria parasite.

(Source: Image from the Centers for Disease Control and Prevention (www.cdc.gov))

The zygote quickly passes through meiosis and divides into a motile form called an ookinete, which has four haploid genomes in its nucleus at this point (Sinden & Hartley, 1985). The ookinete breaks through

the mosquito's mid-gut wall and develops into an oocyst on the outer side (Siciliano *et al.* , 2020). In the oocyst, several rounds of mitosis take place, and numerous sporozoites are produced by sporogony (Araki *et al.* , 2020). Oocyst formation is followed by mitotic divisions, which ultimately result in the formation of sporozoites. When sporozoites leave mature oocysts, they go through the haemolymph to the salivary glands, where they invade the secretory cells. Sporozoites are passively transported to the salivary glands by the haemolymphs by the mosquito hemocoel, less than 20% of the sporozoites enter the hemocoel, and the remaining 80% are eliminated from the haemolymph and within the salivary gland (Pinto *et al.* , 2008; Hillyer *et al.* , 2007). The process of development of sporozoites from the stage of oocyst takes around 10 to 14 days. The sporozoites enter the salivary gland of the mosquito, after which mosquito becomes ready for transmission of malaria infection to the next target person for her blood meal.

Malaria Diagnosis

Quick and accurate diagnosis followed by effective therapy is the main strategy in malaria control (Gerstl *et al.* , 2010). As per the WHO's guidelines all patients with suspected malaria should have their diagnosis confirmed by microscopy or by rapid diagnostic test (WHO, 2010a) before starting the drug treatment considering the feasibility of field level implementation of malaria diagnosis method. In malaria diagnosis methods, malaria parasites or antigens are detected depending upon the methods used.

Malaria Microscopy: Malaria microscopy is the most conventional method where both thin and thick peripheral blood smears are stained and examined (Ngasala *et al.* , 2008) by trained laboratory technician. While the giemsa-stained microscopic parasite identification has been considered the most accurate way to diagnose malaria over the past century (Norgan *et al.* , 2013; Bejon *et al.* , 2006) the rapid staining procedure, JSB (Jaswant Singh-Bhattacharji) stain is mostly used in Indian malaria control programme (NCVBDC, 2002). In the programme setting, wherever microscopy facility is available, blood test of all suspect malaria patients must be done with a follow-up blood test after six hours of the initial test if the first test comes out negative. To set up and maintain reliable and efficient malaria microscopy diagnosis facility at the level of the Primary Health Centre, health system infrastructure, well trained health workers, regular provision of reagent, supervision and quality control are necessary (Payne, 1988). In spite of the shortcoming of missing malaria parasites in endemic countries, microscopy is still regarded as the "gold standard" for diagnosing malaria having sensitivity of detecting 50-500 parasites per mL of blood (Feleke *et al.* , 2017) and allows identification of parasite species and density quantification (Kyabayinze *et al.* , 2008).

Rapid Diagnostic Test (RDT): Considering the limitations in malaria microscopy where highly skilled microscopist is needed, WHO recommends Rapid Diagnosis Test (RDT) as the alternative method for malaria diagnosis (Ugah *et al.* , 2017; Kim *et al.* , 2008). RDT is based on the principle of antigen detection, and the procedure is simple and straight forward as the user has only to spot the patient's blood onto the test strip's base and wait only for about 20 minutes to get the result (Guthmann *et al.* , 2002).

The use of RDT in malaria diagnosis at field settings has increased enormously in the past decade and over 300 million RDTs are used annually at a cost of 100 million dollars (World Health Organization, 2015b). By employing species-specific antigens for lactate dehydrogenase or aldolase, these quick assays may distinguish between the presence or absence of PfHRP2, which is present in *P. falciparum*, as well as other species. WHO recommends for the reliable RDTs which should detect approximately 100 parasites/mL of blood. Many RDTs have low sensitivity/specificity for detection of *P. knowlesi* (Foster *et al.* , 2014). Indian Programme has been using now bivalent RDT for detection of Pv and PF,

Quantitative Buffy Coat (QBC): QBC for malaria diagnosis depends on micro-centrifugation, fluorescence, and density gradient of infected red blood cells. While microscopy detects at the level of 50-100 parasite /mL, QBC detects five parasites /ml blood and gives scope for easy interpretation of malaria parasites specially in case of low parasitaemia (Tangpukdee *et al.* , 2009).

Nucleic Acid Amplification Tests (NAAT): Currently many newer diagnostic tools are available for the research and epidemiological studies. Several Nucleic Acid Amplification Tests (NAATs) have been employed for the above purposes. The sophisticated NAATs allow for species identification with use of species-specific primers or melt curve analysis, detection of mixed infections and have excellent sensitivity, to detect sub-microscopic and sub RDT level parasitaemia down to 1 parasites/mL (Fitri *et al.* , 2022) and permit earlier detection of infections compared to other diagnostic methods.

Polymerase Chain Reaction (PCR) also a nucleic acid amplification based test is 100 times more sensitive than microscopy and with its excellent sensitivity, can identify all five species of the malaria parasite at a very low level of sub-microscopic parasitaemia like 0.2 parasites per mL of blood. Despite all advantages, due to high cost, special lab set up and trained staff, PCR is hardly employed in the field settings and mostly limited to research settings for the study on the malaria epidemiology.

Loop mediated isothermal amplification assay (Lamp assay) is a nucleic acid amplification under isothermal conditions, is a highly compatible with point-of-care (POC) analysis (Kashir & Yaqinuddin, 2020).

Truenat is a chip-based micro PCR test can detect malaria parasite at five parasites/ mL of blood) both for *P. falciparum* and *P. vivax*. This can be used for active surveillance and epidemiological intervention in addition to malaria diagnosis (Nair *et al.* , 2016).

Clinical Symptoms and Signs

The clinical symptoms and signs usually appear 10–15 days after the bite of the infective Anopheline vector mosquito among nonimmune individuals. The fever, headache, and chills are the first symptoms and difficult to recognize as malarial symptoms. The typical clinical illness pattern consists of six-hour fever paroxysms that reach a temperature of 41 degrees Celsius, followed by sweating and defervescence. Besides the above symptoms, other common symptoms are, myalgia, nausea, and vomiting. Occasionally there may occur loose motion, abdominal pain, and cough. When the disease progresses, some patients may develop classic malaria paroxysm with bouts of illness alternating with symptom free period (Weiland, 2023). Before the regular periodicity emerges, the fever may persist for a few days. Among the children, the clinical symptoms vary widely. Symptoms may be non-specific like lethargy, malaise, cough, nausea, vomiting, abdominal cramp and somnolence (Zekar & Sharman, 2023; Fletcher & Beeching, 2013). Classical pattern of malaria fever is rarely seen in children.

Severe Malaria: Severe malaria typically occurs due to delayed treatment of uncomplicated malaria. Nearly all deaths from severe malaria result from infections with *P. falciparum*, although *P. vivax* and *P. knowlesi* can also cause severe disease (Price *et al.* , 2007; Kochar *et al.* , 2005). The manifestations of severe malaria include cerebral malaria (abnormal behavior, impairment of consciousness, seizures, coma, or other neurologic abnormalities), severe anemia due to hemolysis (destruction of the red blood cells), hemoglobinuria (hemoglobin in the urine), acute respiratory distress syndrome (ARDS) (inflammatory reaction in the lungs that inhibits oxygen exchange), low blood pressure caused by cardiovascular collapse, Acute kidney injury (AKI), metabolic acidosis (excessive acidity in the blood and tissue fluids) often in association with hypoglycaemia and multi organ failure (MOF) (Ashley *et al.* , 2018; Silva *et al.* , 2017; Schumacher & Spinelli, 2012; Breman, 2009). Severe malaria in children may mislead as meningitis, encephalitis. The clinical features of severe *falciparum* malaria in children are like the symptom and signs of severe sepsis (Schumacher & Spinelli, 2012).

Malaria treatment: There are very few drugs available for the malaria treatment. Based upon the chemical structures, presently available Anti-malaria medicines are broadly categorized in three groups: i) Aryl Amino alcohol compounds (Quinine, quinidine, chloroquine, amodiaquine, mefloquine, halofantrine, lumefantrines, piperaquine, tafenoquine), ii) Antifolate compounds (“antifols”) (pyrimethamine, proguanil, chlorproguanil, trimethoprim) and iii) Artemisinin compounds (artemisinin, dihydroartemisinin, artemether, artesunate) (Arrow *et al.* , 2004).

Chloroquine: A 4-aminoquinoline derivative of quinine was developed for the first time in 1934 and has been historically the drug of choice both for treatment of uncomplicated malaria and chemoprophylaxis in wide scale throughout the globe. The drug has minor gametocidal properties and mostly affects erythrocytic asexual stages and the side effects are uncommon and mild. The drug is available both in oral and injectable form. Chloroquine could save millions of lives when malaria was ravaging the entire planet. But due to widespread global drug resistance, it is no longer used to treat *falciparum* malaria (Arrow *et al.* , 2004). In India the most prevalent *P. vivax* infection only is being treated with chloroquine as the species is still sensitive to chloroquine (Anvikar *et al.* , 2014).

Quinine/ Quinidine and Artemisinin: derivatives are the two primary medications used to treat severe malaria. Quinine first isolated from cinchona bark in 1920 and useful to treat severe malaria caused due to any *Plasmodium* species. *P. falciparum* respond to quinine very well. It is available both in oral and injectable form and can be used in infants and pregnant women. Common side effects are nausea, mood change, blurring of vision and ringing in the ear, but generally these symptoms resolve after the treatment ends (Arrow *et al.*, 2004). The antimalarial principle of **Artemisinin** was first isolated by Chinese scientists from *Artemisia annua* (sweet wood) in 1972 (Arrow *et al.*, 2004). There are three derivatives of Artemisinin; artesunate, artemether, arteether, and dihydroartemisinin but there is no evidence that one variety of the Artemisinin compound is better than others (McIntosh & Olliaro, 2000). Artemisinin compounds have a faster clearance of parasites and fever resolution, and artemisinin also reverses coma. Artemisinin derivative drugs are effective when given parenterally, orally, and also by suppository. There are no serious adverse effects recorded in humans. They are effective against multidrug resistant malaria (White, 1994). Parenteral artesunate is the most rapidly acting compound among the artemisinin derivatives and is associated with more rapid clearance of parasitaemia than treatment with cinchona alkaloids (Arrow *et al.*, 2004).

Artemisinin based combination therapy: Artesunate when used in combination with another long acting drug, is effective in the treatment of non-severe malaria and the combination of artesunate with mefloquine is the most effective (Nosten, 1994). Artemisinin-based medications were initially advised as second-line treatments in malaria-endemic areas, but at present they are used as the first-line treatments in most severe malaria cases and Artemisinin monotherapy is no more advised (WHO, 2010b). Artesunate derivatives are not recommended to be used in uncomplicated malaria during the first trimester of pregnancy, except when effective alternative therapy not available. But in case of severity artesunate has been recommended to use in such cases. Except occasional hypersensitive reactions, the Artemisinin derivatives are safe and well tolerated (WHO, 2010a). For the treatment of uncomplicated *falciparum* malaria, India uses two types of ACTs i.e., Artemether + lumefantrine in Northeast states and Artesunate + sulfadoxine – pyrimethamine in other states.

Primaquine belongs to the 8-aminoquinolines, which kill mature gametocytes of *Plasmodium falciparum*, developing parasites of all species in the liver and the dormant hypnozoites of *Plasmodium vivax* and *Plasmodium ovale* and have weak asexual stage activity and very weak activity against *P. falciparum* (Ashley *et al.*, 2014). The World Health Organization recommends single dose of primaquine (PQ) for radical cure for *Plasmodium falciparum* malaria and a 14-day course of primaquine (0.25 mg/kg/day, giving an adult dose of 15 mg/day) to eradicate the liver stage of the *P. vivax* parasite. Primaquine is likely to cause haemolysis in G6PD-deficient patients. The National Drug policy of India, 2013 has allowed to use of single-dose PQ treatment by community-level health care givers as it is well tolerated. PQ is not administered to pregnant women and infants (WHO, 2010a; Anvikar *et al.*, 2014).

Tafenoquine is a potential gametocidal drug belong to 8-aminoquinoline like primaquine has got global approval to be used for radical cure in *P. vivax* malaria. Tafenoquine can be administered as a single dosage for radical cure or as weekly chemoprophylaxis, as it is slowly eliminated from the body (Llanos-Cuentas *et al.*, 2019).

Other drugs like proguanil, mefloquine, doxycycline, clindamycin, azithromycin are also used as antimalaria drugs, but due their weaker effect, not used as primary drugs (Van Eijk & Terlouw, 2011; Snow *et al.*, 2001).

Chemoprophylaxis in India: Doxycycline is used as a prophylactic medicine for short-term travellers, while MQ for long-term travellers (NVBDCP, 2013). Though MQ is less expensive and administered once per week, many clinicians fear to administer it as it can induces psychiatric side effects, such as anxiety, panic attacks, paranoia, persecutory delusions, dissociative psychosis, and anterograde amnesia and linked to violent and suicidal behaviour (Ahmad *et al.*, 2021). Use of MQ should be avoided in cerebral malaria due to neuropsychiatric problems associated with the drug (Anvikar *et al.*, 2014).

Malaria Epidemiology Malaria transmission depends upon climatic factors e.g. temperature, rainfall, and relative humidity (Craig *et al.*, 2004) which influence the growth and multiplication of vectors and parasites. Depending upon favourable climatic factors, malaria transmission is perennial or seasonal. *P.*

falciparum infection is mostly seen in tropical and subtropical areas (Zekar & Sharman, 2023) while *P. vivax* in temperate and subtropical areas (Howes *et al.*, 2016), *P. ovale* in West Africa, *P. malariae* in South America, Asia, and Africa and *P. knowlesi* is confined to South-East Asia (Jeyaprakasam *et al.*, 2020).

Malaria – Global Scenario

There were 224 million estimated cases of malaria worldwide in 2015 which reduced to 200 million cases with 400,000 fatalities in 2017. As per the World Malaria Report 2022, an estimated 247 million malaria cases and 619,000 deaths were recorded in 84 malaria endemic countries in 2021, out of which over 95% of global malaria burden was reported from WHO'S African region, only 2% was contributed by WHO South East Asia region and European region is malaria free since 2015 (WHO 2014, 2015, 2017, 2022). The economic burden due to malaria suffering is huge, Governments spent \$1.2 billion (28.2%) of all malaria spending in 2016, increasing 4.0% annually since 2000. The source of malaria spending varied depending on whether countries were in the malaria control or elimination stage. (Haakenstad *et al.*, 2019). Unprecedented success in malaria control was noticed in the last decade, when around 1.5 billion cases and 7.6 million deaths could be averted. There is a reduction by 78% in 2020 in comparison to 2000 i.e., from 23 million cases in 2000 reduced to about 5 million in 2020. The proportion of malaria cases due to *P. vivax* reduced from about 18.5 million in 2000 (8%) to 4.5 million in 2020 (2%). Malaria annual case incidence per 1000 population at risk per year reduced from 81 in 2000 to 59 in 2015 and 56 in 2019, which increased again to 59 in 2020. Malaria case incidence in WHO South-East Asia region reduced by 83%, i.e., from about 18 cases per 1000 population at risk annually in 2000 to about three per 1000 population at risk in 2020. Globally, malaria deaths reduced steadily over the period 2000–2019, i.e., from 896,000 in 2000 to 558,000 in 2019. Most of the cases (82%) and deaths (95%) averted were in the WHO African Region, followed by the WHO South-East Asia Region (10% cases and 2% deaths). The percentage of total malaria deaths among under five children reduced from 87% in 2000 to 77% in 2020 (WHO, 2021). The malaria mortality rate reduced from 150 in 2000 to 56 in 2019 per 100,000 populations at risk, but again malaria deaths increased to 62 in 2020. The increase of malaria deaths in 2020 to that of 2019 was estimated to be 12%, An estimated 47,000 (68%) deaths out of the additional 69,000 deaths is attributed to disruption of health services due to COVID-19 pandemic (WHO, 2021).

Malaria – Indian Scenario

Malaria is an age-old problem in India. As per Sir H. Patrick, India experienced over a million deaths per annum from malaria during the colonial era and there was a significant variation in the contemporary and historical malaria ecosystem of India (Kumar *et al.*, 2007). As per the WHO 1990 report, malaria was the problem in many parts of India between 1950–1970. The Bhore Committee in 1946, recommended in their health survey report to give special attention to diseases like malaria, TB, Smallpox, leprosy, plague, cholera, venereal diseases, filariasis and mental illness (Bhore, n.d.). According to historical records (WHO country office for India), the highest case load of malaria in India was in 1950, with an estimated 75 million cases and 0.8 million deaths due to malaria each year (Das *et al.*, 2012a). After the intervention through the National Malaria Control Programme (NMCP) in 1953, malaria cases dropped from 75 million as estimated in 1950 to only 2 million by 1958. Then due to the National Malaria Eradication Programme (NMEP), launched in 1958, there were only approximately 100,000 cases of malaria annually in 1965, which was extremely low in comparison to the estimated 75 million cases and 800,000 deaths annually during 1950s. Unfortunately, after that, this remarkable gains could not be sustained and malaria started to resurge in many foci even during the maintenance period (Akhtar *et al.*, 1977; Sharma, 1996b). In 1976, there were 6.4 million parasite positive cases (Sharma, 1987) which was the highest resurgence of malaria incidence recorded by the NMEP at the peak of Global Malaria Eradication Programme (Nájera *et al.*, 2011). Since then, there have been numerous reports of malaria resurging across the nation (Sharma, 1996b). With many ups and down, malaria cases gradually decreased to 1.6 million with around 1000 death in 2009 (WHO, 2010b).

Around 275.5 million (22%) of India's population live in high malaria transmission areas (>1 case per 1000 population), 838.9 million (67%) lived in low transmission areas (0–1 malaria case per 1000 population) and 137.9 million (11%) live in malaria free areas i.e. 0 case per 1000 population (WHO, 2014). Out of 128 million malaria tests of suspected patients, around 0.88 million cases (53%) were recorded with *P. falciparum* infection and 47% with *P. vivax* infections. The incidence of malaria cases in India accounted

for 85% in the WHO's Southeast Asia Region (WHO, 2015b). Around 698 million population (more than half of the world population at risk of malaria, India accounted for 6% of all malaria cases, 6% of malaria deaths and 51% of the global *P. vivax* malaria burden in 2016 (WHO, 2017). In 2016, out of globally reported 23990 (1600-46500) malaria deaths, India reported 331 deaths. As per the World Malaria Report 2022, the deaths associated with malaria remained largely similar over the past 3 years (77 in 2019, 93 in 2020 and 90 in 2021), with India accounting for 82.4% of all malaria deaths in the WHO South-East Asia Region in 2021. Positively, India is among the five High Burden High Impact (HBHI) nations to showcase drastic decline in deaths, although their contributions to the malaria burden within HBHI countries is still substantial (WHO, 2022).

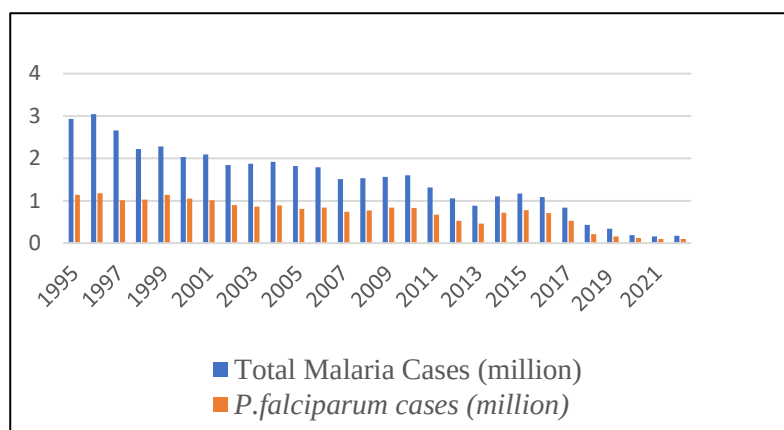


Figure 3. Malaria cases in million in India 1995 to 2022 (source NCVBDCP)

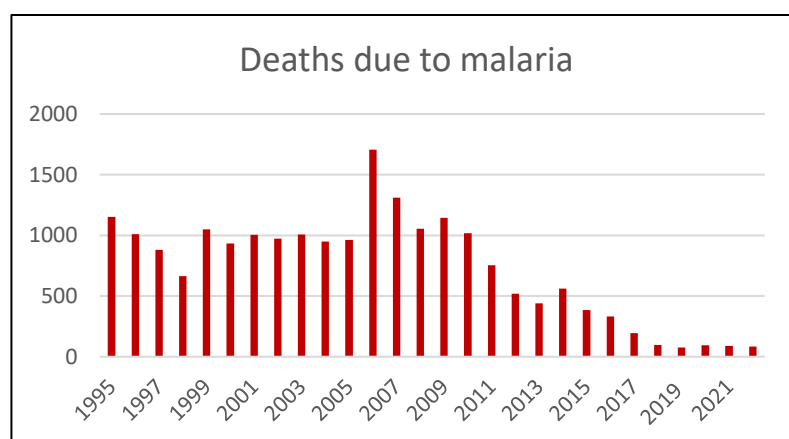


Figure 4. Malaria deaths in India 1995-2022 (source NCVBDCP)

The burden of malaria in India is complex because of the highly variable malaria eco-epidemiological profiles, transmission factors, and the presence of multiple *Plasmodium* species and influence of nine established Anopheles vectors. (Dash et al 2007, 2008, Das. et al., 2017b).

Underreporting of malaria cases and deaths have been highlighted by many investigators. As per Hay *et al.*, malaria incidence in India is in between 9-50 times greater than the reported cases (Hay *et al.*, 2010). The Lancet study, contradicted the reported number of malaria deaths in India by the NCVBDCP and WHO (Dhingra *et al.* , 2010) which reported that death due to malaria in India was 20500 per year out of which maximum deaths (around 50000) occurred in Odisha state, followed by Chhattisgarh, Jharkhand and Assam. According to the WHO's estimation (Source: WHO SEARO website), India had 15 million cases of malaria and 19,500-20,000 deaths per year, compared to 2 million cases and 1,000 reported deaths reported by National programme of India. Estimated malaria mortality in India at 46,800 in 2010 (Murray *et al.* , 2012). The WHO estimates around 4 to 5 million malaria cases and 9-10 thousand deaths due to malaria in India since 2019 to 2021(WHO, 2022). As NCVBDCP, India reported only 0.38 million malaria cases in 2019, 0.18 million cases in 2020 and 0.16 million cases in 2021, it has been suggested that India

should raise the concerns on methodology for the estimation or try to get close to estimation in reporting (WHO, 2022).

In India, the high risk populations are mainly ethnic tribes living in the forested pockets of the states like Odisha, Jharkhand, Madhya Pradesh, Chhattisgarh and the North-eastern states contributing bulk of morbidity and mortality due to malaria in the country (WHO, 2020). Presently all population groups in the country, regardless of gender and age are affected by malaria infection, although children and pregnant women are at the higher risk. Tribal malaria shares significantly to the malaria burden of India. Between 2000 and 2020, the malaria incidence and mortality in tribally dominant areas decreased at an average annual rate of 4.3%, which is consistent with the outstanding progress realised in malaria control at the country level during this period. Additionally, the findings indicated a drop of malaria incidences in tribally dominant areas between 2016 and 2020 (Singh *et al.* , 2023).

Malaria Parasites in India

Out of five malaria parasites, historically, *Plasmodium vivax* infection was predominant (>53%) in India. After the spread of drug-resistant *Plasmodium falciparum* in the 1990s, the prevalence of both *P. vivax* and *P. falciparum* remained equivalent at the national level for a decade (Anvikar *et al.* , 2016). By 2014, the proportion of *P. vivax* decreased to 34% nationally, but with high regional variation. In 2014, *P. vivax* accounted for around 380,000 malaria cases in India; almost a sixth of all *P. vivax* cases reported globally (WHO, 2015b). *P. vivax* remained resistant to control measures, particularly in urban areas as urban malaria is predominantly caused by *P. vivax* and subject to outbreaks, often associated with increased mortality, and triggered by bursts of migration and construction. In 2022, there were 146,559 numbers of malaria cases, out of which 82,523 were *P. falciparum* cases in India. *P. falciparum* cases increased as high as 58.3% and the major contribution was from Odisha, Jharkhand, Meghalaya, Madhya Pradesh, Andhra Pradesh, Nagaland, Maharashtra, West Bengal and Tripura which accounted for 91% of malaria cases and 98% *P. falciparum* cases of the country (NCVBDCP Annual report 2022). A few cases of *P. malariae* (2%) have also been reported. *P. ovale* earlier reported in central India (Anvikar *et al.* , 2016) has been detected in Odisha state where it is found to be widely distributed (Bal *et al.* , 2021). Recent investigations revealed *P. knowlesi* infected malaria cases in Andaman and Nicobar islands, Bihar, Delhi and Utar Pradesh (Tyagi *et al.* , 2013; Chaturvedi *et al.* , 2021).

Malaria Vectors in India

Out of around 450 Anopheles species, at least 60 are known to be human malaria vectors (Manguin *et al.* , 2008) and of these, six Anopheles species; *Anopheles (An) culicifacies*, *An. s. fuviatilis*, *An. minimus*, *An. baimaii*, *An. stephensi* and *An. sudaicus* (now known as *An. epiroticus*) are implicated as primary vectors transmitting malaria in different eco-geographical regions of India (Baruah *et al.* 2024). In addition, the secondary/local vectors *An. annularis*, *An. philippinensis*, and *An. varuna* transmit malaria along with either one or two major vectors in different parts of the country (Subbarao *et al.* , 2019). *An.culicifacies* is the main primary vector responsible for 60-65% of the malaria burden in India (Dash *et al.* , 2008). In hilly and forest areas *An. fluviatilis* plays primary role in transmitting malaria and *An. culicifacies* plays the secondary role. In plain areas *An. culicifacies* plays the primary role while *An. fluviatilis* plays secondary role. In certain plain areas, *An. culicifacies* is found to be the only vector (Subbarao & Sharma, 1997;Singh *et al.* , 1999; Nanda *et al.* , 2000). Both these vectors rest and bite indoors. *An. culicifacies* predominantly zoophilic and the per man hour density (PMHD) is higher in cattle sheds than in human dwellings (Nanda *et al.* , 2000).

Malaria – the economic loss in India

Economic loss was huge in India due to the malarial sufferings. Sinton estimated that there was loss of INR 42 core in India due to malaria in 1935 (source Sinton's; treatise).The progress of India was greatly hampered due to low economic progress (Dash *et al.* , 2007) . In 1994, Shiv Lal and others estimated that if malaria would have been allowed to transmit without any control activities from the 1947 level, there would have been an expenditure of INR 76,660 million (US Dollar 1,670 million) for medication, medical advice, hospitalization, and absenteeism (Sharma, 1996a). It has been estimated that every rupee invested in malaria control in India has produced a direct return of INR 19.70 and the estimated man-days saved were 1,328.75 million per year (Dash *et al.* , 2007, 2008). The total economic burden from malaria in India could be around US\$ 1940 million and the major burden comes from lost earnings (75%), while 24% comes

from treatment costs. When estimated malaria burden and malaria related economic loss is huge, the public expenditure in malaria programme through the NCVBDCP, by the Indian Government is in the declining trend (Gupta & Chowdhury, 2014).

Malaria Control Strategy

Global experiences: As there was no clarity regarding the cause of malaria till Sir Ronald Ross's historical discovery of malaria transmission in 1887 (Sinden, 2007), there could not be any ideal therapy for the treatment of malaria illness in many centuries. Based on the hypotheses of malaria transmission revolving around swamps, bad air (Miasma theory) and insect vectors, preventive measures were adopted by people to protect themselves by various personal protection and environmental management measures which they trusted to be fit.

The global experiences show that the journey of malaria control and elimination is mixed with hope and despair facing the challenges of technical, operational, and financial (Shretta *et al.*, 2017). Over the years in almost all the countries, malaria control strategy was focusing on to clear the parasite load among the malaria infected patients along with reducing the anopheline mosquito vector population both at larval stage and adult stage along with reducing the man mosquito contact by implementing mass scale personal protection measures (Gachelin *et al.*, 2018). The major malaria control strategies adopted under the National malaria control programmes of almost all countries are: 1. Early Detection and Prompt Treatment (EDPT). In countries like India, it is also being said as Early Diagnosis and Complete Treatment (EDCT) to emphasize the full course drug compliance which includes radical treatment to clear the gametocytes responsible for malaria transmission from man to mosquito. Antimalaria drugs are given to the patients as per the National Drug Policy of the country. 2. Vector Control is to reduce the vector population and for which different methods are implemented like (i) Chemical Control; use of Indoor Residual Spray (IRS) with insecticides recommended under the National malaria control programme, use of chemical larvicides like Abate in potable water, malathion fogging during outbreaks etc. ii) Biological Control; use of larvivorous fish in ornamental tanks, fountains, stagnant water bodies etc. and use of biocides. (iii) Personal prophylactic measures that individuals/communities can take up like use of mosquito repellent creams, liquids, coils, mats etc., screening of the houses with wire mesh, use of bed nets treated with insecticide, wearing clothes that cover maximum surface area of the body etc. 4. Strengthen local capacities in basic by imparting training on malaria control strategies and field level implementation. 5. Community Participation is very important aspect by which breeding sites of Anopheles mosquitoes in and around the habitation can be easily identified and eliminated by undertaking feasible and appropriate measures. 6. Environmental Management & Source Reduction methods like filling of the breeding places and proper covering of stored water and channelization of the water from the potential breeding sources. 8. Monitoring and Evaluation to understand the gaps and take corrective measures. 8. Operational research are inbuilt to the programme to assess the drug and insecticide resistance, impact of change in ecology and climate change which help to modify the key malaria intervention strategy (Moonen *et al.*, 2010; Gachelin *et al.*, 2018; NVBDCP, 2016).

Global Malaria Eradication Programme (GMEP)

The WHO under the GMEP efforts launched the world-wide National Malaria Control Programme (NMCP) in 1955 as per the Ninth World Health Assembly (WHA) resolution. The NMCPs were established in most of the affected regions of the Middle East, India, Southeast Asia, islands of Western Pacific including Indonesia and Philippines. The logistical approach towards malaria eradication was based on the specific results of a malaria control effort in Greece, which provided proof of the almost complete cessation of malaria transmission in selected areas.

Intervention with DDT spray : The GMEP (*Malaria Eradication*, 2020) emphasised on vector control by DDT residual spraying and malaria surveillance. There was spectacular reduction in malaria cases in India and Sri Lanka (the then Ceylon) with the DDT residual spraying and treating the malaria patients by Chloroquine. By the mid-1960s, the GMEP was highly successful in eliminating malaria from several regions of the world. After getting enormous success of malaria eradication in nine European countries between 1900 to 1945, WHO launched GMEP in all endemic countries outside of Africa in 1955 with the availability of chloroquine for treatment and DDT for vector control (Shretta *et al.*, 2017). The GMEP expert committee recommended a four-phased regimen for the implementation by the National Malaria

Eradication Programmes (*Malaria Eradication*, 2020) which included a "preparatory phase," during which a National Malaria Eradication Service (NMES) was created to organise and carry out all the three phases of malaria control programme; i.e. the attack phase. Consolidation phase and maintenance phase.

However, GMEP fell far short of its mark of global eradication, partly because it failed to build on its achievements and adapt its interventions to different malaria levels. The discovery of resistance to DDT warned the malaria community that DDT, would only be effective in malaria control for a short period and underscored the significance of a timeline for the worldwide effort to eradicate malaria. After the huge success of malaria eradication programmed by NMEPs, malaria resurged by early 1970s in southern Asia and western Pacific regions due to resistance of the parasite to Chloroquine and vectors to DDT. There was emergence of multidrug resistance to falciparum malaria and total resistance to Chloroquine which was available at cheaper price. Since mid-1960s, Chloroquine resistance became widespread across the tropics of Asia and the Western Pacific and then into Africa from a focus of origin in Southeast Asia.

The development of Artemisinin derivative from the traditional Chinese herbal medicine 'Qinghaosu' was a great contribution to the world's antimalaria therapy when falciparum malaria resistance to chloroquine was spreading rapidly. The Antimalaria campaign of China had a strong foot which controlled malaria early though had little trouble in the late 1960s and early 1970s due to the political turmoil. Recently China could get the malaria elimination certificate from the World Health Organisation on 30th June 2021 due to its robust surveillance and vector control measures (Wu *et al.* , 2022).

Over the decades, most of the poorest countries could access to effective malaria diagnosis, Anti-malaria medicines and personal protection methods like IRS and ITN/LLIN which could reduce man mosquito contact and prevent malaria transmission.

The GMEP got set back in many countries by 1969. In the 22nd World Health Assembly at Boston held during 8-25 July 1969, it was demanded that countries need to change their course of eradication plan with local appropriate strategies and not fully relying upon DDT spraying as relationship between DDT exposure and reproductive effects in humans was suspected, based on the animal studies. In 1972, Environmental Protection Agency (EPA) of USA issued a cancellation order for DDT based on its adverse environmental effects, such as those to wildlife, as well as its potential human health risks. Malaria upsurged in countries like India, Sri Lanka who could show drastic decline in malaria cases in 1960s, as it happened in Africa and Latin America. Attack on the DDT spraying was continued by numerous environmentalists who opposed DDT use for its environmental hazard. The attack became more pronounced after the publication of the book 'Silent Spring' by Rachel Carson in 1962 (Carson, 2002), where it has been apprehended that DDT and its metabolite (DDD and DDE) accumulate in the animal body fat. The Stockholm Convention in 1995 took decision to develop a legally binding instrument to control certain chemicals which pose major threat to human health and environment which included DDT. Despite the opposition to allow DDT for spraying to control malaria, later an exemption was secured for production of DDT and its use in public health for vector control following the recommendation and guidance of the World Health Organization (Rehwagen, 2006) and decision has been taken to phase out DDT to stop using in malaria control programme.

Intervention using Insecticide treated nets (ITN): ITN is a net that "repels, disables, and/or kills mosquitoes coming into contact with insecticide applying on the netting material (Lengeler, 2004). As the effect of DDT was under suspicion, ITN became the most powerful tool for personal protection method to prevent mosquito bites and control malaria globally, particularly in the malaria endemic countries in Africa and Asia (Hill *et al.* , 2006). Since 1940s, when chloroquine and DDT spraying were introduced, ITNs have been the preferred method for preventing malaria, and ITN could take significant role since the mid-1990s, both in global and national malaria initiatives (Hill *et al.* , 2006). ITNs are very effective in reducing the childhood malaria mortality and morbidity (Lengeler, 2004) and sufficiently safe in children and even in new-born (Lu *et al.* , 2015). Widespread use of ITNs was advocated by Roll Back Malaria, but for universal access to ITNs, major financial and operational inputs are needed (Lengeler, 2004). To start with, short lasting ITNs were introduced, where plain mosquito nets were impregnated with pyrethroid insecticides having insecticidal effect for 6-12 months which became cumbersome to implement in mass scale as the nets required reimpregnation at regular intervals.

Long-lasting insecticidal nets (LLINs) became the alternative improved tool to short acting ITNs for large scale operation. In LLINs the insecticidal effects last for around three to five years. LLINs have played an important role in reducing malaria burden over the past decade and presently is the core prevention tool, widely used by people at risk of malaria (WHO, 2015b). Most national malaria control programs have now adopted the universal coverage of LLIN with mass distribution campaigns conducted at regular interval (WHO, 2017). In Sub-Saharan Africa (SSA) due to massive scale LLIN implementation, within 15 years, 663 million out of total malaria cases could be averted and 67–73% of these has been attributed to the extensive distribution and use of LLINs. LLINs have both direct and indirect benefits in protecting population from mosquito bites. Even at higher level of Pyrethroid resistance, LLINs provide enhance protection than conventional nets (Unwin *et al.*, 2023). In communities where the combination of ITN and IRS with non-pyrethroid insecticides is used shows positive association in reducing malaria prevalence (Pryce *et al.*, 2022).

Hope for Malaria Vaccine: Malaria vaccine is a long expectation and is under process of development since 1960, by the vaccine scientists. It is expected with high hope that vaccine can bring a halt to the malaria transmission and the disease manifestation. After several effort in making the vaccine possible, now it has come to a stage to full fill the hope of relief. The revolution in molecular biology could identify the specific genes coding for the specific antigens – thus the subunit vaccine could be made possible. Following three stages of malaria parasite's life cycle have been targeted by the vaccine; pre-erythrocytic stage, erythrocytic stage and parasites' mosquito stage (sexual stage). Vaccine against the proerythrocyte stage intends to prevent the infection by blocking the invasion or development of sporozoites or development of the parasite in the liver cells. The vaccine against the erythrocytic stage intends to reduce the parasite density in human blood and thus can reduce the malaria related complications and deaths. Vaccine directed against the sexual stage of the parasite intends to block the parasite's development in the Anopheline mosquito body. The World Health Organisation has recommended following two vaccines for prevention of malaria amongst the children, RTS, S/A/ASO1 (RTS,S) is the first malaria vaccine approved by the WHO and its brand name is Mosquirix. The HOS has recommended this vaccine for sub-Saharan Africa and other regions with moderate to high malaria transmission. The second recommended vaccine for endemic countries is R2/Matrix-M. WHO says, both the malaria vaccines are complementary tools to other malaria control measures and countries can chose the vaccine from amongst the above two, considering the cost, availability and other factors.

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LYMPHATIC FILARIASIS ELIMINATION IN INDIA: AN OPTIMISTIC APPROACH

P K Srivastava¹, Anju Viswan K², Sagya Singh³

¹Ex-Joint Director, National Vector-Borne Disease Control Programme, Delhi

²Scientist C, ICMR-VCRC, Koraput Field Unit, Odisha

³State Consultant (Entomology), NCVBDC, Government of Jharkhand, India

Abstract

The elimination of Lymphatic Filariasis in India started following deliberations in a meeting at Delhi in 1996 and piloting of Mass Drug Administration (MDA) with DEC. Launching of Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000 after 50th World Health Assembly (WHA) resolution in 1997 for Elimination of Lymphatic Filariasis (ELF) as a global public health problem by 2020 created a movement for ELF. In India, ELF campaign was launched during 2004 with annual single dose of Mass Drug Administration (MDA) in identified endemic districts with the slogan of Freedom from Filariasis. The various challenges experienced during different years of MDA implementation, the programme guidelines were amended for from time to time for improvement like replacement of loose DEC tablets with strip packing and co administration of Albendazole with DEC and introduction of triple drug therapy with addition of ivermectin along with DEC and Albendazole. The emphasis on Morbidity management and patient care has improved the performance of programme. The reported coverage of MDA was improved and consequently microfilaria prevalence started declining in many districts though some foci from non-endemic districts also started reporting mf prevalence. India's significant progress towards ELF has been well recognised by WHO, however, some new districts were added due to presence of mf rate above threshold during resurvey. The experience of success and challenges and the intensified approach towards ELF in a mission mode with priority and commitment to sustain achievements made so far is suggested so as to realize the goal of SDG and prove that the approach has to be optimistic.

Keywords: ELF, MDA, TAS, LF, IDA, MMDP

Introduction

French surgeon Jean-Nicolas Demarquay in 1863 described for the first time that the microfilariae live in the blood stream (Grove, 1990). In 1868, Microfilaria was found in Urine by Wucherer and in 1878, Patrik Manson speculated that disease may be transmitted by mosquito. In India, "Susruta Samhita" mentioned about the disease in 6th century BC and in 7th Century AD "Madhava Nidhana" described signs & symptoms of this disease. In 1709, Clarke called elephantoid legs in Cochin as "Malabar legs" as every house was having affected persons with swollen legs (Clark, J., 1773). Lewis in 1872 discovered Microfilariae (mf) in the peripheral blood in Calcutta, India (Sasa, 1976; Otsuji Y, 2011). In India, the National Filaria Control Programme (NFCP) has been implemented since 1955 and was covering 227 towns with anti-larval activities and detection cum treatment of LF cases. The revised elimination strategy with annual mass drug administration with DEC was piloted in 13 endemic districts of 7 states and based on its result, the annual MDA was expanded in all endemic districts (Biswas, 1996).

Lymphatic Filariasis-A Disease with Social Stigma

Lymphatic filariasis (LF) is a parasitic disease caused by microscopic, thread-like worms which are classified as nematodes (roundworms) of the family Filarioididea. The technical name for Filariasis is Lymphatic Filariasis. Generally, the word 'Filariasis' and 'Filaria' are being used inter-changeably-but Filariasis refers to disease and Filaria refers to parasite. There are 3 types of these thread-like filarial worms namely *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*.

LF is transmitted by mosquitoes and the Vectors (Mosquitoes) are *Culex*, *Anopheles*, *Mansonia* and *Aedes*. The adult worms only live in the human lymph system. The lymph system maintains the body's fluid balance and fights infections. The most common manifestations of the disease are lymphoedema and hydrocele.

Clinical Manifestations

Adult worms nest in the lymphatic vessels and disrupt the normal function of the lymphatic system. The worms can live for approximately 6–8 years and during their life time, produce millions of microfilariae (immature larvae) that circulate in the blood. Vector mosquitoes are infected with microfilariae by ingesting blood when biting an infected host. Microfilariae mature into infective larvae within the mosquito. When infected mosquitoes bite people, mature parasite larvae are deposited on the skin, from where they can enter the body. The larvae then migrate to the lymphatic vessels where they develop into adult worms, thus continuing a cycle of transmission.

Symptoms

Lymphatic filariasis infection involves asymptomatic, acute and chronic conditions. The majority of infections are asymptomatic, showing no external signs of infection while contributing to the transmission of the parasite. A small percentage of persons develop lymphedema (limf-ah-DE-ma). This is caused by improper functioning of the lymph system which results in fluid collection and swelling. This mostly affects the legs, but can also occur in the arms, breasts, and genitalia. Most people develop these symptoms years after being infected. These asymptomatic infections still cause damage to the lymphatic system and the kidneys and alter the body's immune system. The swelling and the decreased function of the lymph system make it difficult for the body to fight germs and infections. Affected persons will have more bacterial infections in the skin and lymph system. When LF develops into chronic conditions it leads to (skin/tissue thickening) of limbs (lymphoedema) or hardening and thickening of the skin, which is called elephantiasis (el-ah-fan-TIE-ah-sis) and hydrocele (scrotal swelling). Men can develop hydrocele or swelling of the scrotum after the death of an adult worm. Such body deformities often lead to social stigma and sub-optimal mental health, loss of income -earning opportunities and increased medical expenses for patients and their caretakers. The socioeconomic burdens of isolation and poverty are immense.

Filarial infection can also cause pulmonary tropical eosinophilia syndrome, although the syndrome is typically found in persons living with the disease in Asia. Symptoms of pulmonary tropical eosinophilia syndrome include cough, shortness of breath, and wheezing. The eosinophilia is often accompanied by high levels of IgE (Immunoglobulin E) and anti-filarial antibodies. The clinical manifestations of LF elephantiasis are shown in figure 1



Figure1. Clinical pictures of LF manifestations

Acute episodes of local inflammation involving skin, lymph nodes and lymphatic vessels often accompany chronic lymphoedema or elephantiasis. Some of these episodes are caused by the body's immune response to the parasite. Most are the result of secondary bacterial skin infection where normal defenses have been partially lost due to underlying lymphatic damage. These acute attacks are debilitating and may last for weeks and are the primary cause of lost wages among people suffering with lymphatic filariasis.

***Wuchereria (W) bancrofti*: The main causative organism for LF**

The name of this worm *Wuchereria bancrofti* is given in the honour of the two scientists *Wucherer* and *Bancroft* who made a considerable contribution in studying the disease caused by these worms.

Systematic Position of *Wuchereria bancrofti*

Phylum: *Nematoda*

Class: *Phasmidia*

Order: *Filaroidea*

Family: *Filariidae*

Genus: *Wuchereria*

Species: *bancrofti*

Life of Cycle *Wuchereria bancrofti*

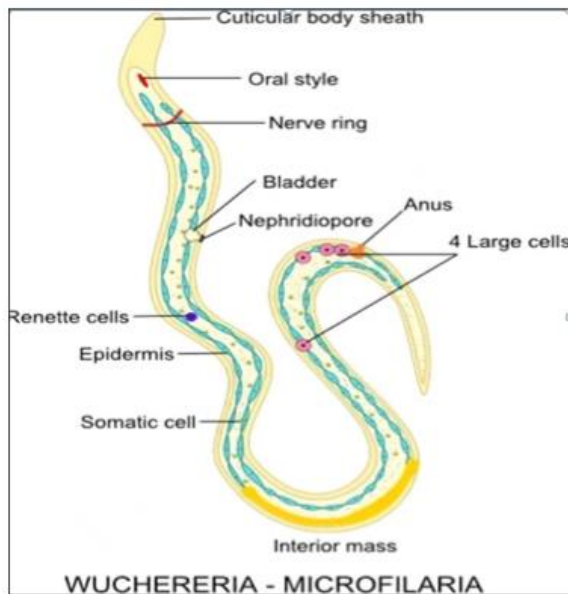
Microfilaria

Mehlhorn, H. (2016) in his book *Human Parasites: Diagnosis, Treatment, and Prevention* described the first stage larva which is called as *microfilaria*. They are colourless, transparent bodies with blunt anterior ends and pointed tails. They are very active and can move forward and backward within the sheath which is much longer than the embryo. They are microscopic and measure about 250–300 µm in length and 6–10 µm in thickness. Its body is covered with a hyaline sheath followed by a cuticula being lined by flattened subcuticular cells or epidermis and an inner column of cytoplasm containing nuclei. Its cuticle has well-marked striations.

Somatic cell or Nuclei appear as granules in the central axis of the body extended from head to tail except for the 5% terminal end of the tip. Their head-end has clear space devoid of granules known as *cephalic space*. Important structures from the anterior end downwards are future mouth or *oral stylet*, *nerve ring*: oblique area devoid of granules, *nephridiopore*, *renette cell*, dark-colored inner mass, and 4 cells of the future anus. They do not undergo further development in the human body unless they are taken up by their suitable host (mosquitoes). Their life span in the human body is probably 70 days.

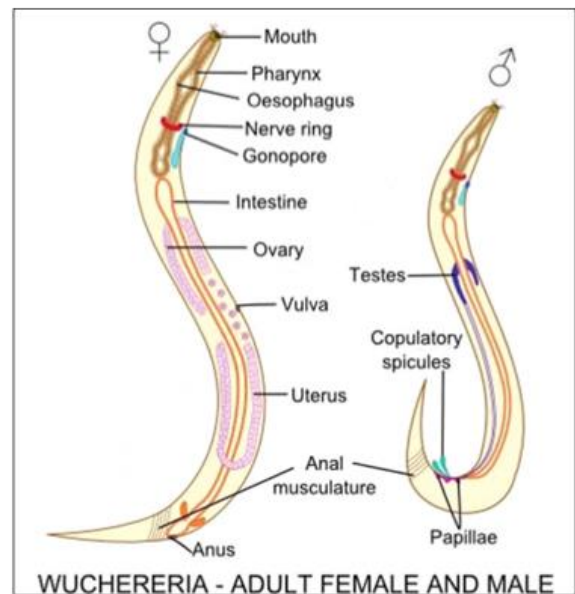
Adult worms

Mehlhorn, H. (2016) described that these are long, hair-like, transparent, translucent, thread-like worms with smooth cuticle and tapering ends. These are filiform and cylindrical in shape with both ends tapering. Sexes are separate with distinct sexual dimorphism. The female is larger (70–100 × 0.25 mm) than the male (25–40 × 0.1 mm). The posterior end of the female worm is narrow and pointed that bears an anus, while that of the male is curved vertically and contains numbers of *genital papillae*, two *copulatory spicules* of unequal length. Males and females remain coiled together usually in the abdominal and inguinal lymphatics and in the testicular tissues. The head end is slightly enlarged. Mouth aperture is simple, without lips. The pharynx is divisible into an anterior muscular portion and a posterior glandular portion. An oesophageal bulb is lacking. The intestine is simple. The vulva of the female is located ventrally in the pharyngeal region and provided with a *pyriform ejector mechanism*.



Wuchereria – Microfilaria

Source: <https://www.cdc.gov/mosquitoes/about/life-cycles/wucheria.html>



Wuchereria – Adult Female and Male Microfilaria

Source: <https://www.cdc.gov/mosquitoes/about/life-cycles/wucheria.html>

The female worm is viviparous and directly liberates sheathed microfilariae into the lymph. The adult worms live for many years, probably 10–15 years or more.

Third stage of the larva (infective form)

Paniker's book of *Medical Parasitology* (2017) described that the third stage larva is the infective form of the parasite is found only in mosquitoes. They are elongated, filariform, measures 1.5mm in length, and 18–23 µm in diameter.

Hosts of *Wuchereria bancrofti*

Wuchereria bancrofti as digenetic i.e. its life history is completed in two hosts

Definitive host: Man. No animal host or reservoir is known for *W. bancrofti*

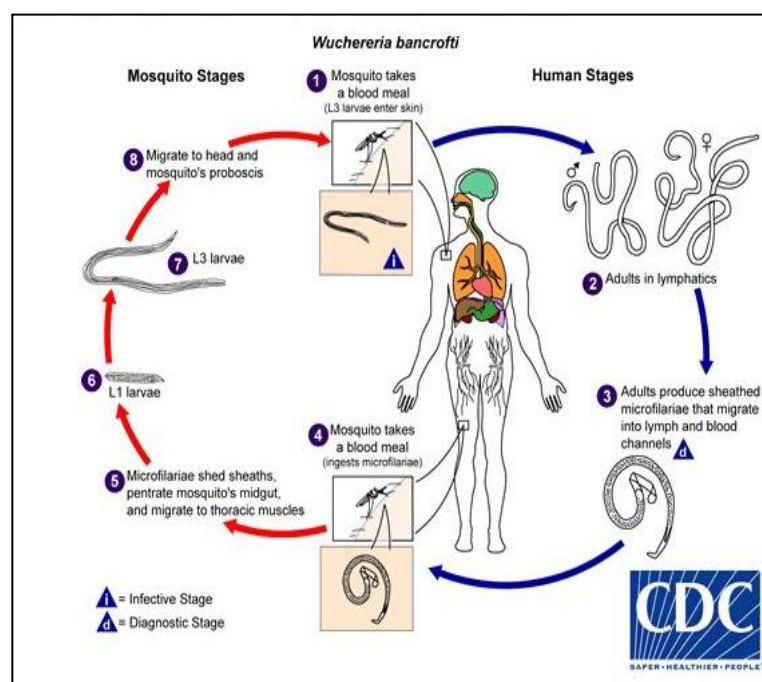
Intermediate host: Female mosquitoes, belonging to genus *Culex*, *Aedes*, and *Anopheles*.

Propagation of *Wuchereria bancrofti*

Copulation takes place when individuals of both sexes are present in the same lymph gland. This as also been mentioned by Odile Bain and Simon Babayan (2004).

Larval development in human: According to Odile Bain (2004), the female worm is viviparous or ovoviviparous, it releases numerous larvae called microfilariae into the bloodstream. The microfilariae are born in a very immature stage. However, they find their way into the lymph vessels, soon enter into blood vessels and circulate with blood showing active movements. They do not undergo further development until sucked by the intermediate host. i.e. female mosquitoes. Microfilariae show nocturnal and diurnal periodicity in the blood of human. During the daytime, the microfilariae tend to stay in the deep blood vessels of human but, at night time they travel near the surface in peripheral or superficial blood vessels. This behaviour enables them to get ingested by the night biting mosquito (*Culex*, *Mansonia* or *Aedes*) which serves as an intermediate host. These microfilariae circulate in the blood for 6 months to 2 years and then die if not taken by a mosquito. The nocturnal periodicity of microfilaraemia by both *W. bancrofti* and *B. malayi* infections are known in mainland of India. The diurnal subperiodic *W. bancrofti* infection was discovered in 1974-75, among aborigines inhabiting Nicobar Group of Andaman & Nicobar Islands

(Kalra, 1974). *Ochlerotatus (Aedes) niveus* (now known as *Downsiomyia nivea*) group of mosquitoes were incriminated as the vectors for *W. bancrofti*.



Development in mosquito: Microfilariae are sucked from the peripheral blood of human. Microfilariae lose their sheath within 2 to 6 hours in the stomach of the mosquito and then they penetrate the stomach wall and within 4 to 17 hours migrates to thoracic muscles where they undergo metamorphosis and grow. In the next 2 days, they become short and thick like sausages having short spiky tails and measures 124 to 250µm in length and 10 to 17µm in diameter, also possess rudimentary digestive tract. These are first-stage larvae (L1). Within next 3 to 7 days, they grow rapidly and moult to become the second stage larvae (L2) which measure 225 to 330µm in length and 15 to 30 µm in diameter. Metamorphosis finally completes by 10-11 days into third-stage filariform larvae (L3) which measure about 1500 to 2000 µm in length and 18-23 µm in diameter. The third stage larvae are actively motile and infective. These larvae migrate to the mosquito's labium (proboscis) and remain coiled.

Infection of the new human host

The infective larvae migrate to the mosquito's proboscis and are deposited on the skin and find their way through a bite wound. The L3 develop to fourth-stage larvae (L4) as they migrate through the human body to the lymphatic vessels and lymph nodes, where they develop into adult worms.

The three species of parasitic worm, *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, have generally similar life cycles. Many factors contribute to the inefficient transmission of lymphatic filariasis. Firstly, microfilariae do not multiply in the mosquito body; hence, the number of L3 is limited by the number of microfilariae ingested. Second, only those mosquitoes that survive more than 10 days will contribute to transmission of the parasites. Those mosquitoes that die before the L3 develops, cannot play a role in the transmission cycle. Third, the L3 are deposited on the skin and have to find their way into the bite wound (rather than being injected with the mosquito saliva like malaria sporozoites).

Diagnosis of Lymphatic Filariasis

Microscopy: The standard method for diagnosing active infection is the identification of microfilariae by microscopic examination. This is not always feasible because in most parts of the world, microfilariae are nocturnally periodic, which means that they only circulate in the blood at night. For this reason, the blood collection has to be done at night to coincide with the appearance of the microfilariae.

Detection of Microfilaria in the Blood: For epidemiological screening, 20 cmm (4-5 drops) of finger-prick blood are taken and dried on a slide, stained and examined under a microscope in accordance with the standard procedure. Advantages of using microfilaria detection in finger-prick blood for initial

assessment include the general availability of materials and trained staff in many filaria endemic districts and the fact that positive specimens are “parasitologically confirmed”. Disadvantages include the need to collect blood at night (between 8.30 pm and 12 midnight) and the labour-intensiveness of preparing and examining slides.

Serologic techniques provide an alternative to microscopic detection of microfilariae for the diagnosis of lymphatic filariasis. Because lymphedema may develop many years after infection, lab tests are often negative with these patients. Immunochromatographic test (ICT) through cards for detection of circulating antigenemia reflecting presence of adult worm has been made cost effective and now being supplied by WHO as Filaria Test Strip (FTS). This gives result in just 10 minutes.

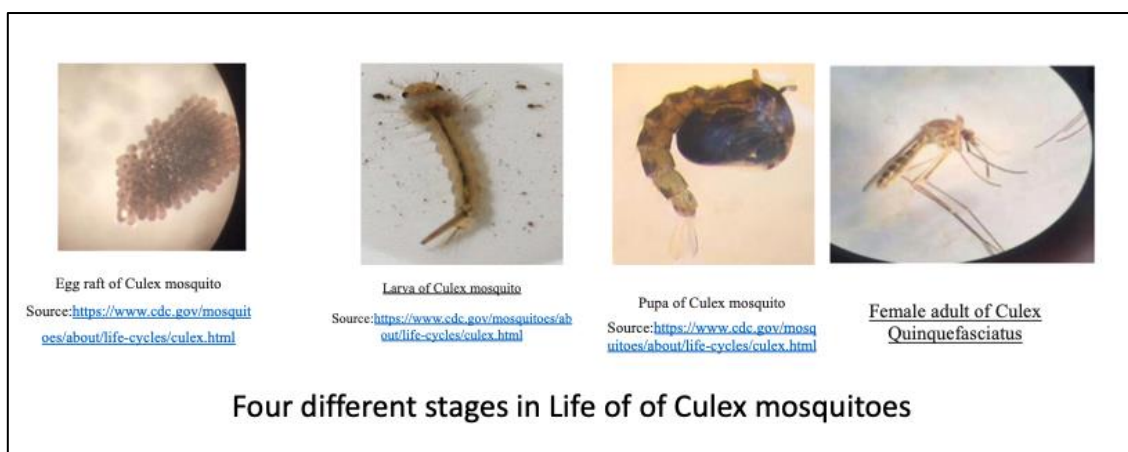
Vectors of LF *Culex (Cx) quinquefasciatus* is one of the major vectors for Bancroftian filariasis in India and is prevalent universally. This was first described by Thomas Say in 1823 from a specimen collected along the Mississippi River in the southern United States. *Bhattacharya & Basu. (2016)* described in his article *The Southern House Mosquito, Culex quinquefasciatus*. The profile of this smart vector *Cx. quinquefasciatus* also creates an ecological bridge between urban, peri-urban and rural areas owing to its presence and adaptability in diverse ecological niches which emerged as a smart vector because of the adaptive fitness, ecological plasticity, invasive behavior, host specificity and high reproductive potential along with expanded immune gene repertoire property at the genetic level. This mosquito possesses the necessary potential to initiate and facilitate the disease transmission by establishing an effective vector-host transmission cycle for diverse pathogens in different environments.

Systematic Position of Vector mosquito (Say, 1823)

Kingdom:	<i>Animalia</i>
Phylum:	<i>Arthropoda</i>
Sub-phylum:	<i>Hexapoda</i>
Class:	<i>Insecta</i>
Subclass:	<i>Pterygota</i>
Order:	<i>Diptera</i>
Suborder:	<i>Nematocera</i>
Family:	<i>Culicidae</i>
Genus:	<i>Culex</i>
Species:	<i>quinquefasciatus</i> (earlier known as <i>pipiens</i> and <i>fatigans</i>)

Life Cycle of *Cx. quinquefasciatus*

Tennyson et.al (2007) described the *Culex* mosquito as probably the most abundant house mosquito in towns and cities of the tropical countries. *Culex* mosquitoes develop in standing water such as polluted ponds, marshes, tanks, street gutters, tin cans, barrels, ornamental ponds, puddles, creeks,



itches, etc. The mosquito goes through four separate and distinct stages of its life cycle – Egg, Larva, Pupa and Adult. Each of these stages can be easily recognized by their special appearance.

Egg: Culex lay their eggs at night. It may lay the every third night during its life span. Eggs are laid together in a raft which contains 100 to 400 eggs. These eggs are stuck together. The eggs of Culex mosquito are cigar shaped and remain erect. Since they are laid in a raft, they lack air spaces and the numerous eggs are glued to each other on the sides and thus remain buoyant. A raft of eggs looks like a speck of soot floating on the water and is about ¼ inch long and 1/8 inch wide. Most eggs hatch into larvae within 48 hour.

Larvae: Mosquito larvae are commonly known as “*wrigglers*”. The larva of Culex mosquito rests obliquely, with the head pointing downwards, the young larva hatches out from the egg after 2-3 days. It has a distinct head but no clear demarcation between thorax and abdomen. It lacks legs. Tufts of bristles occur in a row on each side. The larva comes to the surface of water to breathe through a tube called respiratory siphon or respiratory tube situated at the end of abdomen. The atmospheric air is drawn in through these respiratory tubes into 2 long main tracheae, running from tail to the head. The larva is semi-transparent and swims in all directions by wave-like movements of the body. The larva eats small aquatic vegetation. Swallowing is aided by tufts of bristles present on other side of the mouth called feeding bristles. During growth, the larva undergoes moulting (shedding its skin) 4 times growing larger after each moult. The stages between moults are called instars. At the 4th instar, the larva reaches a length of almost 1/2 inch. When the 4th instar larva moults it becomes a pupa in about 2 weeks.

Pupa: Pupa Mosquito pupae are commonly called “*tumblers*”. The pupa of Culex mosquito is lighter than water and, therefore, floats on the surface. It takes oxygen through 2 breathing tubes called *trumpets*. The pupa is the non-feeding stage of development, but pupae are mobile, responding to light changes and moving (tumble) with a flip of their tails towards the bottom or protective areas. The pupa is comma-shaped and made up of *cephalothorax* (fused head and thorax) and the slender abdomen. When the pupa is about 2 days old, the eyes, antennae, legs and the wings are slowly formed that can be visible through the transparent outer covering. The respiratory trumpets of Culex mosquito are long and narrow. The pupa undergoes metamorphosis to form the (imago) adult.

Adult: The metamorphosis of the mosquito into an adult is completed within the pupal case. An adult mosquito emerges out by splitting the pupal cover. Initially the newly hatched mosquito rests on the floating discarded skin, and then rests on the surface of the water for a short time to allow itself to dry and all its body parts to harden. The wings have to spread out and dry properly before it can fly. Culex adult mosquitoes doesn't have spotted wings and it sits parallel while resting. Abd Sahar (2020) described that the adult's length of Culex is between 3.96 and 4.25 mm and it is are made up of a head with two large compound eyes, a thorax, a pair of scaled wings, and six jointed legs. The thorax, legs, and wings are covered with dark brown scales. The abdomen is covered with black scales and some of which are white, and the anal cercus is withdrawn. The adults rest on the surfaces in such a way that the thorax and head are parallel to the surface and the hose may form a small angle with the surface. The antenna of the male is *plumose*, easily visible near the eye and the antenna of the female is *pilose*. According to him male mosquitoes last less than a week and the females may live for a couple of months and that is only with ideal conditions depending on how much warmth and moisture. Sometimes females have to wait for about a day for their reproductive parts to develop completely. At the optimum temperature of 25°C to 30°C, the eggs hatch within 24 to 48 hours. The youngest stage is the first instar larva which moults to

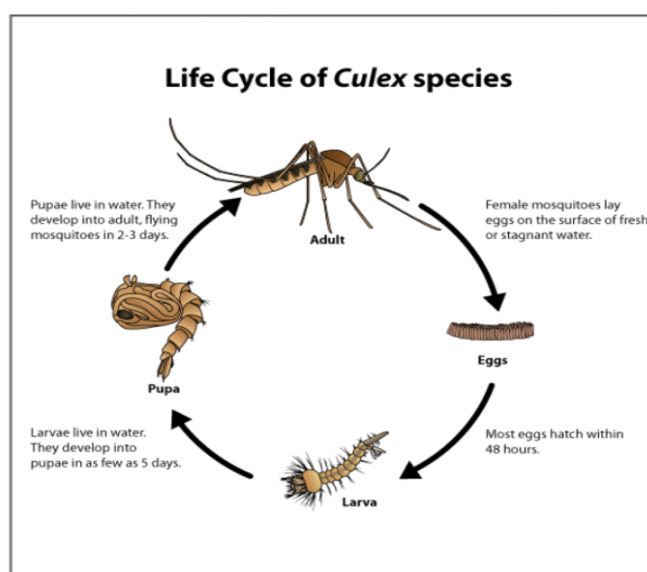


Figure 2. Life Cycle of Culex Species
Source: <https://www.cdc.gov/mosquitoes/about/life-cycles/culex.html>

subsequent instars each within 24-48 hours at optimum temperature. There are four instars in the larval stages, and all the instars are voracious eaters, taking anything and everything of microscopic size into the buccal cavity by instant vibration of its feeding brushes. The larvae feed on organic material in the water and require between 5 to 8 days to complete their development at 30°C. They are mainly bottom feeders but may feed from the surface also. Towards the end of the fourth instar they stop eating and undergo moulting to give rise to a comma shaped pupa, which lasts up to 24-48 hours at optimal condition. Pupae do not feed but are very active, respiring through its pair of breathing trumpets. After 36 hours (at 27°C), pupa emerges into an adult mosquito, through a longitudinal slit formed between the two trumpets. The entire cycle from egg to emergence of adult is completed in 7-10 days.

Culex mosquitoes feed at night with peak biting in late night or third quarter of night. They are both endophagous and exophagous as well. They are highly anthropophilic but feed on birds and other domestic animals too. They rest in dark corners of walls, on hanging objects, cobwebs, inside shoes, cupboards, under cots, tables and chairs etc. While males are usually weak fliers, the average flight range of female *Cx. quinquefasciatus* is about 2-3 km.

Mansonia (Mn) Species: The important vector species are *Mn. annulifera*, *Mn. uniformis* and *Mn. Indiana*. The first one is the primary vector, whereas the latter ones are of secondary importance. These species are found throughout year and their breeding is restricted to fresh water ponds, lakes, swamps & channels with floating vegetation. *Pistia stratiotes* is the most preferred plant for breeding of *Mn. annulifera* but *Salvinia* plant is preferred by *Mn. uniformis*. *Mansonia* lay eggs in clusters of 80-120 on under surface of floating water plants. Tips of pupal breathing trumpet is modified for attachment. *Mn. Annulifera* is endophilic & endophagous, rest in dark corners of house whereas, *Mn. indiana* remains mostly confined to cattle sheds. They are active throughout night with peak biting activity in first and second quarters of night. *Mn. annulifera*, is highly anthropophilic but *M. uniformis* is more zoophilic. They have limited flight range.



Global Disease Burden

Globally it was estimated that a population of about 1.25 billion in 83 endemic countries were at risk of LF. Out of these, 1/3rd of risk population lived each in India and Africa. Rest of the population lived in different parts of world viz., Parts of Asia, Pacific and Americas. The proportion of cases in WHO South East Asia Region was about 64% whereas it was about 32% in African region. European region was free of LF transmission.

At the start of GPELF, 81 countries were considered endemic for LF requiring for preventive chemotherapy (main strategy for elimination of LF), however, epidemiological data reviewed later indicated that preventive chemotherapy and transmission control (PCT) was not required in 10 countries hence the implementation of PCT was sustained in 71 countries.

Disease Burden in India

The National Centre for Vector Borne Diseases Control (NCVBDC) is the nodal agency responsible for implementing the Elimination of Lymphatic Filariasis (ELF). The NCVBDC implements the ELF under the ambit of National Health Mission (NHM). The National Programme initially in 2004 based on historical data and reports of National Filaria Control Programme identified 250 districts in 20 states and union territories with a population of about 600 million living at risk of LF. Subsequently, the programme was further scaled up to 345 districts. In the year 2018, Accelerated Plan for Elimination of Lymphatic Filariasis (APELF) and triple drug therapy with Ivermectin + DEC + ALB (IDA) was introduced as a pilot in 5 districts of five states. As of December 2023, 76 districts across 10 states have conducted at least one round of IDA.

Elimination Concept

The International Task Force for Disease Eradication identified lymphatic filariasis as one of the six infectious diseases to be “eradicable” or “potentially eradicable and World Health Assembly (WHA, 1997) passed the resolution in 1997 for Elimination of LF (Ottesen et al., 1997) as a global public health problem by 2020. India became a signatory to this resolution with commitment towards elimination. Subsequent to Global Alliance to Eliminate Lymphatic Filariasis (GAELF) meeting in 2000 at Spain, Global Programme to Eliminate Lymphatic Filariasis (GPELF) was formulated by WHO with setting a goal of elimination by the year 2020. Elimination of lymphatic filariasis is possible by stopping the spread of the infection through preventive chemotherapy and transmission control (PCT). The two main objectives of GPELF (Ottesen, E A 2000) were to progressively reduce and ultimately interrupt the transmission of lymphatic filariasis; and to prevent, reduce disability in affected persons through disability alleviation and appropriate management.

Elimination Strategy

The WHO recommended preventive chemotherapy strategy for lymphatic filariasis elimination is mass drug administration (MDA). The objective of the Mass Drug Administration (MDA) is to interrupt the transmission by reducing the microfilaria density in the community. However, efforts should be made to reduce the breeding of the mosquitoes by adopting various vector control measures. The strategy for elimination of lymphatic filariasis was based on following two pillars:

Annual Mass Drug Administration (MDA) of single dose of Diethylcarbamazine (DEC) + Albendazole for minimum of 5 years to the eligible population (except pregnant women, children below 2 years of age and seriously ill persons) to interrupt transmission of the disease.

Home based management of lymphoedema cases and up-scaling of hydrocele operations in identified CHCs/ District hospitals /medical colleges

WHO recommends the following MDA regimens

- Albendazole (400 mg) alone twice per year for areas co-endemic with loiasis;
- Ivermectin (200 mcg/kg) with albendazole (400 mg) in countries with onchocerciasis;
- Diethylcarbamazine (DEC) (6 mg/kg) and albendazole (400 mg) in countries without onchocerciasis; and
- Recently revised as ivermectin (200 mcg/kg) together with DEC (6 mg/kg) and albendazole (400 mg) to expedite the process of achieving interruption in countries without onchocerciasis and where other programmatic conditions are met.

Current status on MDA

As per WHO, 9.7 billion cumulative treatments were delivered from 2000 to 2023, to more than 943 million people at least once in 71 countries, considerably reducing transmission in many places. The population requiring MDA has declined by 58.6% (871 million) where infection prevalence has been reduced below elimination thresholds. Twenty-one countries and territories (Bangladesh, Brazil, Cambodia, the Cook Islands, Egypt, Kiribati, Lao People’s Democratic Republic, Maldives, Malawi, Marshall Islands, Niue, Palau, Sri Lanka, Thailand, Timor-Leste, Togo, Tonga, Vanuatu, Viet Nam, Wallis and Futuna, and Yemen) are now acknowledged as achieving elimination of lymphatic filariasis as a public health problem. By 2023, 14 countries had successfully implemented recommended strategies, stopped large-scale treatment and are under surveillance to demonstrate that elimination has been achieved. Preventive chemotherapy is still required in 39 countries and within 6 of these countries, MDA has not yet been delivered to all endemic areas as of the end of 2023.

Programme Implementation in India

To realize the goal set for LF elimination in India, Government of India decided in 2003 to cover all endemic districts under elimination strategy and ELF campaign was launched in the year 2004 by starting annual MDA duly inaugurated by the then Union health minister and Union minister of health for State in Thane and Rae Bareilly simultaneously. The operational guidelines (NVBDCP 2005 & 2009) and training manuals were framed and disseminated across various states and UTs. The National Filaria day was decided as 5th June but was shifted to 11 November considering state's request, preparatory time, logistics of drugs, finance and also the convenient climatic condition for operational purposes. While the programme was being upscaled, many decisions on replacement of loose DEC tablets with strip packing and co administration of Albendazole with DEC were taken during 2006. During 2007, all the 250 identified LF endemic districts were brought under MDA but DEC+ Albendazole was administered only in Tamil Nadu and Kerala. In 2008, two-drug administration was extended to 4 southern states and from 2009 onwards, all districts were brought under coadministration (Srivastava and Dhillon, 2008).

The national average of mf prevalence dropped from 1.3 per cent to 0.4 percent by 2016 and out of 256 endemic districts, about 200 districts reported average mf prevalence below 1% (Srivastava & Dhillon, 2008, Srivastava et al., 2014). This significant achievement was subjected to independent appraisal and assessment by Joint Monitoring Mission. In addition, every year the post MDA assessment by medical colleges, regional offices and research institutions was undertaken which was made as inbuilt component of programme, though some states could not get it done due to resource constraints both physical and financial.

The ELF has made considerable progress since its inception in the year 2004 with significant reduction in and Daman & Diu, Tamil Nadu, Lakshadweep, Andaman & Nicobar Islands that have stopped the disease burden in 6 states/union territories namely Goa, Puducherry, Dadra & Nagar Haveli and Daman MDA and a few states are close to elimination status. & Diu, Tamil Nadu, Lakshadweep, Andaman & Nicobar Islands that have stopped MDA and a few states are close to elimination status.

The WHO has now aligned its global target of LF elimination by 2030 in accordance with Sustainable Development Goal (SDG). Indian Programme has also developed the enhanced five pronged strategy with new interventions to further accelerate the progress towards elimination.

By the year 2023, the number of endemic districts (Implementation units) were increased to 345 in 21 endemic States and Union Territories, of which 170 districts observed MDA as 138 districts stopped MDA and cleared Transmission Assessment Survey 1 (TAS1). The remaining 37 districts are under assessments.

Post MDA surveillance

The states broadly achieving the target required to evidence it through transmission assessment survey (TAS) which facilitates the decision to continue or discontinue MDA. The WHO revised guidelines in 2011 to provide clearer and more feasible methodologies to national programmes on monitoring and epidemiological assessment (WHO, 2005 and WHO 2011). This revised guidelines on monitoring and evaluation and WHO document on strategic plan (WHO 2010) since then has been used in programme for Transmission Assessment Survey (TAS). The programme also developed its TAS guidelines and disseminated to all states (NVBDCP, 2013; Srivastava et al., 2014). The population in an IU is considered to no longer require MDA once the criteria have been met in both sentinel and spot-check surveys and TAS. A TAS is considered "passed" when the number of children who test positive for LF infection is below the allowed critical cut-off value, which represents the prevalence below which transmission is considered unsustainable.

Indian programme on LF elimination started tasting the success (Srivastava PK et al., 2014) when Goa, Thiruvallur of Tamil Nadu and Daman Diu passed first TAS during 2013-14. There were many success stories as many districts (Dhariwal et al., 2015) started reporting microfilaria prevalence below 1% and cleared TAS successfully.

Entomological surveillance especially Xeno-monitoring, also known as xeno-surveillance, is being emphasized in LF elimination programmes. This indirect approach plays a crucial role in monitoring LF transmission and guiding control efforts. Xeno-monitoring directly detects LF parasites within mosquito vectors using polymerase chain reaction (PCR) techniques. This method is crucial during post-Mass Drug Administration (MDA) surveillance to identify potential resurgence of infections. During xeno-monitoring, mosquito samples are analysed in pools with for prevalence of infection in the area.

Morbidity management and disability prevention (MMDP)

Morbidity management and disability prevention are vital for improving public health and are essential services that should be provided by the health care system to ensure sustainability. Surgery can alleviate most cases of hydrocele. Clinical severity and progression of the disease, including acute inflammatory episodes, can be reduced and prevented with simple measures of hygiene, skin care, exercises and elevation of affected limbs. People with lymphoedema must have access to continuing care throughout their lives, both to manage the disease and to prevent progression to more advanced stages. The GPELF aims to provide access to an essential package of care for every person with associated chronic manifestations of lymphatic filariasis in all areas where the disease is present, thus alleviating suffering and promoting improvement in their quality of life. Goals toward the elimination of lymphatic filariasis will be achieved if affected people have access to the following essential package of care:

- Treatment for episodes of adenolymphangitis (adl)
- Guidance in applying simple measures to manage lymphoedema to prevent progression of disease and debilitating, inflammatory episodes of adl
- Surgery for hydrocele
- Treatment for infection.

In India, home based morbidity management (WHO, 2004; Srivastava and Dhariwal, 2010) was also scaled up and mostly line-listing of lymphoedema and hydrocele cases was made in all endemic districts enumerating around 1.3 million cases. The patients are being provided with the recommended minimum package of care to alleviate suffering and improve the quality of life. Under the programme, a cumulative total of 2,13,208 hydrocelectomies were reportedly performed till end of 2023 to improve the condition of the affected people.

Vector Control inclusion

Mosquito control is a supplemental strategy supported by WHO (WHO 2013). It is used to reduce transmission of lymphatic filariasis and other mosquito-borne infections. Depending on the parasite-vector species, measures such as insecticide-treated nets, indoor residual spraying or personal protection measures may help protect people from infection. The use of insecticide-treated nets in areas where *Anopheles* is the primary vector for LF enhances the impact on transmission during and after MDA. Historically, vector control in select settings has contributed in control of lymphatic filariasis in the absence of large-scale preventive chemotherapy (Das et al., 2002)

Conclusion

The dramatic research advances have led to new understanding about the severity and impact of the disease and new diagnostics and monitoring tools and most importantly new treatment tools and control strategies (WHO Bulletin 1997-75) helped in conceptualizing the elimination of LF. The new strategy aimed both at transmission control through community wide (mass) treatment programmes and at disease control through individual patient management. Annual single dose co-administration of two drugs (Albendazole with Ivermectin or DEC) and now triple drug IDA (Ivermectin + DEC+ Albendazole) has evidenced in significant reduction of microfilaria prevalence thereby reducing at risk population.

New approaches on preventing bacterial super infection may reduce the suffering of affected persons and reduce the number of acute attacks.

In 2020, GPELF set the following goals for the new NTD Road Map (2021–2030) and announced that 58 (80%) endemic countries have met the criteria for validation of elimination of LF as a public health problem, with both sustained infection rates below target thresholds for at least 4 years after stopping MDA and providing the essential package of care in all areas with known patients. It was also stated that 72 (100%) endemic countries implement post-MDA or post-validation surveillance in the districts after clearance of TAS. WHO has also listed the milestones towards validation of elimination as: Stopping the spread of infection through mass drug administration in all endemic areas (100% geographical coverage); Reduction of infection below a threshold at which transmission is not sustainable in all endemic areas and stopping of MDA and its evidence by sustained reduction of infection below the threshold no earlier than 4 years after stopping MDA; ensuring availability of recommended minimum package of care in all areas of known patients (100% geographical coverage) to alleviate suffering through MMDP and supplement with vector control in defined areas. In view of the significant progress made globally and in India towards achieving elimination of LF, it may be concluded that the mission is optimistic and may be achieved with the same enthusiasm and commitment by political leadership, policy makers, administrators, implementers (service providers), community (recipients) subject experts and researchers of academic and scientific institutions.

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DENGUE IN INDIA: STATUS AND PUBLIC HEALTH CHALLENGES

**Kalpana Baruah*, Amit Katewa, Gavendra Singh, Nandini Arora,
Rinku Sharma and Tanu Jain**

National Center for Vector Borne Diseases Control
Directorate General of Health Services
Ministry of Health and Family Welfare
Government of India, Delhi

*Corresponding author: drkalpanabaruah@gmail.com

Abstract

Dengue fever is a significant public health concern in India, with outbreaks occurring annually, particularly during the monsoon and post-monsoon season. The disease is transmitted primarily by *Aedes* mosquitoes. In urban areas, *Aedes aegypti* is the main vector whereas in semi-urban and rural areas, *Aedes albopictus* plays a vital role in transmission. Symptoms of dengue include high fever, severe headache, joint and muscle pain, rash, and in severe cases, it may be fatal without proper management. India has seen a rising number of cases in recent years, exacerbated by urbanization, climate change, insufficient vector control measures and other factors. This Chapter deals with status of Dengue, its epidemiology, factors responsible for geographical spread, control programme and challenges for prevention and control.

Introduction

Dengue fever is a significant public health concern in India, with outbreaks occurring annually, especially during the monsoon season. It is a viral illness caused by the Dengue virus (DENV), which is transmitted to humans primarily through the bite of infected female *Aedes* (*Ae.*) mosquitoes. These mosquitoes are diurnal (day biter) and most active during early morning and dusk time. Dengue fever typically presents with high fever, severe headache, pain behind the eyes, joint and muscle pain (often referred to as "breakbone fever"), nausea and vomiting, skin rashes and fatigue and weakness. In some cases, the infection can progress to moderate and severe forms, which can be life-threatening and require immediate medical attention. Since isolation of virus from India for the first time in 1945, it has spread to all States and Union Territories except Ladakh. All four Dengue virus serotypes have been isolated from the Country. All age groups of both the genders are affected. Though the number of cases shows an increasing trend in recent past, the Case Fatality Rate (deaths per 100 cases) is very low due to better case management. Earlier, urban areas were more affected, however in last few years, cases are reported from rural and semi-urban areas as well due to various compounding factors. Climate change including altered rainfall patterns and rising temperatures, has also contributed to the year-round transmission, geographical spread and intensity of disease making it more unpredictable in both endemic and non-endemic areas.

Global status

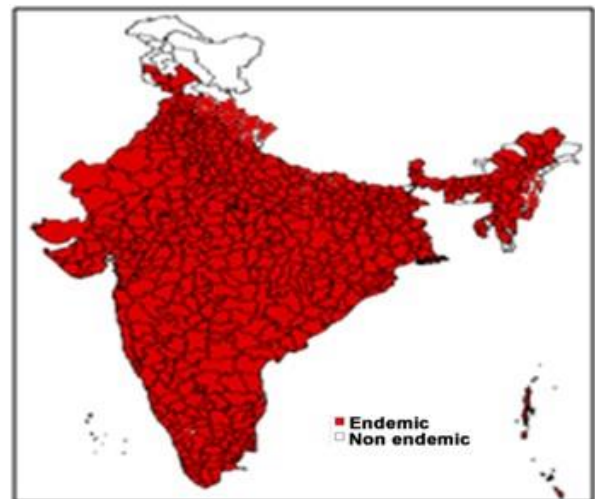
Dengue is a rapidly growing global public health threat, with an estimated 390 million infections annually, affecting millions across tropical and subtropical regions of the world. Dengue has been identified as one of the 17 neglected tropical disease by WHO as mentioned in their first report on neglected tropical diseases (2010) (WHO). The disease is endemic in over 129 countries, particularly in Southeast Asia, the Pacific Islands, Latin America, and parts of Africa and the Middle East (WHO 2009, 2010, 2011). In recent decades, the frequency and geographic spread of dengue have increased, driven by various factors. Efforts to control dengue have focused on vector management, vaccine development, and public health initiatives, but the

increasing resistance of mosquitoes to insecticides, combined with limited resources in many endemic regions, has made effective control challenging. The World Health Organization (WHO) has prioritized dengue as a global health issue, and ongoing research into vaccine development and novel vector control methods is key to reducing the disease burden. Dengue is also listed among the 40 emerging diseases of global importance (Vijayakumar et al. 2005; Suaya et al. 2009).

Dengue in India

The history of dengue in India spans over a century, and its evolution from a neglected disease to a major public health concern reflects the country's changing socio-economic, environmental, and health dynamics. Although the disease likely existed in India for centuries, it was only during the 20th century that dengue became a recognized and important epidemic, and in the 21st century, it reached endemic status in many regions of the Country. It remains a major public health challenge in India, with cases fluctuating year-on-year, particularly during the monsoon season when the *Aedes aegypti* mosquito thrives. The country reports fluctuating numbers of dengue cases annually, with outbreaks often occurring in urban and peri-urban areas, although rural regions are increasingly affected as well (Barde et al. 2015). While the number of cases has been rising in recent years, the actual incidence is believed to be on higher side. The government has been implementing vector control measures and public awareness campaigns, but challenges remain in controlling mosquito populations due to factors like rapid urbanization, climate change, and inadequate waste management systems.

Dengue virus was isolated in India for the first time in 1945. The first evidence of occurrence of dengue fever in the country was reported in 1956 from Vellore district in Tamil Nadu (Chatterjee et al. 1965; Arunachalam et al. 2010). The first dengue hemorrhagic fever (DHF) outbreak occurred in Calcutta (West Bengal) in 1963. Currently, all States/Union Territories (except Ladakh) are endemic for Dengue. During 1996, one of the most severe Dengue outbreaks occurred with 16,517 cases and 545 deaths (10,252 cases and 423 deaths were from Delhi alone). In 2006, the country witnessed another outbreak with 12,317 cases and 184 deaths. The incidence of dengue is increasing in the last few years. Outbreaks were also reported during 2010, 2012, 2013, 2015, 2016, 2017, 2019, 2021, 2023. Though the number of Dengue cases show an increasing trend in the Country, however, the case fatality ratio (CFR- deaths per 100 cases) has declined from 3.3% in 1996 to 0.1% in 2023 due to better case management as per the updated National guidelines (Source: NCVBDC data).



The disease mostly exhibits a seasonal pattern at country level. Cases start to increase with the onset of monsoon, continue throughout the wet months, reaches on peak during post-monsoon and then declines. This pattern is not uniform at State level due to diverse eco-epidemiological variants within States, which is evident from the perennial transmission in Southern and Western states (Chakravarti and Kumaria 2005).

Virus

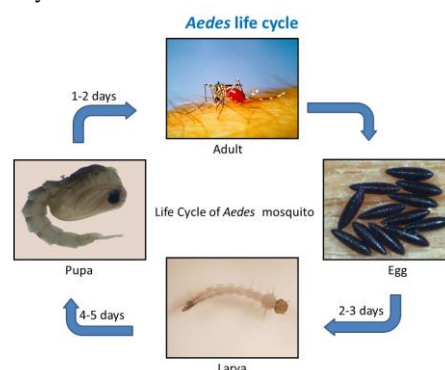
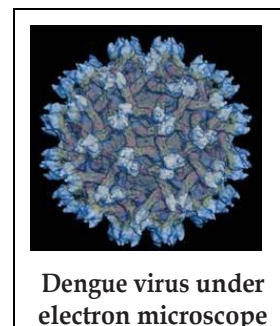
Dengue virus (DENV) is *Flavivirus* under family *Flaviviridae*. The virus has an envelope (lipid bilayer) derived from the host cell containing the E protein (envelope protein) which plays a critical role in receptor binding and fusion with the host cell membrane. Inside the envelope, the viral genome is a positive-sense RNA, which encodes several proteins, including the capsid (C), which surrounds the RNA genome, and non-structural proteins involved in replication. The virus has an icosahedral symmetry, with the E proteins arranged on the surface in a way that forms a characteristic spiky appearance.

There are four distinct serotypes of dengue virus known as DENV-1, DENV-2, DENV-3, and DENV-4. These are genetically distinct but closely related. A person can be infected by each serotype separately, immunity to one serotype typically lasts for life, but only against that particular serotype.

Vector

The *Aedes* mosquitoes transmitting Dengue also transmit Chikungunya, Zika and Yellow Fever which mainly breed in artificial containers with stagnated water. Dengue is mainly transmitted by *Ae. aegypti* mosquito and also by *Ae. albopictus*. In India, both species are prevalent. The *Ae. aegypti* is a major vector throughout country but in peninsular areas and North-East, the *Ae. albopictus* is an established vector of Dengue (Rigau-Perez et al. 1988; Gubler 1997; Gubler 1998; Kyle et al. 2008; Nathan et al. 2009). Other than peninsular States, the number of *Aedes* breeding sites increase during monsoon and post-monsoon period only.

Vector breeding containers varies from place to place and different areas depending on ecology, human behaviour, cultural practices, industrialization and human habitats. The major types of usual breeding habitats like domestic water holding/storage containers, desert coolers, flower vases, earthen pots, refrigerator trays, money plants, Tyres, junk materials, construction sites, leaf axils, latex collection cups etc. There are unique breeding habitats for both *Ae. aegypti* and *Ae. albopictus* in many areas.



Breeding sites for *Ae. aegypti*

Breeding sites for *Ae. albopictus*

A few breeding sites of *Aedes* mosquitoes (Source: NCVBDC unpublished data) are as given below:

In view of risk due to Dengue, *Aedes* mosquitoes received special attention of entomologists, public health specialists, policy makers and sanitary workers. It is important to identify the vector and the vulnerable time in the life cycle for correct forecasting of population increase under known environmental conditions. This information provides a sound basis for the planning of strategies for effective prevention and control.

Transmission cycle

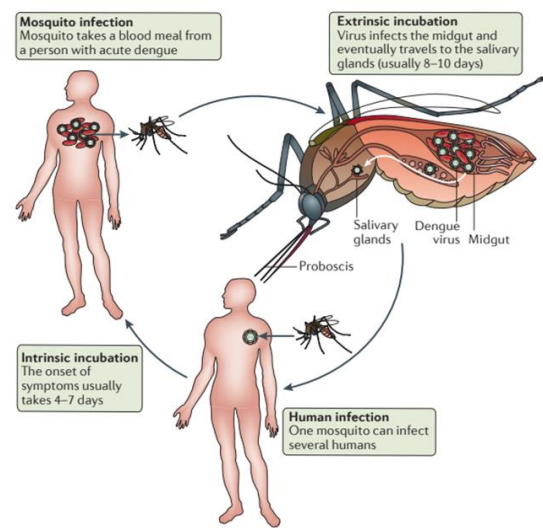
The disease is spread when a mosquito bites a person who is already infected with the virus and then bites another person. Once the mosquito ingests the virus, it replicates in various tissues including the mosquito's salivary glands. This is crucial because the virus needs to reach the salivary glands in order to be transmitted when the mosquito feeds on a human. The mosquito bites an uninfected person, injecting

the virus into their bloodstream through the mosquito's saliva which contains proteins that prevent blood clotting and allow for the feeding process.

The virus incubates in human body for 4-10 days before symptoms begin to appear. If a human develops dengue, the virus may be taken by female *Aedes* mosquitoes if they are bitten during the period when the virus is present in their bloodstream (typically 4-5 days after the onset of symptoms). After the mosquito bites the infected human and ingests the blood, the cycle repeats, allowing the mosquito to transmit the virus to other humans.

The transmission cycle in brief is as below:

The mosquito is the primary vector for dengue transmission. Humans are the reservoir of the virus and serve as the source of infection for mosquitoes. The mosquito-to-human transmission cycle is necessary for the spread of the disease.



Dengue Transmission Cycle

Source: Guzman et al. 2016

Infected Human → *Aedes* Mosquito (through bite) → Virus replication (in mosquito's body) → Uninfected Human (bite) → Uninfected Human becomes infected and cycle continues

Factor responsible for geographical spread

Dengue spread is influenced by a combination of environmental, socio-economic, and biological factors. The primary vector of dengue, the *Ae. aegypti* mosquito, thrives in warm, humid environments and breeds in stagnant water, making poorly managed urban areas, rural communities, and places with inadequate sanitation prime hotspots for transmission. Climate change is exacerbating the situation by creating warmer temperatures and more erratic rainfall patterns, expanding the mosquito's breeding season and geographical range. Rapid urbanization and population growth, coupled with insufficient waste management and water storage practices, provide ample breeding sites, especially in informal settlements (Baruah et al. 2020; Baruah et al. 2021). Additionally, the movement of people due to global travel, migration, and trade helps spread the virus across regions, making outbreaks more widespread (Cummings et al. 2004). Socio-economic factors, such as lack of public awareness, limited access to healthcare, and inadequate vector control infrastructure, also contribute to the spread and severity of the disease, hindering effective prevention and response efforts.

The urban areas are more vulnerable for Dengue transmission as enormous vector breeding sites are created due to the construction related activities, erratic water supply leading to improper water storage practices, sub-optimal solid waste management and increased use of non-biodegradable plastics. Currently, urbanization is increasing at a rapid pace and it is expected that there will be many new cities during next few years. As per Census 2011, urban population was 31.16% of the total population, which is projected to be 46% by 2030.

Population movement plays a significant role in the spread of dengue fever. The movement of people between rural and urban areas, as well as across borders, facilitates the transmission of the virus. Migrants and travellers often introduce the virus to new areas earlier unaffected which can lead to localized outbreaks. Additionally, festivals, religious gatherings, and travel during peak monsoon months, when mosquitoes are most active, further increase the risk of dengue spreading to non-endemic areas. Population movement combined with insufficient vector control measures, accelerates the geographic spread of

dengue, making it harder to contain outbreaks and posing challenges for timely and effective public health interventions (Baruah et al. 2020).

Disease control

The National Center for Vector Borne Diseases Control- NCVBDC (earlier National Vector Borne Disease Control Programme- NVBDCP) is a key public health initiative in India under Ministry of Health and Family Welfare, Government of India to control and eliminate vector borne diseases including Malaria, Dengue, Chikungunya, Japanese encephalitis, Kala-azar and Lymphatic filariasis. Its history, a National Malaria Control Programme (NMCP) was launched in 1953 for malaria control in endemic areas and the programme was modified to a countywide National Malaria Eradication Programme (NMEP) in 1958 in view of spectacular success of NMCP. In 2003, the programme was renamed as NVBDCP (involving Dengue and other VBDs) and NCVBDC in 2021 (Source: NCVBDC).

Country's dengue control programme primarily focuses on vector control and public awareness to reduce the spread of the disease. Key strategies include eliminating mosquito breeding sites by managing stagnant water in domestic and public areas, regular fogging and spraying of insecticides, and the use of larvicides in water bodies where mosquito larvae are found. The government conducts awareness campaigns to educate communities about preventing mosquito breeding by eliminating discarded containers, maintaining clean water storage, and using personal protection measures like mosquito nets and repellents. Additionally, surveillance systems are established to monitor cases and identify outbreaks early. The programme is supported by state and local health departments with ongoing efforts to improve diagnostic facilities and healthcare responses for both mild and severe dengue cases. However, challenges such as rapid urbanization, inadequate sanitation, and inconsistent implementation of control measures in rural and urban slums continue to hinder the effectiveness of these programs.

Till date, there is no specific medicine to treat dengue infection or any vaccine to prevent infection. Cases are managed symptomatically (based on symptoms). National guidelines for clinical management of Dengue fever have been developed which proved helpful in effective case management to minimize the Case Fatality Rate (deaths per 100 cases) in the Country (Source: NCVBDC).

Vector control

Vector control is the sole effective method for prevention and control of *Aedes* borne disease. For *Aedes* control, different methods are being used which are as per the National Guidelines highlighting key elements including Vector Surveillance and Vector control. Also, guidelines for Integrated Vector Management including measures for *Aedes* control are implemented by States and Union Territories (Srivastava et al. 2014). The main measures for *Aedes* control include entomological surveillance; source reduction; environmental modification; use of larvicides, growth regulators, adulticides, larvivorous fishes; personnel protection (mosquito nets, repellents, protective clothing) and bye-laws (legislation). Under chemical control measures, larvicides are recommended in permanent big water containers where water has to be conserved or stored because of scarcity of water or irregular and unreliable water supply. Adulticides are recommended only during emergencies and outbreak situations to cut down or interrupt transmission.

The vector control measures are also supported by the focused awareness activities (including print/digital/social media, rallies, drawing/printing competitions, observation of 'National Dengue Day' on 16th June and 'Anti Dengue Month' during July every year throughout the Country and involvement of local & community leaders).

Vaccine

Dengue vaccines have been under development since the 1940s, but a development of a tetravalent vaccine which simultaneously provides long-term protection against all serotypes is still awaited (Gupta et al. 2012). Globally, there are currently 2 licensed dengue vaccines. Both are tetravalent live-attenuated vaccines but differ in the extent of chimerization and the genome backbone, as well as in efficacy and safety. These are not yet approved for use in Country for want of more locally generated data. ICMR initiated a first-ever Phase III clinical trial for Indigenous dengue vaccine. It is a tetravalent dengue vaccine strain originally developed by National Institutes of Health (NIH). Phase 1 and 2 clinical trials of the Indian vaccine formulation were completed in yielding promising results. The Phase III trial will evaluate the efficacy of this vaccine involving healthy adult participants as per protocol (Source: ICMR).

Challenges in Dengue control

Dengue control faces several challenges, including the complex biology and behavior of *Ae. aegypti* mosquitoes, which are highly adaptable to urban environments and thrive in close proximity to human populations. Mosquitoes are capable of developing resistance to insecticides, making chemical control less effective over time. For Aedes mosquito, limited insecticide-based interventions are available for vector control. But over the years, development of insecticide resistance in both the vector species is posing a major challenge for the effectiveness of vector control strategies (Kumawat et al. 2021). The absence of a universal vaccine and the presence of multiple dengue virus serotypes further complicate efforts, as immunity to one serotype does not protect against others, and secondary infections can increase the risk of severe disease. Additionally, the lack of widespread access to healthcare in endemic regions, combined with inadequate public awareness and vector control efforts, hinders effective management. Environmental factors, such as climate change, can also influence mosquito distribution and abundance, potentially leading to more frequent outbreaks (Chandy et al. 2013). Furthermore, the asymptomatic nature of many dengue infections means that people may unknowingly spread the virus, making containment even more difficult.

Climate change: Climate change is intensifying the spread of dengue by creating more favourable conditions for *Aedes* mosquitoes, the primary vectors of the disease. Rising temperatures, increased rainfall, and more frequent flooding due to climate change extend the mosquitoes' breeding season and expand their geographic range, allowing them to thrive in new areas previously considered too cool or dry for their survival (Rochlin et al. 2013; Tuladhar et al. 2019). Warmer temperatures accelerate the mosquitoes' development and increase the virus replication rate within them, leading to higher transmission rates. Additionally, erratic weather patterns, such as more intense storms and prolonged droughts, create stagnant water pools in urban and rural areas, further providing breeding grounds. These changes in climate patterns, combined with inadequate vector control and public health infrastructure, are making dengue outbreaks more frequent and more severe, especially in regions where the disease was not previously a significant concern.

Insecticide resistance: Insecticide resistance in *Aedes* mosquitoes is a growing concern requiring urgent attention. Resistance develops through selection pressure created by prolonged usage of a particular insecticide (Sumitha et al. 2023). Resistance to commonly used insecticides, such as Organophosphates, Pyrethroids, and Temephos has been observed in several regions across the country. This resistance can complicate efforts to control mosquito populations and reduce the transmission of mosquito-borne diseases. The key factors contributing to insecticide resistance in *Aedes* mosquitoes are overuse of Insecticides due to repeated and excessive use; inadequate surveillance without proper monitoring and genetic adaptation in mosquitoes with high reproductive rates and short lifespans (accelerating the process of natural selection).

Co-infection and Co-morbidity: Dengue coinfection occurs when a person is simultaneously infected with dengue virus and another pathogen, such as another arbovirus (e.g., Chikungunya or Zika), a bacterial or parasitic infection. Coinfection can complicate the clinical presentation as symptoms may overlap or worsen, making diagnosis and treatment more challenging. Dengue fever comorbidity risk is also increased in individuals with chronic conditions such as diabetes, hypertension, and cardiovascular diseases, as these can lead to complications like bleeding or shock during severe dengue. Immunocompromised individuals are at greater risk for severe disease due to a weakened immune response. Previous dengue infections with a different serotype increase the risk of severe outcomes through antibody-dependent enhancement (ADE). Additionally, young children, the elderly, and individuals with obesity or liver disease are more susceptible to complications. Pregnant women are at particular risk, with potential adverse effects on both the mother and fetus. These comorbidities make dengue management more complex, emphasizing the need for early intervention and careful monitoring in at-risk populations.

Inter-sectoral Coordination for Dengue: Intersectoral coordination is crucial for effective dengue control, as the disease's spread is influenced by factors beyond the health sector, including urban planning, water management, sanitation, and environmental factors. For successful dengue prevention and control, collaboration is needed between multiple sectors such as health, municipalities, environment, education, transport, and agriculture. Local government bodies must work with health departments to identify mosquito breeding sites, implement vector control measures like the elimination of stagnant water, and improve waste management. Public awareness campaigns, led by educational and community organizations, play a vital role in informing the public about dengue prevention practices. Additionally, climate and weather forecasting agencies can provide data that helps anticipate outbreaks and guide preventive actions. By integrating efforts across different sectors, the impact of dengue can be significantly reduced, ensuring a more comprehensive and sustainable approach to control and prevention.

Community participation: Community participation is crucial for effective dengue control, as local engagement can significantly reduce mosquito breeding sites and prevent the spread of the virus. Communities can actively participate by eliminating standing water around homes, such as in containers, tires, and gutters, which are prime mosquito breeding grounds. Public education campaigns can raise awareness about dengue symptoms, the importance of mosquito protection, and proper water management practices. Local residents can also help monitor mosquito populations, report breeding sites to health authorities, and collaborate with health workers for targeted vector control efforts. By fostering collective responsibility and ensuring that prevention measures are integrated into daily routines, community participation strengthens dengue control efforts, making them more sustainable and widespread.

Dengue and Health Impact Assessment: Health Impact Assessment (HIA) for dengue in context of construction bye-laws, faces challenges due to insufficient integration of vector control measures in urban planning and construction regulations. In many areas, construction activities inadvertently create environments conducive to mosquito breeding, such as open water storage or poorly managed waste. Existing bye-laws often lack specific guidelines for preventing mosquito habitats, such as ensuring proper drainage and proper storage of water in covered containers. There should be provision for access to roof top to check and eliminate the *Aedes* breeding in overhead tanks and other containers lying on rooftop. Moreover, rapid urbanization and rampant construction, where compliance with formal bye-laws may be limited, intensify the spread of dengue. Also, the existing regulations do not adequately address mosquitogenic conditions in project areas such as improperly designed drainage systems, open water storage, or unregulated waste disposal. These deficiencies can lead to an increase in *Aedes* mosquito populations, elevating the risk of dengue outbreaks in project areas. As a result, the effectiveness of public health measures in reducing dengue transmission is compromised and the potential for outbreaks remains high in these areas.

Vaccine Development: Dengue vaccines have been under development since the 1940s, but a development of a tetravalent vaccine which simultaneously provides long-term protection against all serotypes is still waited (Gupta *et al.* 2012). The development of an effective vaccine is complex due to the need to achieve good efficacy for all four serotypes which have the ability to cause disease. All four serotypes of Dengue virus are known to circulate or co-circulate in many areas of the Country. In addition, Antibody-dependent enhancement (ADE) induced by pre-existing antibodies against DENV, creates an impediment for vaccine development since neutralizing antibodies need to be generated to all serotypes of dengue to confer protection (Kirkpatrick *et al.* 2016). The desirable characteristics of a dengue vaccine include acceptable short & long term safety; long term protection against all four serotypes; even if infections are not prevented, the vaccine must have the ability to reduce the risk of severe disease; and vaccine must be effective against all ages and population groups. While a dengue vaccine has been developed, it is not approved for use under public health programme.

Paradigm shift: The dengue transmission paradigm has undergone a significant shift, evolving from a primarily urban, seasonal concern to a more widespread, perennial, and geographically diverse issue affecting both urban and rural areas, as well as plains and even high-altitude regions. Historically, dengue was considered an urban disease, predominantly affecting cities with high population densities, poor sanitation, and rapid urbanization. Its transmission was largely seasonal, peaking during the monsoon months when rainfall created ideal breeding conditions for *Ae. aegypti* mosquitoes. However, dengue is spreading in rural areas due to a combination of environmental, socioeconomic, and infrastructural factors that create ideal conditions for mosquito breeding (Arunachalam *et al.* 2004). In these areas, poor waste management, inadequate water storage practices, and limited access to sanitation infrastructure often result in standing water, which serves as breeding sites for *Aedes* mosquitoes.

The transmission has also shifted from seasonal to perennial pattern in many States, as the mosquitoes' adaptability to varying climates, temperature changes, and human behaviours has increased. The rise in temperature and altered rainfall patterns due to climate change has enabled mosquitoes to breed and thrive throughout the year, making outbreaks more frequent and unpredictable. Dengue is increasingly spreading in hilly areas due to a combination of climatic and socio-environmental factors. Traditionally, these regions were less affected by dengue, but rising temperatures and erratic rainfall patterns linked to climate change are creating more favourable conditions for *Aedes* mosquitoes to breed in higher altitudes (Baruah *et al.* 2021). The construction of new settlements, increased tourism, and urbanization in previously remote hilly areas have also introduced new sources of stagnant water, such as water tanks, containers, and poorly managed infrastructure, providing ideal mosquito breeding sites. Additionally, limited public health awareness, inadequate vector control measures, and challenges in accessing healthcare further complicate the response to outbreaks in these areas. As a result, dengue cases are becoming more frequent in hilly regions, affecting both locals and tourists, and challenging traditional methods of prevention and control.

Conclusion

Dengue prevention and control in India require a multi-faceted approach that combines effective vector control, public awareness, improved healthcare infrastructure, and strong surveillance systems. While the government has made significant strides through initiatives like the National Center for Vector Borne Diseases Control (NCVBDC), challenges such as rapid urbanization, inadequate sanitation, and climate change continue to exacerbate mosquito breeding and dengue transmission. To achieve sustained success, it is crucial to implement community-based interventions, promote personal protection measures (such as the use of bed nets and repellents), and ensure timely diagnosis and appropriate treatment for dengue cases. Additionally, collaboration between health authorities, local governments, and communities is essential to curb outbreaks, particularly in vulnerable and high-risk areas. With continued efforts, innovation in vector control tools, community engagement, adequate intersectoral convergence, availability of effective vaccine and drug, the burden of dengue may be reduced in near future.

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CHIKUNGUNYA FEVER AND ITS ASSOCIATED PUBLIC HEALTH CHALLENGES IN INDIA

Kalpana Baruah*, Gavendra Singh, Amit Katewa, Nandini Arora, Rinku Sharma and Tanu Jain

National Center for Vector Borne Diseases Control
Directorate of General Health Services
Ministry of Health and Family Welfare
Government of India

*Corresponding author: drkalpanabaruah@gmail.com

Abstract

Chikungunya is an *Aedes* borne disease caused by an Arbovirus. It was initially seen in the early 1950s at the boundary of Tanzania and Mozambique. Due to the ease with which its vectors propagate, the virus has spread to India, Europe, and recently it arrived in the Caribbean, eventually extending into North, Central, and South America. The most common clinical manifestations are abrupt fever, polyarthralgia, headache, maculopapular rash, myalgia, nausea and vomiting. Severe joint pain and stiffness have been known to incapacitate some patients from a few days to several months after infection. Presently, all States and Union Territories (except Ladakh) are endemic for Chikungunya. The re-emergence of the Chikungunya virus and its spread to newer places has encouraged the development of new preventive, diagnostic, and treatment strategies. In this chapter, authors discussed brief history of Chikungunya virus, vector, challenges and expanding geographic distribution a public health challenge.

Introduction: Chikungunya is an arboviral disease transmitted by bite of infected female *Aedes aegypti* and *Ae. albopictus* mosquitoes. Chikungunya virus (CHIKV) belongs to genus Alphavirus and family Togaviridae. In the Swahili language, Chikungunya means "which contorts or bends up." It refers to the contorted (or stooped) posture of patients afflicted with severe joint pain (arthritis), a most common feature of the disease. It is a debilitating and a non-fatal viral illness. It causes fever and severe joint pain, which is often debilitating and may persist for several months, or even years. Other symptoms include muscle pain, headache, nausea, fatigue and rash. Joint pain is often debilitating and can vary in duration. The word 'Chikungunya' has been derived from a description in the Makonde language, meaning "that which bends up", of the contorted posture of people affected with the severe joint pain and arthritic symptoms associated with this disease. CHIKV belongs to the "New World" group of alphaviruses, which mainly cause musculoskeletal inflammatory diseases such as arthralgia, arthritis, and myalgia, classified as 'arthritis virus' including Chikungunya, Ross River virus (RRV), Ballmarch Forest virus (BFV), Group Sindbis and Mayaro (Mayv) (Hamid et al. 2018; Chung and Chlebicki 2013). It was originally described in the early 1950s after an outbreak in a Swahili village on the Makonde plateau that lies between Tanzania and Mozambique in Africa. It was first described during an outbreak in southern Tanzania in 1952 and has now been identified in nearly 40 countries in Asia, Africa, Europe and in the Americas.

During inter-epidemic periods, the CHIKV is consented through an enzootic cycle in places where it is endemic (such as Africa and Asia) and an urban cycle when the virus reaches the cities. In the enzootic cycle, continuous transmission of the pathogen occurs between wild animals and vectors. Non-human primates (NHP) serve as the reservoir for the virus, and the mosquitoes from the *Aedes* genus are the vectors responsible for the transmission of the virus. It is known that the CHIKV can use animals such as buffalos, rodents, and birds as hosts, but the critical host for enzootic circulation is not known with certainty. A spillover of the virus can occur when people who live in rural areas close to these virus reservoir cycles are bitten by an infected mosquito. If these people then travel to urban areas in which there are viable vectors,

more people could almost certainly be infected. In urban areas, the virus is transmitted by mosquitoes of the *Ae. aegypti* and *Ae. albopictus* species. It is preserved and further spread in an autochthonous cycle in which continuous transmission occurs between mosquitoes and humans.

Global Scenario

In 1952 the first CHIKV was reported in the Makonde Plateaus region in Tanzania. The virus was isolated from serum of several febrile patients and mosquitoes in 1953 by RW Ross. Then in 1958 the first laboratory confirmed CHIKV outbreak in Asia was reported in Bangkok, Thailand. Many countries in South East Asia, along with counties located in Central, Southern and Western Africa continued to report sporadic outbreaks up to 1980. However, activity dwindled in the latter half of the 20th century. Chikungunya epidemics display secular, cyclical and seasonal trends. There is an inter- epidemic period of 4-8 years (sometimes as long as 20 years). Outbreaks are most likely to occur in the post-monsoon period when the vector density is very high. Chikungunya outbreaks typically result in many cases, but deaths are rarely encountered. CHIKV was first identified in the United Republic of Tanzania in 1952 and subsequently in other countries in Africa and Asia. Urban outbreaks were first recorded in Asia in the 1970s, but since 2004, outbreaks of CHIKV have become more frequent and widespread. The first local, mosquito-transmitted Chikungunya cases in the Americas were reported in late 2013, after which large outbreaks occurred affecting most of the countries in the region. Chikungunya has now been reported in 118 countries in Asia, Africa, Europe and the Americas (Source: WHO). In Asia, CHIK virus strains were isolated in Bangkok in the 1960s; from various parts of India; Sri Lanka in 1969; Vietnam in 1975; Myanmar in 1975 and Indonesia in 1982.

Chikungunya has been known since 1952, with its first ravage in East Africa followed by numerous epidemics in Asia, including the Philippines (1954, 1956, and 1968), Thailand, Cambodia, Vietnam, India, Burma, and Sri Lanka. A distinctive feature of the Chikungunya virus is that it causes explosive outbreaks before apparently disappearing for a period of several years to decades. Re-emergence of the disease was documented in Kinshasa, the Democratic Republic of the Congo, 1999-2000, after more than 39 years with an estimated infection of 50,000 persons. Since then, frequent epidemics were noticed in Java (2001-2003), the islands of the South Western Indian Ocean (during the end of 2004), and Comoros islands (January-March 2005) involving 5,000 persons. Later, the virus circulated in other islands of the Indian Ocean, i.e., Mayotte, Seychelles, Reunion, and Mauritius. Of all the islands in the Indian Ocean, Réunion, with a total population of 7,70,000, was the most affected. The infection was thought to be imported from the Comoros islands.

Chikungunya situation in India

From the Country, the virus was first isolated in Calcutta (present Kolkata) in 1963. A major epidemic of Chikungunya fever was reported during the last millennium, viz.; 1963 (Kolkata), 1965 (Puducherry and Chennai in Tamil Nadu, Rajahmundry, Vishakhapatnam and Kakinada in Andhra Pradesh; Sagar in Madhya Pradesh; and Nagpur in Maharashtra). After the outbreak of Chikungunya infection in 1971, sporadic cases continued to be recorded during 1973 in Barsi, Solapur district, Maharashtra. The last outbreak of Chikungunya infection in the 20th century occurred in India in 1973.

The CHIKV activity appeared to decline, and no outbreak was reported until 2005. A study in Calcutta (Kolkata) in 1994 showed a 4.3% seroprevalence of the Chikungunya virus out of 389 sera tested. The highest seropositivity was observed in the age group of 51-55 years, and no Chikungunya antibodies were detected in young and adults. In 2006, after an inactivity of 2-3 decades, the disease re-appeared in the country in unprecedented magnitude, affecting millions of people in 16 States/UTs and incapacitating many with crippling disabilities for a varied period. Initially, when the disease was observed in some parts

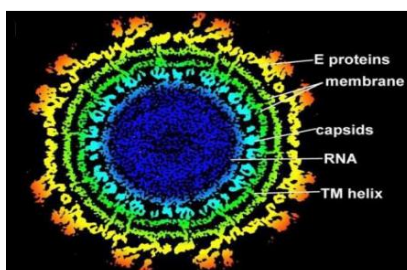
of Karnataka and Andhra Pradesh, it was thought to be Dengue, but the incapacitating arthralgia raised the doubt. In January 2006, the outbreak was confirmed as Chikungunya with laboratory findings with 13.9 million clinically suspected and 2001 laboratory-confirmed cases. Subsequently, World Health Organization confirmed the re-occurrence of Chikungunya fever in the Country. The outbreak had an attack rate of 4-45%.

Since then, transmission has been ongoing in various parts of the country. The re-emergence of Chikungunya may be attributable to various social, environmental, behavioural and biological factors (Khan et al. 2015). Lack of herd immunity might have probably led to its rapid spread across several states. Chikungunya cases have been reported from various parts of the country since 2006, but cases gradually declined until 2014. However, the disease showed an upward trend since 2018. Presently, Chikungunya is endemic in all States and Union Territories (except Ladakh) of the Country (Source: NCVBDC). Till date, no report of mortality directly attributable to Chikungunya has been received from any State/Union Territory.

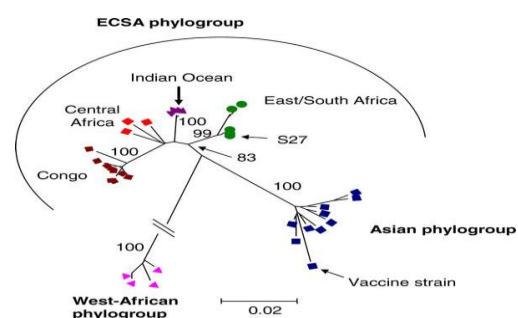
Epidemiology

The evolution of the virus, globalization and climate change may have contributed to the spread of CHIKV. 2005-2006 saw the most severe outbreak on Reunion Island, affecting nearly 35% of the population. Since 2005, cases of Chikungunya fever have spread mainly in tropical and subtropical regions, eventually reaching the Americas through the Caribbean Island. Today, CHIKV is widely spread worldwide and is a global public health problem. In addition, the lack of a preventive vaccine and approved antiviral treatment makes CHIKV a major global health threat (Escobar et al. 2017; Tandale et al. 2009; Haese et al. 2016; Kumar et al. 2017; Epelboin et al. 2017).

CHIKV is a RNA virus belonging to family *Togaviridae*, genus *Alphavirus*. Viral RNA codes for both structural (three) and (four) non-structural proteins (Zhang et al. 2011). Phylogenetically, two different types of CHIKV have been found African and Asian types. While the African type has a natural host among monkeys where the Sylvatic focus is maintained, in Asia, no Sylvatic focus has been identified. The cycle is maintained in man through transmission from infected individual to a health host. Usually, all ages are equally susceptible to infection at least in the early part of the outbreak. Though no death is reported in man severity of infection has been found to be more among young laboratory animals which even may result in death. Manifestations of the disease are immunologically related and are known to affect the connective tissues and skeletal muscles. Vertical transmission has been reported from the outbreaks.



Structure of Chikungunya virus
(Source: NCVBDC guidelines)



Chikungunya virus phylogroup
(Source: Schuffenecker et al. 2006)

Disability-Adjusted Life Years (DALY): It is an important measure used in global health to quantify the burden of diseases. It is particularly useful for assessing the overall impact of diseases like Chikungunya. Although chikungunya is not typically fatal, but can lead to significant severe long-term disability and

health issues making it a disease that disproportionately affects the DALY. The understanding of relationship between DALY and Chikungunya helps to highlight the disease's public health impact, particularly in terms of the suffering it causes and the strain on healthcare systems. Chikungunya outbreaks can cause a substantial number of DALYs due to the prolonged nature of its symptoms. The extended period of disability, particularly in affected populations (such as the elderly or those with underlying health conditions), leads to significant morbidity. Chikungunya can lead to a loss of productivity, as many patients are unable to work for extended periods due to joint pain. This results in both direct healthcare costs and indirect costs associated with reduced economic productivity, which compounds the DALY burden.

Vector

Aedes mosquitoes are responsible for transmitting several viral diseases including Chikungunya, Dengue, Zika and Yellow Fever. These mosquitoes predominantly breed in artificial containers that collect stagnated water. In India, both *Ae. aegypti* and *Ae. albopictus* are prevalent, with *Ae. aegypti* being the primary vector throughout most of the country. However, in the peninsular regions and North-Eastern States, *Ae. albopictus* is more common as a vector for Dengue.

Ae. aegypti and *Ae. albopictus* (Asian tiger mosquitoes) are day biting vector mosquitoes originated in Africa (Mattingly 1957; Hawley 1988). The biology of *Ae. albopictus* breeding was more in the peridomestic container breeding habitats as surveyed. *Ae. aegypti* is the main vector mosquito for the transmission of Dengue and Chikungunya and *Ae. albopictus* could possibly be acting as a secondary vector mosquito (Puppy et al. 2009; Gupta et al. 2012; Samuel et al. 2014). *Aedes* vector mosquito's density caused by the man-made containers (socio-economic variables), the natural breeding habitats, environmental risk factors, and climate determinants (Selvan and Jebanesan 2016).

The abundance of *Aedes* mosquitoes and their breeding sites increases during the monsoon and post-monsoon periods, which are critical times for controlling their spread. These breeding sites vary depending on local ecology, human behaviour, cultural practices, industrialization and can include domestic water storage containers, flower vases, earthen pots, refrigerator trays, discarded tires, construction sites, and even leaf axils and latex collection cups (Futami et al. 2020; Laporta et al. 2023). Both species have unique breeding preferences in different areas, which further complicates vector control efforts. Understanding these diverse breeding habitats is essential for developing effective strategies to reduce mosquito populations and mitigate the spread of the diseases they transmit. The common breeding sites for *Ae. aegypti* and *Ae. albopictus* are as shown below:

***Aedes aegypti* breeding sites**



***Aedes albopictus* breeding sites**



Transmission cycle

Chikungunya is transmitted by infected female *Aedes* mosquitoes. The intrinsic incubation period in humans is typically 2-12 days during which the virus replicates without causing symptoms leading to the onset of fever, joint pain and rash. The extrinsic incubation period in mosquitoes is the time it takes for the virus to incubate within the mosquito after it feeds on an infected person, typically lasting 7-12 days depending on environmental factors. Once the mosquito completes this period, it can transmit the virus to newer human hosts through its bite. The *Aedes* mosquitoes are most active during daylight hours, contributing to the spread of the virus during peak feeding times typically during early morning and late afternoon.

Outbreaks of chikungunya are most likely to occur during the post-monsoon period, when the density of the *Aedes* mosquito vectors is particularly high due to the increased availability of breeding sites, such as stagnant water in containers. This surge in mosquito population significantly accelerates the transmission of the virus. During epidemic periods, humans serve as the primary reservoir for the chikungunya virus, as infected individuals become the source of the virus that mosquitoes pick up through feeding. The key agents contributing to chikungunya fever are the virus (which causes the disease), the vector (*Aedes* mosquitoes, which transmit the virus), and the host (humans, who harbor the virus and facilitate its spread). These three components-virus, vector, and host-interact in a cycle that drives the transmission of chikungunya, particularly during times of high vector density.

Vector Control: Vector control is the principal method available for controlling *Aedes* borne diseases. A wide range of vector control tools can be broadly classified into chemical and non-chemical based tools. These vector control methods targeting larval stages by chemical, biological larvicides and predator species or by removing suitable habitat modification or manipulation. Tools targeting the adult vectors function by killing the vector by space spraying and reducing vector contact with human and/or animal reservoir hosts with use of topical repellents and house screening. There are also several novel vector control tools under development, e.g., genetic manipulation of mosquitoes, bacterial infection of vectors like *Wolbachia*, and insecticide treated eave tubes. Under the programme, only recommended insecticides are used.

Larval control: Larvicides insect growth regulators are used to minimize the risk of Chikungunya transmission. Non-chemical method the habitat manipulation and source reduction also help in reducing the vector density.

Adult control: For control of adult *Aedes* mosquitoes, recommended adulticides are used during emergencies and outbreak situations to interrupt the transmission for further spread.

Use of Repellents: Chemicals that prevent a vector from entering a space occupied by a potential human host to reduce encounters between the vector and the host are known as 'Repellants'. They are typically used against *Aedes* mosquitoes. These are available in various types including coils, creams, passive emanators and impregnated fabric etc.

Challenges in Chikungunya control

Urbanization: The rapid urbanization has led to overcrowded cities with inadequate infrastructure and sanitation facilities. Many urban areas, particularly slums, suffer from poor waste management and lack of proper drainage systems, which encourage the accumulation of stagnant water in discarded containers, tires, and open drains. These conditions create ideal environments for mosquitoes to breed, exacerbating the spread of Chikungunya in densely populated urban centers.

Globalization, Travel and Transport: Increased travel and trade have played a significant role in the spread of Chikungunya in the Country. Travelers returning from endemic areas may bring the infection to non-endemic areas and lead to outbreaks. With the movement of people across state and national borders, the

virus is rapidly disseminated, and urban areas with high connectivity become focal points for intense transmission.

Climate and Weather Conditions: India's climate significantly contributes to the spread of Chikungunya, as it provides an ideal environment for the *Aedes* mosquitoes that transmit the virus. These mosquitoes thrive in warm temperatures and high humidity, which are common throughout the year. During the monsoon season, the country experiences heavy rainfall, leading to the accumulation of stagnant water in open containers, discarded tires, and other water-holding objects, which serve as perfect breeding grounds for mosquitoes. The abundance of these breeding sites in both urban and rural areas creates a favourable environment for the mosquitoes to proliferate. Furthermore, the seasonal rise in temperature during the post-monsoon period accelerates the mosquito reproduction cycle and enhances the replication of the Chikungunya virus within the mosquitoes. Warmer temperatures speed up the mosquito life cycle, allowing them to breed and spread the virus more rapidly. This combination of high rainfall, increased humidity, and elevated temperatures significantly boosts the likelihood of mosquito-borne transmission of Chikungunya, contributing to the frequency and severity of outbreaks across the Country.

Limited vector control tools: Vector control measures are mainly concentrating on larval source management which is a difficult and laborious task especially in plantation areas where *Ae. albopictus* breeds. On the other hand, the source reduction activities for elimination of *Ae. aegypti* in urban areas becomes difficult due to refusal by the community for entry of health workers, inaccessible rooftops and multistoried buildings.

Insecticides Resistance: The resistance of *Aedes* mosquitoes to commonly used insecticides has been a growing challenge in controlling Chikungunya transmission. Mosquitoes have developed resistance to pyrethroid-based insecticides, which are commonly used for vector control which may have reduced the effectiveness of mosquito control measures.

Inadequate Infrastructure: In many areas, particularly rural and underserved urban areas, healthcare infrastructure is often inadequate. Limited vector control tools, overburdened diagnostic and management facilities hampers the response to Chikungunya outbreaks. In such areas, people may not seek medical attention promptly, which leads to delays in diagnosis and check on further spread of the virus as infected individuals continue to move in unaffected areas.

Increased Population Density: The high population density in urban areas facilitates the spread of vector-borne diseases like Chikungunya. Overcrowding increases human-mosquito contact, particularly in areas where mosquito control measures are poorly implemented. In these environments, the virus can spread rapidly from one individual to another as mosquitoes easily find new hosts to feed on, leading to widespread outbreaks in a short period of time.

Socio-economic Factors: Socio-economic conditions are closely linked to the spread of Chikungunya. People living in poverty, particularly in urban slums or rural areas, often live in substandard housing. These conditions make it more difficult to implement mosquito control measures effectively. Moreover, individuals in these communities often lack access to healthcare and preventive tools making them more vulnerable to infection.

Lack of Community participation: Public awareness about Chikungunya and its prevention is a critical factor in controlling the spread of the disease. Unfortunately, community is unaware of the importance of controlling mosquito breeding sites and the need for personal protection, such as using insect repellents and wearing protective clothing. This lack of awareness, coupled with a tendency to ignore basic preventive measures has contributed to the persistence of Chikungunya outbreaks.

Genetic Adaptation of Mosquitoes: The genetic adaptation of *Aedes* mosquitoes has made it easier for them to survive and proliferate in various environments, including urban and suburban areas. In recent years, *Ae. albopictus* and *Ae. aegypti* mosquitoes have adapted to different climatic conditions and have expanded

their range across the Country. This broader distribution of mosquito species increases the risk of Chikungunya transmission in both rural and urban areas.

Lack of robust intersectoral coordination: Effective community engagement is paramount in combating Chikungunya like Dengue, however, several challenges persist. The government has initiated several strategies to enhance community engagement. Behaviour Change Communication (BCC) strategies, including mass media campaigns and community meetings, aim to change behaviour related to dengue prevention. Every year, 16th May is observed as 'National Dengue Day' and July as 'Anti-Dengue Month' across the Country to raise awareness and promote preventive measures. Still lack of awareness and education about preventive measures, low risk perception, coordination issues result in inconsistent ownership and hinder active community involvement.

Conclusion: Chikungunya remains a significant public health challenge in India, primarily due to its rapid transmission by *Aedes* mosquitoes in both urban and rural areas. The disease burden is exacerbated by various challenges including inadequate vector control measures, limited public awareness, and insufficient healthcare infrastructure in some areas. While the government has made notable progress through National Center for Vector Borne Diseases Control (NCVBDC), challenges such as rapid urbanization, inadequate sanitation, and the impacts of climate change result in geographical spread and repeated outbreaks of Chikungunya. Despite efforts to combat the disease, the periodic outbreaks highlight the vulnerability of the population, especially in the monsoon and post-monsoon period when proliferation of mosquito population occurs. Therefore, Chikungunya prevention and control in the Country require a robust mechanism integrating effective vector management, strengthening of healthcare infrastructure, improved surveillance systems, community's ownership and effective inter-sectoral coordination.

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ZIKA TRANSMISSION: CHALLENGES FOR PREVENTION AND CONTROL

Akhtar Nasreen¹, Gupta Sanjeev Kumar¹, Singh Kuldeep¹, Baruah Kalpana², Singh Himmat¹

¹ ICMR-National Institute of Malaria Research, Delhi

² National Centre for Vector Borne Disease Control, Delhi

Abstract

ZIKA virus (ZIKV) has now become a global phenomenon. Since 2007, evidence of ZIKA transmission has been reported in over 72 countries and territories. The transmission of ZIKA has made the World Health Organization categorize the situation under the ambit of a health emergency. *Aedes* mosquitoes are the principal way of transmission of the virus to humans. ZIKV can be spread by transplacental, perinatal, and body fluids. ZIKV infection is often asymptomatic and those with symptoms present minor illness after 3 to 12 days of incubation, these symptoms are characterized by a mild and self-limiting disease with low-grade fever, conjunctivitis, widespread pruritic maculopapular rash, arthralgia and myalgia. This situation is serious because there appears to be a highly tangible link between infection during pregnancy and the occurrence of microcephaly and Guillain-Barré syndrome. In the context of this emergency, this chapter intends to discuss the prevention and control strategies such as avoiding travel to infected areas, being careful of mosquito bites, taking precautions to reduce the risk of sexual transmission, and seeking medical care for any acute illness with rash or fever.

Keywords: ZIKA, diagnoses, prevention, treatment, awareness.

Introduction to Zika Virus

Zika virus (ZIKV) is a mosquito-borne Flavivirus belonging to the Flaviviridae family. It is an emerging pathogen that is spreading rapidly across the world, raising concerns at the forefront of global healthcare (Ayres, 2016). The virus is closely related to other members of the Flavivirus genus (positive-sense, single-stranded RNA viruses), including the dengue virus (DENV), West Nile virus (WNV), yellow fever virus (YFV), tick-borne encephalitis virus (TBEV), and Japanese encephalitis virus (JEV) (Lazear and Diamond, 2016). Zika virus infection during pregnancy is a cause of microcephaly, Guillain-Barré Syndrome (GBS) and other congenital abnormalities in the developing fetus and newborn. Zika infection in pregnancy also results in pregnancy complications such as fetal loss, stillbirth, and preterm birth (Basarab *et al.*, 2016). Microcephaly is characterized by at least two standard deviation reductions in brain volume intellectual and motor disabilities and behavioural issues (Petersen L. R. *et al.*, 2016). Multiple developmental factors, such as genetic, environmental, and infectious exposure, during pregnancy, are known to contribute to the onset of prenatal microcephaly. GBS, on the other hand, is a rare autoimmune disorder of the peripheral nervous system which could result in muscle weakness, paralysis, or even death (Lazear and Diamond, 2016). It was suggested that the rise in the number of prenatal microcephaly and GBS cases could potentially be attributed to increased awareness and/or misdiagnosis (Mlakar *et al.*, 2016).

Epidemiology

ZIKV was first isolated from the blood of a febrile sentinel rhesus monkey in the Zika Forest of Uganda in 1947 (Dick *et al.*, 1952; Lanciotti *et al.*, 2008; Haddow *et al.*, 2012). In the following years, ZIKV was isolated from various species of *Aedes* mosquitoes (Mlakar *et al.*, 2016; Slavov *et al.*, 2016). The first case of ZIKV infection in humans was reported in Nigeria in 1954 (MacNamara, 1954). Since then, several outbreaks have been reported, prominently in the African and Southeast Asian regions (Pan American Health Organization, 2016b). The first major ZIKV outbreak occurred in 2007 on Yap Island, Federated States of Micronesia, with approximately 75% of the population being affected within 4 months (Saiz *et al.*, 2016). Subsequently, in 2013 and 2014, the ZIKV epidemic was reported in the French Polynesia, Cook

Islands, Ester Islands, and New Caledonia. After many ZIKV outbreaks, WHO declared Zika, a Public Health Emergency of International Concern on February 1, 2016, and called for active surveillance in countries where no outbreaks were reported initially, including India. In India, Zika outbreak occurred in 2017 and after that many positive cases were found in states of India, viz. Gujarat (Ahmedabad), Delhi, Rajasthan, Madhya Pradesh, Kerala, Uttar Pradesh etc. (Akhtar et al., 2022). Zika virus is primarily transmitted by the bite of an infected mosquito from the *Aedes* genus, mainly *Aedes aegypti*, in tropical and subtropical regions. *Aedes* mosquitoes usually bite during the day, peaking during early morning and late afternoon/evening. This is the same mosquito that transmits dengue, chikungunya and yellow fever.

Molecular Classification

Through ZIKV genome sequencing and phylogenetic analysis of several human isolates, three geographically distinct lineages of ZIKV have been reported, including the East African, West African, and Asian strains. The Asian genotype is subdivided into Southwest Asia, Pacific Island, and American strains, and is responsible for most outbreaks (Bernardo-Menezes et al., 2022, Singh et al., 2019). It is postulated that ZIKV originated in the Eastern region of Africa and then spread toward the West (Lanciotti et al., 2016). In the late 1960s, the first Asian ZIKV was isolated in Malaysia and then subsequently, the virus spread across Southeast Asia (Marchette et al., 1969). It is postulated that the Asian ZIKV strain emerged as a result of the accumulation of mutations in the ZIKV genome, hence, introducing new molecular interacting partners with the host cell factors and changes in disease pathogenicity, vector competence, and epidemic potential (Lazear and Diamond, 2016; Wang et al., 2016; Weaver et al., 2016). At this point, it must be noted that when compared to other RNA viruses, fixation of a mutation in the genome of an arbovirus faces greater constraints due to the presence of important genes required for replication in mammals and invertebrates (Holmes, 2003; Hamel et al., 2015).

Virology and Pathogenesis

ZIKV, a biosafety level-2 pathogen, has an enveloped positive-sense, single-stranded RNA genome with a size of approximately 10,676 bp and is known to be closely related to the Spondweni virus (Charrel et al., 2016; Wong et al., 2016). ZIKV virions are approximately 60 nm in size and spherical (Charrel et al., 2016). The ZIKV genome encodes for a single polyprotein (approximately 3400 amino acids) that is subsequently processed by host and viral proteases into ten different proteins, consisting of three structural and seven nonstructural proteins. Currently, little information is available regarding ZIKV pathogenesis, as compared to other members of the Flavivirus genus. Generally, arboviruses (mosquito-borne viruses) are known to replicate in dendritic cells and subsequently disseminate to the lymph nodes and bloodstream (Diamond et al., 2004). Within the cells, replication of Flaviviruses is known to occur in the cytoplasmic region however, ZIKV antigens have been observed within the cell nuclei. Hence, it is suggested that ZIKV replication may differ from that of other Flaviviruses (Buckley and Gould, 1988).

Symptoms

Up to 80% of human, ZIKV infections appear asymptomatic, with a small subset of cases presenting with mild clinical symptoms similar to other flaviviral and influenza infections (Marano et al., 2016; Shah and Kumar, 2016). Among those who do, they typically start 3–14 days after infection, are generally mild including rash, fever, conjunctivitis, muscle and joint pain, malaise and headache, and usually last for 2–7 days (Buathong et al., 2015). Thus, the diagnosis of Zika virus infection requires laboratory confirmation.

Mode of Transmission

1. **Mosquito-Borne Transmission:** ZIKV transmission to humans occurs primarily through bites of an infected, day-dwelling female *Aedes aegypti* or *Aedes albopictus* mosquito, similar to the transmission of chikungunya virus (CHIKV) and DENV. *A. aegypti* mosquitoes are confined to the tropical and subtropical regions, hence, limiting ZIKV transmission potential (Petersen L. R. et al., 2016). However, *A. albopictus* mosquitoes are known to be geographically distributed throughout the tropical, subtropical, and temperate regions, hence, allowing for greater transmission potential (Thomas et al., 2012). The exact incubation period for ZIKV before the mosquito becomes capable of transmitting the virus is yet unknown. However, according to

Hayes (2009), the extrinsic incubation period of ZIKV in mosquitoes is suggested to be approximately 10 days.

2. **Blood Transfusion-related Transmission:** Zika virus can be spread by blood transfusion. However, there have been no confirmed cases of Zika virus being spread by blood transfusion in the United States. Zika virus may be detected in some human cells and tissue-based products for months after infection. You may need to delay blood and tissue donations following Zika virus infection (CDC, May 2024).
3. **Maternal Transmission:** Zika virus can be transmitted from a person who is pregnant to their fetus during pregnancy (congenital transmission) or around the time of birth (perinatal transmission). **Congenital or intrauterine (in utero)** transmission of the Zika virus occurs when a pregnant person is infected with the Zika virus before delivery, and the virus passes to the fetus. **Perinatal** transmission of the Zika virus occurs when a pregnant person is infected with the Zika virus within approximately 2 weeks of delivery, and the virus passes to the infant at or around the time of delivery. When an infant acquires Zika virus infection perinatally, the infant may develop symptoms such as maculopapular rash, conjunctivitis, arthralgia, and fever (NCEZID Report on Transmission of Zika virus, 2024).
4. **Transmission by Physical Contact:** Zika virus can be passed through physical contact from a person who has Zika virus infection to their partner(s). Zika virus can be passed through sex, even if the infected person does not have symptoms at the time. It can be passed from a person with Zika before their symptoms start, while they have symptoms, and after their symptoms end. The virus may also be passed by a person who carries the virus but never develops symptoms (NCEZID Report on Transmission of Zika virus, 2024).

Mode of detection

There is relatively limited literature describing the performance characteristics of diagnostic tests for ZIKV. This section describes the methods that have been used to identify ZIKV infections in Human specimens and adult *Aedes* mosquitoes.

1. **Viral culture method:** Culture-based methods for ZIKV detection are used in laboratories for research purposes only for the development of antibodies against ZIKV but are not generally available for clinical purposes. The Zika virus can be cultured in both mosquito and mammalian cells (Baronti *et al.*, 2014).
2. **Antibody detection method:** Evidence of human ZIKV infection was obtained primarily via the identification of ZIKV neutralising antibodies in human serum ((Dick, 1952). As ZIKV spreads throughout the areas of the world where DENV infection is endemic, detection of DENV IgM antibodies in patients with a dengue-like illness, particularly in the absence of DENV nonstructural protein 1 (NS1) or DENV RNA, should prompt consideration of the possibility of ZIKV infection. While DENV serologic tests are widely used, assays for ZIKV antibodies are currently available only through public health laboratories such as the CDC laboratories.
3. **Antigen detection method:** Unlike in the DENV diagnostics field, where NS1 antigen assays are widely available, antigen assays for the diagnosis of acute ZIKV infection have not yet been developed.
4. **Viral RNA detection method:** Zika virus diagnosis is primarily based on the detection of viral RNA by reverse transcription polymerase chain reaction (PCR) from clinical samples. Specific assays have been published for Asian and African Zika viral strains targeting the envelope gene or NS5 region (Lanciotti *et al.*, 2008). There are several commercial assays available for Zika virus genome detection. A pan-flavivirus by PCR assay and subsequent sequencing analysis can be used as an alternative screening or confirmatory test for possible ZIKV infection.

Treatment

There are no antiviral treatments available for Zika virus disease. Treatment is generally supportive and can include rest, fluids, and the use of analgesics and antipyretics. Because of similar geographic distribution and symptoms, patients with suspected Zika virus infections also should be evaluated and managed for possible dengue or chikungunya virus infection. Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) should be avoided until dengue can be ruled out to reduce the risk of

haemorrhage. People infected with Zika, chikungunya, or dengue virus should avoid mosquito exposure during the first few days of illness to prevent other mosquitoes from becoming infected and reduce the risk of further transmission. Carefully monitor people who are pregnant with laboratory evidence of Zika virus infection. Closely manage these patients during pregnancy and carefully evaluate live-born infants for clinical features associated with intrauterine infection.

Prevention

No vaccine or preventive drug is available for the Zika virus. All travellers to areas with possible Zika virus transmission should take steps to avoid mosquito bites during the day and night to prevent Zika virus and other vector-borne infections. Patients with possible exposure to the Zika virus should be advised to reduce the risk of sexual transmission through abstinence or using condoms.

Control Measures

1. **Vaccine Development:** At present, there are no vaccines or medications specifically approved for Zika virus (ZIKV). Nevertheless, several candidates are in the process of clinical trials. Various platforms have been assessed for vaccine development, including DNA, mRNA, viral vectors, virus-like particles (VLPs), inactivated viruses, live attenuated viruses, peptide and protein-based vaccines, as well as passive immunization utilizing monoclonal antibodies (MAbs) and vaccines aimed at vector-derived antigens. These vaccine candidates have demonstrated the ability to elicit targeted humoral and cellular immune responses, as well as to decrease viremia and viral RNA levels, both in laboratory settings and in living organisms (Wang *et al.*, 2022).
2. **Antiviral Therapeutics:** Since ZIKV vaccine clinical trials are yet underway, more efforts are being put toward developing antiviral therapeutics against ZIKV for immediate control (Lazear and Diamond, 2016).
3. **Preventative Strategies:** Collective responsibility and engagement for integrated vector management, particularly through the removal of stagnant water and use of insecticides (diethyltoluamide/ethyl butylacetylaminopropionate)/larvicides, is greatly emphasized due to the lack of vaccines and antiviral therapeutics against ZIKV (Lazear and Diamond, 2016; Pan American Health Organization, 2016a). Although, *Ae. aegypti* and *Ae. albopictus* are primarily responsible for the current ZIKV outbreak, vector control strategies and vector-pathogen interaction of all possible mosquito species are advised to be considered owing to ZIKV's ability to evolve (Ayres, 2016). It is also recommended to wear long-sleeved shirts and long pants, even potentially insecticide-impregnated clothing, in order to minimize vector contact (Basarab *et al.*, 2016; Weaver *et al.*, 2016). In addition, men who have a recent travel history to ZIKV endemic regions are advised to refrain from having unprotected sexual intercourse with their pregnant partner (Oster *et al.*, 2016). For asymptomatic travelers returning from ZIKV endemic regions, barrier contraception for 28 days is recommended (Wong *et al.*, 2016).

Conclusion

The role of the *Aedes* mosquito in the spread of Zika virus (ZIKV) has attracted significant global interest. Factors such as high human population density, a lack of immunity, and viral mutations contribute to the virus's spread. Furthermore, global warming has exacerbated the situation. While there are various methods of ZIKV transmission, prioritizing mosquito control is essential. Therefore, it is crucial to implement effective mechanical, biological, and chemical strategies to prevent or reduce mosquito breeding and manage their populations. Although chemical methods can lead to resistance and bioaccumulation issues, there has been a recent trend towards the use of biopesticides. Numerous plant extracts and essential oils have demonstrated efficacy in mosquito control. Additionally, the use of bacteria like *Wolbachia pipientis* and alpha-proteobacterium can help mitigate virus transmission, as these bacteria can be transferred both horizontally and vertically. Genetic engineering approaches, such as employing siRNA to target testes-specific genes, may also be utilized to induce sterility in male mosquitoes. The application of fungi for controlling *Ae. aegypti* mosquitoes shows promise, but further investigation is necessary to assess their viability, infectivity, and spore longevity in natural environments. Utilizing fish for mosquito predation appears to be a viable option; however, careful

selection of fish species is essential to avoid issues related to invasive behavior and disruption of aquatic ecosystems.

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VISCERAL LEISHMANIASIS (KALA-AZAR) VECTOR BIONOMICS, CHALLENGES AND GAPS FOR VECTOR CONTROL IN INDIA

Rudra Kumar Das Gupta

Formerly Joint Director

National Centre for Vector Borne Diseases Control, Delhi 110054

B 406, Sagar Apptt. C -58/15, Sector 62 NOIDA 201301

E mail: rkdasgupta2003@yahoo.com

Visceral Leishmaniasis (Kala-azar) is a disease caused by the parasite *Leishmania donovani* and is transmitted in India subcontinent by the bite of the Sandfly vector *Phlebotomus argentipes*¹. Visceral leishmaniasis is a slow progressing disease and often recognized late. The disease presents with fever of long duration (more than two weeks) with splenomegaly, anaemia, progressive weight loss. Visceral Leishmaniasis in India exists in two forms, namely, Visceral Leishmaniasis or (Kala-azar) and Post Kala azar Dermal Leishmaniasis (PKDL). Without timely treatment the disease is fatal. *Leishmania donovani*, a parasite mainly residing in bone marrow, spleen and liver. No intermediate host is known in India (i.e. humans are the only hosts).

Kala azar infection is manifested in two forms. Initial infection leads to acute disease, i.e. Kala-azar. Later, skin manifestations of Kala azar infection, known as Post Kala azar Dermal Leishmaniasis may appear after variable periods ranging up to a few years. PKDL is a sequela of visceral leishmaniasis that appears after patients have apparently been cured of visceral leishmaniasis. PKDL has also been reported in patients without a history of visceral leishmaniasis.

Visceral Leishmaniasis (Kala-azar) has been a serious medical and public health problem in India sub-continent since historical times. The disease is prevalent mostly among lower socio-economic groups of the population in rural areas. It could often be labeled as a disease associated with poverty since the living habits of these marginalized populations facilitate vector breeding and malnutrition with lowered immunity make them predisposed and susceptible to infection. Although all age groups are affected, the most vulnerable groups include children and young adults that represent economically productive sections. The incidence is recorded throughout the year with no specific seasonality in view of variable incubation periods.

VECTORS

In India, so far three vectors also known as Sandflies have been incriminated as vectors of Leishmaniasis².

1. *Phlebotomus argentipes* as only known vector of visceral leishmaniasis.
2. *Phlebotomus papatasi* as the vector of anthroponotic of urban cutaneous leishmaniasis.
3. *Phlebotomus salehi* as the vector of rural (zoonotic) cutaneous leishmaniasis.

Sandfly belong to Order Diptera of class Insecta (Phylum Arthropoda) that are characteristically recognized for one pair of functional wings and rudimentary clubbed shaped second pair called "Halteres"- the organs for maintaining equilibrium. It is characterized by 'V' shaped wings. The vectors of Leishmaniasis belong to group of insects commonly called as sandflies. Sandflies are grouped in two sub-families namely Psychodinae and Phlebotominae. Only the members belonging to family Phlebotominae are transmitting agents for different types of leishmaniasis.

The sandflies are usually associated with warm climate. About 500 or more species are distributed all-over the tropics and sub tropics particularly in those parts of the world having at least one month

temperature of 20°C³. Only very few species are considered to be the vectors and even fewer have been incriminated as vectors. The identification of the species of sandflies solely depends upon the close examination of mounted specimen particularly the head and genitalia which bear the characters of taxonomic importance.

The adult sandfly is a small fuzzy, delicately proportioned fly usually 1/4th of the size of the mosquito. The length of sandfly body ranges from 1.51 to 3.5 mm. The males and unfed females can pass through mosquito net easily. The elongated wings are hairy, held erect or 'V' shaped on the abdomen and are bigger than the size of the body. The body, wings and legs are heavily clothed with long hairs. The sandfly could be spotted easily because of the posture of the wing which are always held vertically erect when at rest. The sub family Phlebotominae family Psychodidae comprises of six genera. Of these, only two genera, namely Phlebotomus and Sergentomyia are found in Indian sub- continent and are represented by about 46 species.

Biology and Bionomics

The sandflies are associated with warm climate and could be grouped into two categories namely species associated with wet zone and the species associated with arid zone and this association further delimits the distribution of different types of leishmaniasis. Napier (1926) suggested ecological factors favorable for transmission of Visceral Leishmaniasis or Kala-azar as:

- Alluvial soil
- High sub-soil water
- Monthly mean maximum temperature below 37 °C
- Monthly mean minimum temperature above 7.2 °C
- Annual rainfall 1250 mm or more
- Mean Annual Relative Humidity of 70%
- Abundant vegetation
- Altitude below 600 metre

Adults Distribution

Ph.argentipes or Sandfly is one of the widely distributed and is essentially the species of wet zone. It is widely distributed alongside of the equator and in India, its distribution primarily could be seen on the eastern half of the country (Bihar, Jharkhand, West Bengal and Uttar Pradesh) and limited distribution in southern and western parts. Though commonly the sandflies are not found at the altitude above 600 metres, sporadic occurrence in India has been recorded in Kasauli⁴ at height of 1200 metres and at 1300 metres in Pauri Garhwal in Himalayas.

High relative humidity, warm temperature, high sub-soil water and abundance of vegetation favours proliferation of *Ph.argentipes* and accordingly, depending on this condition seasonal prevalence vary from area to area. In India the species is found throughout the year in majority of areas of prevalence with complete or fewer numbers in winter months. With the onset of warm weather coupled with humidity the density increases till June with sudden decline due to high temperature. It is again followed by increasing trend reaching maximum during and just after the monsoon rain. This trend has been reported in Bihar and Jharkhand with a minor peak in March/ April and major peak in August / September in densities of *Ph.argentipes*.

Feeding Behaviour

All species of sandflies feed on plant sugar and the females often feed on vertebrate host including man. The females of genus Phlebotomus feed on mammals whereas the Sergentomyia feed on reptiles.



Photo: *Ph. argentipes* (sandfly) vector of VL

zoophilic, it has been observed by various workers that the anthropophilic index largely depends on the sampling. For example, the samples collected from human habitation showed 69.6% anthropophilic index whereas the samples collected from cow sheds in the same area showed only 21.6% anthropophily. The same was true in the case of bovid blood index which was 96% in the cowsheds and 44% in the human dwellings. This indicates clearly that *P. argentipes* are primarily indiscriminate (opportunistic) feeder and the type of blood meal largely depends on availability of host in its immediate vicinity.

Flight Range

Though named as sandflies, these insects are not capable of flying very long distances. Their usual mode of flight pattern is a series of short erratic hopping in which the fly usually covers a distance less than 1/2 metre. Further, the dependence of the most of the vectors on humidity or their survival limits their movements. However, this distance may be even less or more depending upon climatic factors.

Resting Sites

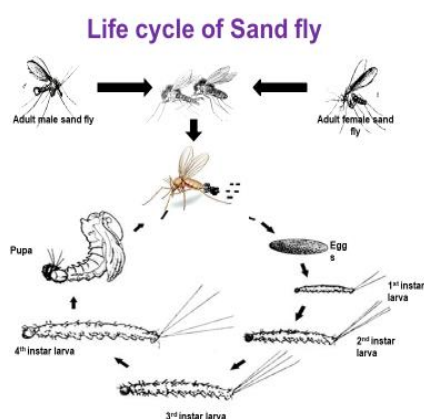
The most favoured resting sites for sandflies include soil cracks and crevices, burrows (rodent burrows), tree holes termite hills, bird tunnels, in earthen mounds, under stone and foliage. In eastern parts of India like Bihar and West Bengal *Ph. argentipes* prefers to rest indoor about 8-10 times higher in cattle sheds dwellings than the human dwellings. In western India, sandflies rest outdoors also in considerable numbers. All these resting places provide them dark and damp shelters where the micro climate & humidity is very high. They usually leave these shelters at dusk and are active in open in the evening and night. Usually sandfly remain throughout in night but they are sensitive to decreasing temperature and air currents. Even a gentle breeze of 1.5-2 metre/seconds may greatly reduce their activity.

Longevity

Very limited studies have been conducted on the longevity of sandflies. In India *Ph. argentipes* have been reported to undergo 5 gonotrophic cycles⁵ under laboratory conditions, duration of each cycle being 4 to 5 days at 26 ± 2 °C i.e. a minimum longevity up to 23- 27 days under laboratory conditions. The longevity under field conditions to be upto 16-20 days in a proportion of natural population. However, longevity in field is directly dependent on ecological factors.

Immature Stages

Eggs: The freshly laid eggs are creamy white in colour which later become dark. The eggs are usual deposited in cracks and crevices with high organic content, humidity and darkness. Sometimes eggs are also found in loose soil. The eggs are glued to the surface through flattened while the convex side faces upwards. The egg shell has sculptors and their size varies from 0.336-0.432mm x 0.096-0.160 mm. A wide range has been observed for total number of eggs laid per female (5-68). The eggs hatch in 3-4 days at 26±2 °C in laboratory.



Larva: The creamy white larva with distinct There are four larval stages.

I instar: The average life is 2-4 days.

II instar: The average life is about 2-5 days.

III instar: The average life is about 3-4 days.

IV instar: The stage last for 4-7 days and transform into pupa.

The total larval period may vary from 11-29 days.

Pupa: The elongated comma shaped pupa is milky white in the beginning and turns brown. It is non feeding stage

lasting for about 6-10 days. The total life cycle from egg to adult is reported to take about 20-36 days with average 26.75 days in *Ph. argentipes* in laboratory.

Framework for entomological surveillance for Kala-azar elimination in India

Entomological surveillance is an essential component of vector borne disease control program including operational activities and research both in disease control and elimination phase. It is important in determining the distribution, population density, larval habitats, and susceptibility to insecticides in order to prioritize VL vector control in terms of time and space.

Specific objectives of entomological surveillance

- To identify Kala-azar vectors in the area and changes in sandfly bionomics
- To monitor density of vectors, temporal variations and other bionomics traits (breeding habitats, biting and feeding habits, resting behaviour, infection rates, parity and longevity)
- To monitor insecticide susceptibility, intensity and mechanisms of resistance
- To suggest appropriate vector control measures/ interventions
- To evaluate the impact of vector control measures
- To monitor quality of insecticide products and equipment

Entomological parameters

- a. Determine the susceptibility status of *Ph.argentipes* to different classes of insecticide in VL endemic states
- b. Determine the impact of IRS on *Ph.argentipes* abundance
- c. Bionomics: resting behavior of vector
- d. Bionomics: outdoor vs indoor abundance
- e. Periodic assessments of IRS coverage and quality

Entomological surveillance criteria in different transmission settings

- Category-1- High endemic blocks: > 1 case/10,000 pop./yr
- Category-2- Low-endemic blocks 0.8 to < 1 case/10000 pop./yr
- Category-3 – Blocks reporting no case in last 3 years
- Category-4 – Non endemic blocks
- Category-5- Hot spots- reporting persistently high number of cases in last 3 years
- Outbreak
- Entomological surveillance in specific scenarios (e.g. prevention of re-establishment of cases in blocks)

Sites for surveillance

- Sentinel sites for routine surveillance
- Randomly selected sites

Periodicity

1. Sentinel sites – fortnightly / monthly surveillance
2. Random sites – fortnightly / monthly surveillance
3. Insecticide resistance monitoring- once a year
4. Focused investigation sites, new focus, persistent high endemic sites

Collection Methods

1. Hand catch (Aspirator)
2. Light traps
3. Sticky traps
4. Cone bio-assay test
5. Baited trap collection (animal baited: cow/ goat)

Outcome

- Inform pragmatic decisions on the basis of entomological findings in Kala-azar elimination
- Mapping of areas (high/ low risk areas) on the basis of Sandfly abundance
- Enable risk area stratification and improved targeting and resource allocation

Entomological Indicators

- **Resting habit** -An important index is the proportion of blood-meals taken on humans followed by resting indoors.
- **Feeding habits** -Feeding habit refers to whether the vector prefer to feed indoors (endophagy) or outdoors (exophagy) and the times of feeding during the night (nocturnal biting cycle).
- **Human-biting rate** -average no. of bites per person per night.
- **Per Man Hour Density (MHD)**-Index is calculated from the daytime hand collection made by the Insect collectors for observing vector fauna in the area, their seasonal prevalence, resting habits (both in-doors and out-doors) & impact of vector control measures.

$$\text{Per Man Density} = \frac{\text{No. sand flies (male \& female) collected}}{\text{No. of Insect Collector} \times \text{Time spent in search in hour}}$$

- **Vector Infectivity Rate (VIR)** It is a proportion of vector females containing developed parasite, for indicating transmission potential in community.

$$\text{VIR} = \frac{\text{No. of infected vector females} \times 100}{\text{Total No. vector females dissected}}$$

- **Human Blood Index (HBI)** -observing feeding preference and biting frequency of vector on men and change in feeding behavior of vectors.

$$\text{HBI} = \frac{\text{No. of vectors showing source of human blood}}{\text{Total no. of vectors whose blood meals have been identified.}}$$

VL (Kala- azar) Elimination Strategies

The strategy for elimination of Kala-azar is a multipronged approach and includes:

- Early diagnosis & complete treatment (EDCT)
- Integrated Vector Management including two rounds of Indoor residual spraying (IRS)
- Advocacy, Communication for behavioural Impact and Inter-sectoral convergence
- Capacity Building
- Supervision, Monitoring and Evaluation

Integrated Vector Management

Indoor residual spray (IRS) is one of the main components of the strategy for breaking the human-vector-human cycle of transmission for killing of parasite. Since the *Phelbotomus argentipes* is the only insect vector responsible for the spread of Kala-azar, keeping the numbers of the insect in check will limit the human vector interface and thus reduce the transmission.

The method of IRS undertaken (two rounds per year with appropriate insecticide) is based on the breeding, resting and biting habits of the Sandfly. The Sandfly breeds in damp dark places which have proliferation of organic matter around. They rest in cracks and crevices during the day especially in the bamboo thatch and mud plasters. Therefore, the spray is 'indoor' with emphasis on cracks crevices and non-cemented structures. Proximity of humans to animals make them more prone to be bitten. Hence cattle sheds are to be sprayed as well. Since the Sandfly can only jump up to six feet above ground level, therefore, spray is to be undertaken up to six feet height in all rooms and full cattle sheds.

Advocacy, Communication for Behavioral Impact and Inter-sectoral Convergence

Awareness of the disease, its clinical features, its treatment options, prevention and other aspects about it are not widely known among the community. Countries have taken IEC/BCC vigorously for sensitization the community on prevention and control of disease. Community based and human to human communication is considered the best methods for spreading awareness.

Capacity building of health program staff

It is necessary to enhance the skills of all varieties of health workers who are associated with this disease. Since it is in the elimination mode, capacity building of all classes of staff/ workers is necessary. The country has built capacity for training of personal engaged in Kala-azar elimination. Training modules developed. In India, National Centre for Vector Borne Diseases Control (NCVBDC), Rajendra Memorial Research Institute (RMRI), Patna (ICMR) are nodal institutes designated for training to medical officers / consultants, supervisors, paramedics and field level workers.

Supervision Monitoring & Evaluation

Supervision and monitoring are the key elements of any good program that has components operative in the field. Supervision and monitoring are two separate entities, but they work hand in hand. Supervision is in fact the continuous evaluation that provides guidance to ensure that a series of tasks are carried out by the concerned staff within a particular unit/ organization and is concerned with operational running. This should be part of the regular management activities. In supervision the most important activity is the visit by the supervisor. Visits by the supervisor enable the health worker to learn about the actual implementation whether carried out properly, supervisory visits assure the healthy workers of continuous and positive support by the program. Supervision should be done in such a way that it motivates the staff working for Kala-azar. It should be done in very supportive manner. Under KA elimination this component is important to know the activities are being done as per guidelines. Under the program monitoring supervision of activities are being carried out by field visits with partners.

Vector Monitoring

Entomological monitoring is an essential component of any vector borne diseases. It is necessary to collect data on abundance, density, and sandfly bionomics to know the continuing effectiveness of vector control measures. Similarly, systematic longitudinal sampling of *Ph.argentipes* populations to monitor the impact of IRS on their abundance, density. Sentinel sites need to be established in areas where indoor residual spraying is routinely performed to ensure control activities are providing much needed dividends in reduction of vector density viz a viz reduction in numbers of Kala-azar cases. The sentinel sites should be functional both indoors and outdoors settings to monitor changes in the behaviour of *Ph.argentipes* that could limit the impact of IRS. The collection of longitudinal data and continued operational research is necessary to know the transmission dynamics.

Need for vector surveillance in Visceral leishmaniasis (Kala-azar) control

One of the four pillars of action identified in the WHO Global Vector Control Response 2017-2030, is the need to strengthen vector surveillance and monitoring, and to improve the criteria for evaluation of interventions⁶. In order to achieve enhanced vector surveillance, the vector surveillance systems need to be integrated with the available health information systems in India to guide vector control, and targets for protection of at-risk populations. The districts and institutions engaged with VL elimination should be carefully monitored and basic and applied research on entomology and vector control. This requires continued involvement of all programme official and stakeholders to sustain control efforts. Therefore, entomological zones in states should be appraised and receive continued support for training by national, state, through ICMR institutions and regional offices networks. What is require is integrated approach, including integrated vector management and robust surveillance tools with a view for maintaining the successes achieved and for sustainability in coupled with foci investigations, spot checks and for epidemiological vigilance.

Gaps and Challenges in elimination

Substantial progress in reducing the number of Kala- azar cases were made by India. India is close for elimination. India has also made progress however, some more efforts will be required.

Due to increase community awareness and active search detection, the number of reported PKDL cases has increased. This is a challenge as affected PKDL person are less motivated to seek treatment. The incentive to patient for loss of wages for Kala-azar and PKDL has been provisioned in India which has improved treatment compliance.

Effective implementation of the interventions is crucial for the success of Kala-azar elimination. The first and foremost that country need to play pivotal role, stewardship and ownership which is lacking. Indoor residual spray efficacy is established but not the effectiveness. District and sub districts are not planning spray in time resulting vector to build density and *inter-alia* transmission risk. The disease occurs among the poorest of the poor. Hygiene and sanitation are one of the challenges in elimination. Most of houses in rural setup particularly in Bihar and Jharkhand built are in such a manner leaving no room to sunlight to enter which allows preferred place for Sandfly to rest. The capacity of staff in Program management needs to be strengthened and training to all level. Though India and other VL countries have started treatment with Liposomal Amphoterecin B (L-AmB), it requires investment like maintaining of cold chain, distribution mechanism, development of SOPs, training to medical and paramedics. HIV-VL co infected patient are posing new challenges to program, which requires tracking of patients, strengthens diagnosis, treatment guideline, inter departmental collaboration etc.

The other challenge is asymptomatic infection, clinical Kala-azar or Post Kala-azar Dermal Leishmaniasis (PKDL). Many infected patients harbor the parasite without any clinical manifestation, but over the time, few of them develop the overt form with clinical sign and symptoms as prolonged fever, large spleen, with bone marrow suppression evolving to anemia, and leucopenia.

In clinical cases, the incubation period between infection and clinical signs generally ranges from 3-6 months, and in some cases over a year. After Kala-azar treatment, a small number of KA patients develops a dermal infection, known as PKDL, while they are otherwise healthy. These people do not develop any symptoms.

Further, there is little knowledge on Kala-azar vector bionomics/abundance, its breeding habits, human blood index (HBI), entomological infection rate and status on insecticide resistance. The transmission dynamics of vector population, epidemiological pattern, correct timing of spray coincide with vector abundance, temperature, rain fall and humidity needs to be studied in all endemic countries to understand vector behavior. In India, the indoor residual spray timing is from February /March (1st round) to May/June (2nd round). This timing was set during 70s under malaria programme. Climate has changed over the years. There is need to conduct a detailed study for transmission cycles to know exact when vector population is being built up and co-relates it with epidemiological pattern of cases in month wise. The vector is showing changes in their behavior also. Therefore, it is the time to modify ongoing vector control measures in order to eliminate the disease successfully from the Indian subcontinent⁷. It is also well understand that *Ph.argentipes* in India cannot hop beyond 1.8meter i.e above 6 feet height⁸. The IRS programme is spraying up to 6 feet height. But this study indicates that statistically significant number of *Ph.argentipes* (blood fed and gravid) are also found beyond 1.8 meter (6 feet) height. If this is the case so, the country need to modify the spray guideline from 6 feet height to total height of wall and cattle sheds.

In India sub-continent, the transmission is anthroponotic. No studies on zoonotic transmission established. This need research to establish the facts that in forest areas whether animals are playing potential role in transmission?

The challenge for the sustained elimination of Kala-azar cases from the community is an emerging of HIV-VL co-infection in the adult population. How decisive are PKDL cases and asymptomatic in maintaining transmission to human. The small pox eradication was successful as there were no asymptomatic carriers and no silent transmission, where as in Kala-azar, a high number of asymptomatic infections in human occur.

There is need to develop new tools to restrict Kala-azar transmission in Indian sub-continent. The tools may be efficacy of rK 39, development of shorter duration of treatment in view of resistance developed in some cases with LAMB and studies on vector susceptible. Need to address vector control strategies.

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JAPANESE ENCEPHALITIS: PATHOGEN, PATHOGENESIS, PREVENTION AND CONTROL STRATEGIES

Neha Srivastava¹, Rajni Kant^{2*} and P K Srivastava³

¹ICMR-Regional Medical Research Centre, Gorakhpur, Uttar Pradesh, India

²Indian Council of Medical Research (ICMR), New Delhi, India

³Ex. National Vector Borne Disease Control Programme, GOI, Delhi

*Correspondence: rajnikant.srivastava@gmail.com

Abstract

Japanese encephalitis virus (JEV), a member of the *Flavivirus* genus in the *Flaviviridae* family, is the leading cause of viral encephalitis in Asia, transmitted primarily through *Culex tritaeniorhynchus* mosquito bites. While most human infections are asymptomatic, approximately 1% develop clinical symptoms, with a case fatality rate of up to 30%. Survivors often suffer long-term neurological or psychiatric sequelae. The history of Japanese encephalitis (JE) dates back to the 1870s in Japan, with the virus first isolated during a severe 1924 epidemic. Advances in virology, including the isolation of the prototype Nakayama strain in 1935 and its reclassification in the *Flaviviridae* family in 1985, have enhanced our understanding of JEV. JE primarily affects children under 15 in endemic areas, with an estimated 68,000–100,000 clinical cases annually, putting over three billion people at risk, particularly in South-East Asia and the Western Pacific. Factors such as climate change, population growth, and urbanization are expanding its geographic range. Despite the absence of specific antivirals, effective vaccines are available, and the World Health Organization (WHO) recommends their inclusion in national immunization programs. This review presents the JE epidemiology, transmission, historical milestones, control strategies, and public health interventions, highlighting the importance of integrated efforts in mitigating its impact.

Introduction

The Japanese encephalitis virus (JEV) belongs to genus *Flavivirus* and family *Flaviviridae*, is the leading cause of viral encephalitis in Asia (WHO, 2024). JEV is primarily transmitted to humans through mosquito bites and *Culex tritaeniorhynchus* is considered as its primary vector (WHO, 2024).

JE virus is transmitted to humans through the bite of infected *Culex vishnui* group of mosquitoes, particularly *Culex tritaeniorhynchus*, which extensively breeds in paddy fields. In India, JE virus has been isolated from *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui* and *Cx. Bitaeniorhynchus*, *Cx. epidesmus*, *Cx. fuscocephala*, *Cx. gelidus*, *Cx. quinquefasciatus*, *Cx. Whitmorei*, *Cx. Infula*, *An. paeditaeniatatus*, *An. barbirostris*, *An. subpictus*, *An. vagus*, *Mansonia (Ma.) annulifera*, *Ma. indiana*, and *Ma. Uniformis* (NCVBDC, 2022)

The main reservoirs of the JE virus are pigs and water birds. In its natural cycle, virus is maintained in these animals. Human is an accidental host and does not play a role in JE transmission. After 9–12 days, mosquitoes transmit disease to other vertebrate hosts. Epidemics occur during monsoon and post monsoon period because the vector density is high due to paddy cultivation. However, in endemic areas, sporadic cases may occur throughout the year. (NCVBDC, 2022)

Although most JEV infections in humans are asymptomatic, around 1% of cases develop symptoms, and the case fatality rate among these can reach up to 30% (Simon et. al., 2023). Furthermore, 30–50% of surviving cases may experience long-term neurological or psychiatric sequelae (WHO, 2024).

Japanese encephalitis-like symptoms were first reported in Japan during the 1870s, marking the beginning of Japanese encephalitis (JE) epidemiology, with major outbreaks occurring roughly every decade (Solomon et al., 2023). In 1924, Japanese encephalitis virus (JEV) was first isolated from a clinical case during Japan's initial recorded epidemic, a severe outbreak with over 6,000 documented cases (Solomon et al., 2003). Between 1933 and 1937, early encephalitis cases in the United States were suspected to be caused by JEV, then termed "type B encephalitis" due to its resemblance to strains from Asia (Solomon et al., 2003). However, cross-challenge and cross-neutralization studies using mice later showed that the virus present in the U.S. was distinct from JEV (Solomon et al., 2003). In 1935, the prototype Nakayama strain of JEV was isolated from the brain tissue of a patient with encephalitis, advancing scientific understanding of the virus (Solomon et al., 2003). By 1985, JEV was officially reclassified into the Flaviviridae family under the genus *Flavivirus*, which includes other notable viruses such as yellow fever (Westaway et al., 1985). The genus name *Flavivirus* originates from the Latin word "*flavi*," meaning yellow, after the yellow fever virus (Westaway et al., 1985). JEV was subsequently recognized as the prototype virus within the JE antigenic complex, which also includes West Nile, Murray Valley encephalitis, St. Louis encephalitis, and Kunjin viruses (Westaway et al., 1985).

JEV leads to an estimated 68,000–100,000 clinical cases annually, predominantly affecting children under 15 in endemic areas (Moore, 2021). Over three billion people live in regions endemic for JEV, particularly in the WHO-defined South-East Asia and Western Pacific Regions (WHO, 2024). Climate change, population growth, and increased urbanization contribute to the further expansion of JEV to non-endemic regions (Singh et al., 2023). Currently, no effective antiviral is available for Japanese encephalitis (JE), and treatment focuses on alleviating severe symptoms and supporting recovery (WHO, 2024). However, effective vaccines are available, and the WHO recommends their inclusion in national immunization schedules in affected regions (WHO, 2024). To improve outbreak preparedness, understanding of the virus, its pathogenesis, challenges in its control and possible solution are essential. This review presents the JE epidemiology, transmission, history, control strategies, and public health interventions.

Japanese encephalitis (JE) virus is found from far eastern Russia, northeastern Asia through China and Southeast Asia to Papua New Guinea and the Torres Strait Islands of Australia and westward into India (Reed, 2018). JE virus causes the highest number of human cases approximately thousands annually and most of them are children (WHO, 2024). JE infects humans and horses and abortion in swine (an amplifying host). Herons and egrets are wildlife amplifying hosts. The virus is transmitted by *Culex* spp. of mosquitoes specifically the *Cx. tritaeniorhynchus* (CDC, 2024). Prevention of disease is mainly through vaccination of humans, horses, and swine (CDC, 2024). Insecticides and integrated pest control measures that include natural compounds (*Bacillus thuringiensis* toxins), larvivorous fishes, and larval habitat modification have been successfully used in China (CDC, 2024; Guo et al., 2020; Sahu et al., 2022). Use of permethrin-impregnated bed netting can also prevent transmission (CDC, 2024).

Genomic Structure of Japanese encephalitis virus, genetic diversity and its geographical distribution

Japanese encephalitis virus is a single-stranded positive-sense RNA virus with genome size of approximately 11 kilobases (Kb) (Sumiyoshi et al., 1987). Structurally, JEV particles are spherical, about 40–50 nanometers in diameter, and have a 30 nm wide core surrounded by a lipid envelope (Lindenbach & Rice, 2003). This envelope is embedded with the envelope (E) protein, a critical antigen that helps the virus penetrate host cells (Lindenbach & Rice, 2003). The viral genome encodes ten proteins (Figure 1), including three structural proteins: capsid (C), membrane (M), and envelope (E). The genome also includes seven non-structural (NS) proteins – NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 – that are crucial for viral replication and immune evasion. NS3 functions as a helicase, and NS5 serves as an RNA-dependent RNA polymerase (RdRp), which is prone to mutation, driving genetic diversity in JEV strains (Lindenbach & Rice, 2003). Phylogenetic studies divide JEV into five genotypes based on the nucleotide sequences of the E protein gene or the full polyprotein gene, with most isolates of genotype I (GI) or genotype III (GIII) (Solomon et al., 2003; Pan et al., 2011). All JEV genotypes are phylogenetically, antigenically, and geographically distinct. Historically, GIII was the predominant genotype, but surveillance over the past two decades has shown a gradual shift, with GI now either dominant or co-circulating with GIII in many endemic and epidemic regions (Pan et al., 2011) (Fig. 2).

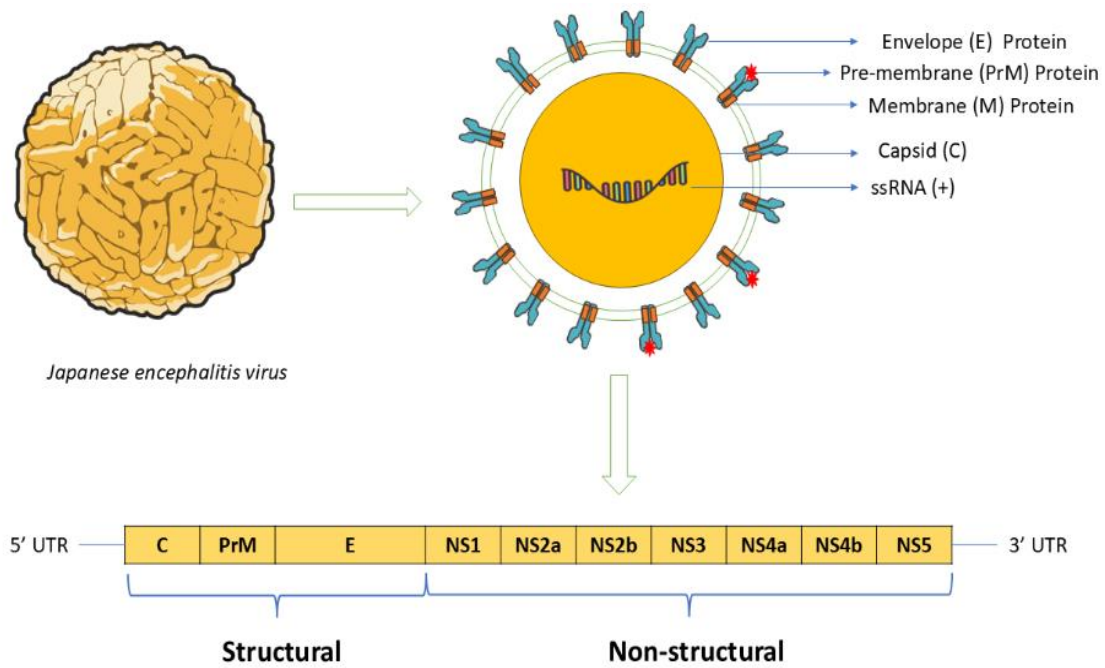


Figure 1. Genomic structure of Japanese encephalitis virus (drawn using BioArt by

Genotype 3 (GIII) was the predominant genotype circulating in Asia until the 1990s, after which Genotype I (GI) became the dominant strain. Genotype 2 (GII) has been detected in outbreaks in Malaysia, Indonesia, Papua New Guinea, and Australia between 1970 and 2000. Genotype 5 (GV), initially isolated in Malaysia in 1952, has since been reported in China and is now also prevalent in South Korea (Srivastava et al., 2023). Genotype 4 (GIV) remains the rarest, having been identified only in mosquitoes from Indonesia and Papua New Guinea (Srivastava et al., 2023).



Figure 2. Geographical distribution of JEV genotypes (I-V) (Map of Asia adopted and modified from Perry Castañeda Library map collection -<https://maps.lib.utexas.edu/maps/asia.html>)

Transmission cycle and pathogenesis of Japanese encephalitis:

Japanese encephalitis virus (JEV) is transmitted to humans primarily by the bites of infected *Culex* mosquitoes, particularly *Culex tritaeniorhynchus* (Fig. 3) (Hurk et al., 2008). The virus is maintained in a natural cycle between mosquitoes and amplifying hosts like pigs and wading birds, with humans and horses serving as incidental, or "dead-end," hosts, as they do not sustain viral levels high enough to infect mosquitoes (Misra & Kalita, 2010). Transmission often occurs in rural, agricultural regions, especially near rice fields and flood-irrigated areas, and can also affect some urban areas. In temperate regions of Asia, JEV transmission is seasonal, peaking in summer and fall, whereas in tropical areas, transmission can occur year-round, often intensifying during the rainy season (Fischer et al., 2010). Pigs play a critical role in JEV ecology, developing viremia that supports both enzootic (ongoing) and epizootic (outbreak) transmission cycles (Le Flohic et al., 2013). While the direct transmission of JEV between pigs through oronasal shedding is not fully confirmed, serological evidence and mathematical models from studies in high-incidence areas, like Cambodia, suggest it may contribute to virus maintenance, particularly when mosquitoes are less active or absent, as in temperate regions (Diallo et al., 2018). To confirm the ecological significance of direct transmission, multi-season studies with virus genetic data would be needed to verify if JEV can persist independently of mosquito vectors.

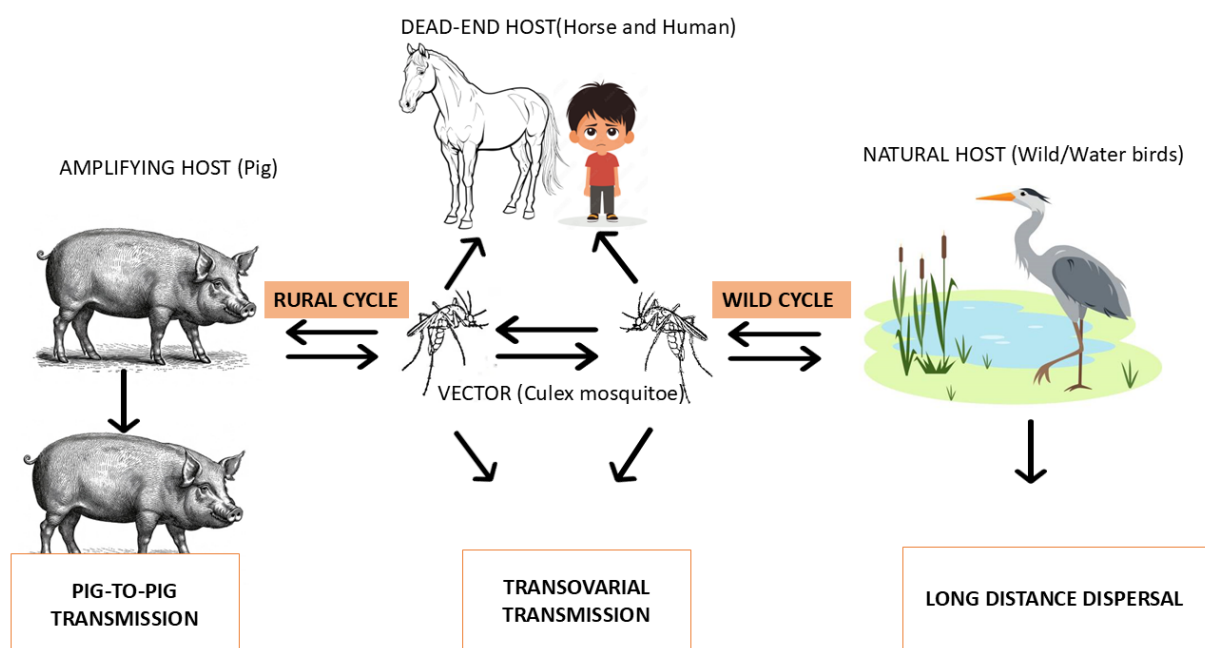


Figure 3. Japanese encephalitis virus (JEV) Transmission cycle

After bite of JEV infected mosquitoes, the virus inoculates intradermally and replicate initially in the skin before entering nearby lymph nodes (Fig. 4) (Wu et al., 2000). During infection caused by viruses of flaviviruses family such as dengue and West Nile virus, the virus replication and movement via lymphatic system to thoracic duct and blood stream are facilitated by langerhans dendritic cells in the skin (Huang et al., 1963). During this phase, called primary viremia, viral particles are disseminated to various extra-neural tissues, including connective tissue, skeletal and smooth muscle, myocardium, lymphoreticular tissues, and both endocrine and exocrine glands. The virus then circulates through the bloodstream and potentially enter the central nervous system (CNS) (Huang et al., 1963; Turtle & Solomon, 2018) (Fig. 4).

Although the exact mechanism of JEV's entry across the blood-brain barrier is not fully understood (Filgueira & Lannes 2019), it is clear that if JEV remains confined to extra-neural tissues, symptoms may be mild or unnoticed. However, if JEV reaches neural tissues, it can result in encephalitis, underscoring the importance of understanding CNS penetration to grasp the virus's severity (Filgueira & Lannes 2019). Studies with electron micrographs show that JEV attaches to endothelial cells in cerebral blood vessels,

where it is transported across the barrier within endocytotic vesicles, likely facilitated by perivascular cells (Filgueira & Lannes 2019). This CNS invasion triggers a combined response of direct viral damage and host inflammatory processes, forming the basis of flavivirus encephalitis pathogenesis (Filgueira & Lannes 2019). The virus attaches to cell membranes using its envelope spikes, dissolving the plasma membrane at the contact point to enter cells. Within the host cell's endosome, JEV fuses with the host membrane. Electron micrograph studies of infected brain cells have shown that viral RNA synthesis occurs in the perinuclear region, while viral replication takes place within the rough endoplasmic reticulum and Golgi apparatus. Once mature, viral particles exit the cell via exocytosis. Extensive hypertrophy of the endoplasmic reticulum and degeneration into cystic structures eventually leads to severe cellular dysfunction (Filgueira & Lannes 2019).

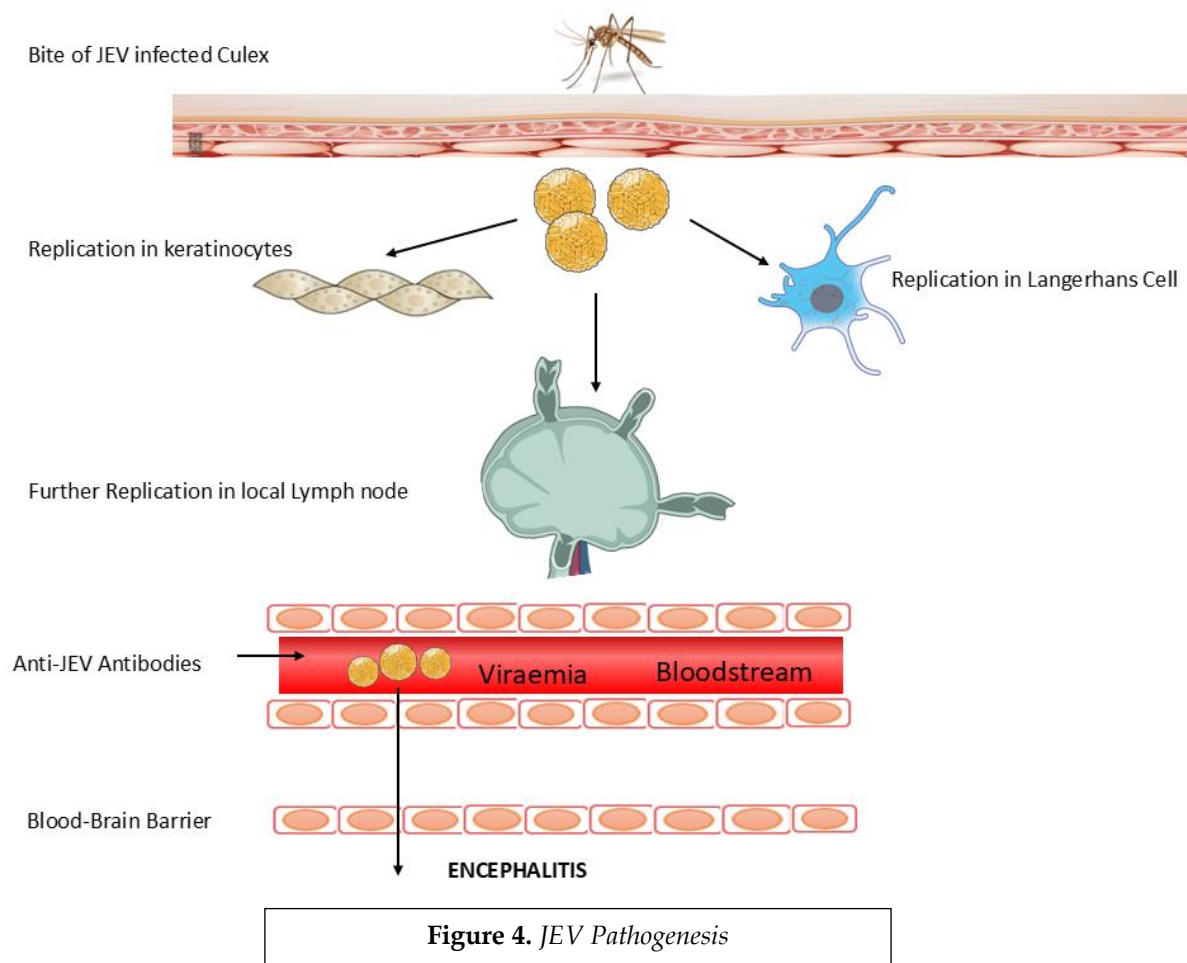


Figure 4. JEV Pathogenesis

JEV Therapeutics

There is no specific antiviral treatment for JE and supportive care are given to patients (WHO, 2024). Severe meningeal symptoms in them are often managed by pain control for headaches and antiemetic treatment for nausea and vomiting. Development of elevated intracranial pressure, seizures, and inability to protect their airway are closely monitored in them (CDC, 2024).

Clinical trials on drugs such as dexamethasone, minocycline, ribavirin, intravenous immunoglobulins (IVIG), and interferon have shown limited or inconclusive benefits for JE patients (Ajibowo et al., 2021). Dexamethasone has been tested to alleviate intracranial pressure but showed no significant impact on mortality rates or patient outcomes (Sahu et al., 2022). Minocycline, known for its anti-inflammatory and neuroprotective effects, demonstrated some benefits in smaller studies, particularly in reducing fever and hospital stay duration (Singh et al., 2016). Ribavirin proved ineffective against early mortality in JE-infected children (Kumar et al., 2009). IVIG showed increased antibody response in a small patient sample, suggesting potential, though larger trials are necessary to confirm efficacy (Rayamajhi et al.,

2015). Interferon also failed to improve outcomes, though further studies with higher doses are recommended (Solomon et al., 2003). Compounds like ouabain from natural sources showed promise in reducing JE viral load in animal studies, indicating a potential direction for future research (Guo et al., 2020).

Currently, vaccination is the most effective prevention method. WHO recommends JE vaccines in endemic regions and for travellers to high-risk areas (WHO, 2024).

JE Vaccine

Japanese Encephalitis (JE) vaccines are available in various types, substrates, and strains, each suited to specific regions and populations. The inactivated vaccine type includes several formulations. For instance, JE-VAX, originally produced using a wild strain in mouse brain substrate, was manufactured by BIKEN in Japan and widely distributed across countries including India, the European Union, Japan, Malaysia, Korea, Taiwan, Thailand, the USA, and Vietnam, though it has since been discontinued (Muangchana et al., 2012; Plesner 2003). Green Cross Corporation in Korea also produced a similar formulation under the brand JEV-GCC (Hegde & Gore, 2017). Other inactivated options use Vero cell substrates; ENCEVAC (Yun, 2015) and JEIMMUGEN (produced by KAKETSUKEN, Japan) (Hegde & Gore, 2017) and JEBIK-V (by BIKEN, Japan) (Hegde & Gore, 2017) are distributed in Japan and South Korea, while TC-JEV is produced by Boryung Pharma in South Korea (Hegde & Gore, 2017).

JENVAC (ICMR-Bharat Biotech initiative) is another inactivated vaccine utilizing the 821564XY strain in Vero cells and is specifically available in India (Hegde & Gore, 2017). Live attenuated vaccines provide an alternative approach, with brands like IXIARO and JESPECT (from Novartis/Intercell in Austria and Commonwealth Serum Laboratories in Australia) using the SA14-14-2 strain grown in Vero cells (Hegde & Gore, 2017). These vaccines are widely used in countries such as India, Australia, the USA, Canada, the European Union, Japan, Hong Kong, Singapore, South Korea, New Zealand, Bangladesh, Bhutan, Papua New Guinea, Latin America, and Pacific Islands (Hegde & Gore, 2017). JEEV, another Vero cell-based live attenuated vaccine by Biological E. Limited, is available in India (Hegde & Gore, 2017), while CD.JEVAX from Chengdu Institute of Biological Products, China, uses PHKC cells and is distributed across India, China, Japan, South Korea, Nepal, Sri Lanka, Hong Kong, and Thailand (Hegde & Gore, 2017). Lastly, the chimeric vaccine IMOJEV (also branded as THAIJEV), developed by Acambis/Sanofi Pasteur, incorporates the JEV protein into the YF 17D strain within Vero cells. This vaccine is utilized in Australia, South Korea, and Thailand, offering broader immunization options for JE-endemic regions (Hegde & Gore, 2017).

JE Prevention and control

Japanese Encephalitis (JE) is primarily a regional health concern, with transmission largely confined to parts of Asia and the Western Pacific (WHO, 2024). This limited spread means that global awareness of JE remains low, despite occasional outbreaks that briefly capture public attention.

Mosquito control is a key element in JE prevention, as the virus spreads mainly through *Culex tritaeniorhynchus* mosquitoes, which thrive in stagnant water near rice paddies (WHO 2024; NCVBDC, 2024). Short-term mosquito control methods—like insecticides and larvicides—can help manage outbreaks but are costly and face challenges from insecticide resistance. More sustainable solutions, such as spraying natural pesticides like neem and introducing larval-eating fish into rice paddies, show promise. In Assam, India, using insecticide-treated nets around pig enclosures significantly reduced JE transmission among both pigs and humans, demonstrating the success of focused interventions (NCVBDC, 2024).

Reducing transmission through animal hosts, especially pigs, which are major amplifiers of the virus, can further decrease human infection risk. Building pig farms away from residential zones and vaccinating livestock in high-risk areas help limit JE's spread. Human vaccination remains the best preventive strategy, though access to vaccines is still lacking in many low-income regions. For those at risk, additional protective measures—such as mosquito repellents, protective clothing, and bed nets—offer some defence (NCVBDC, 2024).

JE in India: Journey of JE Vaccination and Policy Development

Since the first confirmed case of JE in 1955 in Vellore, Tamil Nadu, the disease has become endemic in 355 districts across 24 states and Union Territories in India (Ghosh et al., 2022). Assam, Uttar Pradesh, and West Bengal report the highest number of JE cases annually. In 2005, focal outbreaks of JE in Uttar Pradesh, Bihar, and Assam intensified during the monsoon and post-monsoon periods caused 5,737 cases and 1,344 deaths in Uttar Pradesh alone (Parida et al., 2006). This posed a major challenge for India's health authorities. The key hurdles in managing JE included a weak surveillance system, limited vaccine supply, inadequate healthcare infrastructure, competing health priorities, and ineffective grassroots communication. Despite these challenges, opportunities emerged to strengthen the JE control program (Ghosh et al., 2022). Indigenously developed ELISA diagnostic kits by ICMR-NIV, Pune, enhanced JE surveillance, and the National Technical Advisory Group on Immunization (NTAGI) played a crucial role in guiding the JE vaccination policy in India (Ghosh et al., 2022; Kulkarni et al., 2018).

In 2000, the Bill & Melinda Gates Foundation funded a project to introduce JE vaccines in India and other South Asian countries, providing valuable early experiences, particularly in Andhra Pradesh, which demonstrated the potential of vaccines to control JE outbreaks (PATH). At the time, the live, attenuated SA14-14-2 JE vaccine from China was becoming more widely available and offered a more scalable alternative to the older inactivated mouse brain-derived vaccine. The SA14-14-2 vaccine had already been used in several countries, including Nepal, India's neighbour, further strengthening the evidence for its broader adoption. The Government of India prioritized JE vaccination as the most effective tool for controlling the disease, citing evidence from countries like Nepal and its own state Andhra Pradesh where the vaccine had successfully reduced JE incidence (Ghosh et al., 2022).

The JE immunization intervention was guided by the National Vector Borne Disease Control Programme (NVBDPC), now known as the National Centre for Vector Borne Disease Control (NCVBDC), and the Indian Council of Medical Research (ICMR), which provided vital epidemiological and serological data to identify high-priority districts for vaccination (Ghosh et al., 2022). Strong public demand, both locally and nationally, made JE vaccination a top priority for the government. Upon recognizing the urgent need for an effective vaccine, the Ministry of Health and Family Welfare (MoHFW) initiated efforts to find a suitable Vaccine for Indian population. The estimated requirement for a successful vaccination campaign was 12–15 million doses, and five key criteria were prioritized: safety, efficacy, affordability, supply stability, and program compliance. Two vaccines were available for consideration at that time: the inactivated mouse brain-derived vaccine and the live attenuated SA14-14-2 vaccine from Chengdu Institute of Biological Products (CIBP), China. The mouse brain-derived vaccine was ruled out due to concerns raised by WHO regarding its safety, limited supply (only a few hundred thousand doses), high cost, and the need for multiple doses with boosters, which complicated compliance. In contrast, the SA14-14-2 vaccine emerged as the preferred choice due to its single-dose, high efficacy (80–90% with one dose, over 98% with two), excellent safety profile, and affordability – 15 times cheaper than the alternative. Supported by an uninterrupted supply commitment and a strong safety record from over 200 million immunizations in China, the SA14-14-2 vaccine was endorsed by WHO (Ghosh et al., 2022).

Now the problem was procurement of SA 14-14-2 from China for which a public-sector government-owned industry named HLL Lifecare Limited (HLL) was identified to import the SA14-14-2 vaccine from China. HLL leveraged its experience in procuring products from China for various programs. However, selecting HLL for this role posed several challenges. Although HLL was primarily responsible for procuring and distributing condoms, it had no prior experience in vaccine licensing or importation as of 2006. Additionally, the World Bank was auditing its ongoing funding to the Indian government, preventing the use of World Bank resources to purchase the vaccine until the audit was completed. As a result, the Government of India had to directly allocate funds from its budget to cover the cost of the JE vaccination program. Due to these circumstances, HLL required technical support from PATH to overcome cultural differences in communication with the CIBP. Despite these challenges, HLL successfully managed the vaccine procurement, coordinated logistics, and placed a final order with CIBP, ensuring the vaccine was imported in time for the planned launch of the JE vaccination campaign in Uttar Pradesh on May 15, 2006 (Ghosh et al., 2022).

The JE vaccination program faced four major challenges: 1) reaching remote areas, including villages cut off by rivers and mountains; 2) achieving high coverage in regions with weak health systems and low

immunization rates; 3) establishing a robust vaccine safety surveillance system to track adverse events following immunization (AEFI), given that the vaccine had been used sparingly in India before; and 4) addressing media concerns about the use of a foreign vaccine without prior bridging studies in India. To overcome these challenges, program managers applied lessons learned from the Pulse Polio campaign, utilizing microplanning, community mobilization, and monitoring. UNICEF and WHO also supported the implementation of safety surveillance and community outreach (Ghosh et al., 2022).

In 2006, JE vaccination campaigns were launched in 11 districts across four states, as part of a 5-year plan (2006–2010) targeting 104 JE-endemic districts. These areas were selected using surveillance, epidemiological, and serological data from NVBDCP and ICMR. However, due to limited vaccine supply the 2006 vaccine rollout was restricted to 11 districts only. The strategy for the campaign involved a one-time vaccination drive for children aged 1 to 15. The campaign comprised five core components. First, program guidelines were developed, covering planning, training, AEFI monitoring, waste disposal, and communication. Second, a multi-tiered training strategy was implemented, with national trainers training state and district trainers, who in turn trained health workers in the program districts. The MoHFW developed a training module covering vaccine administration, AEFI monitoring, communications, and waste management, with additional training for medical professionals and media personnel. Third, vaccine logistics were addressed, drawing on lessons from previous polio immunization programs. Vaccines were transported from Chengdu to Delhi, where HLL managed customs clearance. Vaccine samples were sent to the Central Drugs Laboratory at Kasauli for potency and quality checks according to WHO technical recommendations. The vaccine was then stored at the Central Medical Stores Depot in Karnal and after passing quality check distributed through a cold chain network across India. Fourth, micro-plans were developed for local adaptation at the block level, and fifth, district task forces were established to oversee AEFI, communication, and cold chain monitoring. The program included three tiers of monitoring: district-level oversight, concurrent monitoring by WHO and UNICEF, and coverage evaluation surveys. The first phase of the JE vaccination campaign (2006–2010) and a mop-up campaign in 2011 reached 118 districts, with an average reported coverage rate of 82% (ranging from 69% to 98%) (Ghosh et al., 2022).

Until 2012, the SA 14-14-2 JE vaccine was used in India. In September 2013, the first indigenous JE vaccine, JENVAC, was licensed in India (PATH). JENVAC was developed jointly by scientists at the National Institute of Virology (NIV), Pune, and Bharat Biotech Ltd. Later, in 2013, the JE immunization introduced under Universal Immunization Programme (UIP) of Government of India. According to Government of India guidelines, two doses of the JE vaccine are included in the UIP. The first dose is administered alongside the measles vaccine at 9 months, and the second dose with the DPT booster at 16–24 months, as of April 2013 (Murhekar et al., 2017; NCVBDC). Routine JE immunization covers 334 out of 355 endemic districts. In addition, adult vaccination has been initiated in 42 districts across Assam, Uttar Pradesh, and West Bengal, where adult JE cases are prevalent. This adult vaccination uses the JENVAC vaccine for individuals aged 15 and above (PATH). In 2020, the SA14-14-2 vaccine was discontinued, and JENVAC was introduced into the UIP for children and adult.

Vector control is another critical strategy targeting the mosquito population. Larval source reduction, achieved by eliminating or treating breeding sites like stagnant water in rice fields, irrigation canals, and puddles, is key. This involves environmental management, proper water storage, and larvicide application. Indoor residual spraying of insecticides in high-density mosquito areas helps reduce mosquito populations and human contact (NCVBDC). Personal protective measures, such as avoiding mosquito bites by wearing long-sleeved clothing, using insect repellents, and staying in screened or air-conditioned accommodations, are essential. Sleeping under mosquito nets, especially in areas lacking insecticide-treated nets, provides additional protection. Locating piggeries at least 4–5 km from human dwellings further reduces risk. Combined efforts in vaccination, vector control, and education are pivotal in combating JE in India. Health education campaigns are crucial in raising awareness about the transmission and prevention of Japanese Encephalitis (JE) and other vector-borne diseases (NCVBDC). One such campaign is "DASTAK," which means "knock the door," and focuses on door-to-door awareness efforts (gorakhpur.nic.in). Launched in February 2018 by the Uttar Pradesh government, in partnership with UNICEF, DASTAK aims to combat Acute Encephalitis Syndrome (AES) and JE in the state. The campaign's objective is to eradicate these deadly diseases by uniting communities in collective action. The slogan, "*Darwaja khatkhatao, AES aur JE ko bhagao*" ("*Knock the door, drive away AES and JE*"), emphasizes

its mission. Implemented across 38 districts, primarily in the terai region, which accounts for around 60% of AES cases in India, DASTAK involves collaboration between various state departments, including health, rural development, and education. The campaign educates the rural population, particularly children, about the importance of clean drinking water, sanitation, vaccination, and early disease treatment. It also promotes preventive measures through mass media communication and engages schools in affected areas. Through these efforts, the DASTAK campaign seeks to reduce the spread of JE and AES and ensure a healthier environment for vulnerable populations, especially in rural regions (gorakhpur.nic.in).

Successful JE prevention and control model: Gorakhpur model

Vaccination initiatives in Japan, South Korea, and Thailand, China, India, and Nepal have shown promising results (Halstead & Jacobson, 2008). However, comprehensive public health measures are still essential to prevent JE in high-risk areas where human and animal populations are exposed to high numbers of mosquitoes. One such effective prevention model is “Gorakhpur JE Prevention Model” in India (Srivastava et al., 2022).

The success of the Gorakhpur Japanese Encephalitis (JE) prevention model is due to robust coordination among various inter-ministerial and inter-departmental stakeholders, including the central and state governments, non-governmental organizations (NGOs), healthcare providers, and the local community (Srivastava et al., 2022). This collaborative model is anchored by a central coordinating body, which includes the Uttar Pradesh State Health Department, the Indian Council of Medical Research (ICMR), the PATH Foundation, and the World Health Organization (WHO) India. This central body brings together representatives from each sector to harmonize objectives and set clear priorities focused on JE, Acute Encephalitis Syndrome (AES), and vulnerable populations, while also ensuring alignment with national health strategies.

Through this coordination, the model develops action plans that emphasize data collection, disease surveillance, and community-led interventions, with cross-sector efforts to address disease determinants such as diagnostics, treatment accessibility, and socio-behavioural factors. A critical component of this approach is the sharing of resources and capacity-building programs, which include workforce training and public awareness campaigns such as DASTAK which is a door-to-door awareness campaign. These initiatives empower healthcare providers and community health volunteers with the necessary skills and knowledge to implement JE prevention measures effectively, further contributing to the model’s success.

In addition, an AES cell, a centralized facility at pediatric department of Baba Raghav Das Medical College Gorakhpur, Uttar Pradesh, India which aimed to streamline the collection, storage, and analysis of clinical details, blood, and cerebrospinal fluid (CSF) samples from AES cases played a vital role in management of severity in AES cases and declining the mortality (Srivastava et al., 2022).

The initiation of prevention strategy was obviously the key for controlling JE in Gorakhpur but efforts of all government and non-government bodies for its effective implementation and sustainability played a major role.

JE through the lens of One Health

The multipronged strategy employed to reduce JE cases represents an early, practical application of what is now termed the *One Health approach*, which has gained prominence in the prevention of infectious diseases. Although the term "One Health" is relatively new but professionals working on JE have effectively utilized its principles for over 70 years. This approach has been useful in understanding JEV transmission dynamics and developing tools to combat epidemics. Despite these successes, the implementation of the One Health framework for JE remains incomplete, with persistent challenges in reducing the natural transmission of virus. Nevertheless, the insights gained from JE interventions provide a valuable template for designing and executing One Health strategies to address other mosquito-borne zoonotic diseases.

Conclusion

In conclusion, Japanese Encephalitis (JE) remains a significant public health concern, particularly in rural areas of Asia, where it disproportionately affects children and vulnerable populations. Despite the

availability of vaccines, challenges such as inadequate access, misdiagnosis, and emerging viral strains continue to hinder effective control and prevention efforts. Ongoing research is essential to enhance understanding of the disease's pathogenesis, develop more effective diagnostics and treatments, and improve vaccination strategies. A collaborative approach involving governments, healthcare providers, and communities like Gorakhpur JE Prevention model is crucial to reducing the burden of JE and preventing future outbreaks, particularly in endemic regions.

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TICKS AND MITES BORNE DISEASES: PREVENTION AND ITS CONTROL

S N Sharma¹ and S K Farhat²

¹Former Joint Director, NCVBDC and Senior Consultant, NCDC, MoHFW, GoI, Delhi

²Research Scholar, Vector Biology Research Laboratory, Central University of Tamil Nadu, Thiruvavur, India

Abstract

Ticks and Mite borne diseases are posing a future public health threat in the country as the exact burden of the diseases is uncertain due to lack of disease surveillance and diagnostic tools at the primary health care delivery system. The feeding nature of the ticks and mites on mammals, and other hosts play a role in the spread of fatal tick-borne diseases like Kyasanur Forest Disease (KFD), Lyme disease, Indian Tick Typhus, Crimean Congo Hemorrhagic Fever (CCHF), Relapsing fever, Rocky Mountain spotted fever, Q fever and mite-borne diseases like Scrub typhus, Scabies, Rickettsial pox, and Dermatitis, with human as accidental hosts affecting across the globe. Moreover, there is also no systematic vector surveillance to understand the dynamics of disease transmission in different eco – geographical settings. There are frequent reports of local outbreaks of KFD, CCHF and scrub typhus in several states and areas of endemicity are increasing from one area to other area.

It is important to understand the distribution and biology of ticks and mites in different hot spots based on the disease reporting. This may help to know the different species of ticks and mites which play role in the transmission of tick and mite borne diseases. In this chapter, an attempt has been made to describe transmission of ticks and mite borne disease, vectors, prevention and control strategy. The role of community and environmental sanitation are important components towards prevention of transmission of these diseases and control in the localized situations. Inter-sectoral coordination is to given priority wherein departments veterinary, agriculture, forest, rural development can work together with the health department in minimizing the vector – human contact.

Key words: Hard ticks, Soft Ticks, flavivirus, Rickettsial, Mites, KFD, CCHF

Introduction

Tick- and mite-borne diseases are global health problems caused by a variety of bacterial, viral and protozoan pathogens, which are responsible for significant morbidity and thousands of human deaths annually. With the similar appearance of ticks and mites to insects, they often confuse with other insects. Ticks and Mites are widely classified into Arachnida class with body divided into two segments namely, cephalothorax and abdomen with eight-legged adults, having no wings and antennae, unlike insects having three body segments viz., head, thorax, and abdomen with six legs and an antenna, and well-developed wings. Next to mosquito nuisance, is the ticks causing major public health concerns (Benelli, 2019) by acting as vectors of many pathogens responsible for mortality and morbidity. Important tick, mite & flea borne diseases include Lyme disease (LD), tick-borne relapsing fever, Babesiosis, Anaplasmosis, Rocky Mountain spotted fever, Bouton Neuse fever, Q fever, Crimean Congo hemorrhagic fever virus, *Rickettsia rickettsii* (Rocky Mountain Spotted Fever), Kyasanur forest disease (KFD), numerous tick-borne encephalitis complex of flaviviruses, Scrub typhus and plague. Tick-borne flaviviruses have been reported to cause significant global losses and health risks, with an annual estimation of India's cost at \$498.7 million. (Ghosh & Nagar, 2014). Tick-borne encephalitis causes between 10,000 and 15,000 human cases every year on both continents. Lyme disease is the most commonly reported tick-borne disease in the United States. In 2017, more than 42,743 confirmed and 382700 probable cases of Lyme

disease were reported to the Centers for Disease Control and Prevention (CDC). Crimean-Congo hemorrhagic fever (CCHF) is the most widespread, tick-borne viral disease affecting humans. The disease is endemic in many regions and 3 billion people at risk, estimated 10,000 to 15,000 cases and 500 deaths each year are reported mainly from endemic regions, such as Africa, Asia, Eastern and Southern Europe. Rickettsial diseases are most covert emerging and re-emerging diseases and are being increasingly recognized in India.

In India, the fatal tick-borne viral diseases, viz., Crimean Congo Hemorrhagic fever (CCHF) and Kyasanur Forest Disease (KFD) were caused by ticks of *Hyalomma analoticum* and *Hemophysalis spinigera*. KFD is a re-emerging disease discovered in 1957 from Shimoga district of Karnataka state; however, in recent years it has moved its territory to seven districts of the state as well as centripetally spread to neighboring Kerala, Goa and Maharashtra states which are sharing borders with the state. CCHF was first reported from Gujarat in 2011; however, in recent years cases were recorded from Rajasthan and Uttar Pradesh states. There are sporadic records available for the occurrence of other tick-borne diseases, viz., relapsing fever, Lyme disease, Ganjam virus disease and Q fever from various parts of the country from time to time. While only few cases of Lyme disease are reported from India, scrub typhus has been reported from many parts of Rajasthan, Jammu & Kashmir, Vellore, Sikkim, Darjeeling, Nagaland, Manipur. Indian tick typhus infection is widely prevalent in the hilly forest tract in different parts of India and sero-epidemiologically the infection has been reported from various parts of the country like Nagpur, Jabalpur, Jammu and Kashmir, Kanpur, Sagar, Pune, Lucknow and Bangalore. In India, major outbreaks of plague occurred in states of Maharashtra (bubonic type) and Gujarat (pneumonic type) in 1994 with 876 cases and 54 deaths.

Presently, the states /UTs are reporting information with regard to Malaria, Dengue, Chikungunya, Filariasis, Japanese encephalitis and Kala-azar at the National level under the overall umbrella of NHM. There have been sporadic reports of Zika, scrub typhus and KFD from different parts of the country. There is no such organized national program as such so far in the country hence regular surveillance is not done.

Kyasanur Forest Disease (KFD)

Kyasanur Forest disease (KFD) is caused by Kyasanur Forest disease virus (KFDV), a member of the virus family Flaviviridae. KFDV was identified in 1957 when it was isolated from a sick monkey from the Kyasanur Forest in Karnataka (formerly Mysore) State, India with 176 suspected cases and 38 confirmed cases (Holbrook, 2012). Since then, between 400-500 human cases per year have been reported and has a fatality rate of 3-10%. Hard ticks (*Hemaphysalis spinigera*) are the reservoir of KFD virus which remains infective throughout life if once infected. A retrospective study conducted in India, from 1957 to 2017, estimated 9,594 cases of KFD within 16 districts. The most significant human outbreaks of the disease were in the years 1957-1958 (681 cases), 1983-1984 (2,589 cases), 2002-2003 (1,562 cases) and 2016-2017 (809 cases). In 2015, KFD appeared in Goa and in 2016, new cases emerged in Belgaum, district of Karnataka state and Sindhudurg district of Maharashtra (Chakraborty et al., 2019). Rodents, shrews, and monkeys are common hosts for KFDV.

Transmission: Transmission to humans may occur after a tick bite or contact with an infected animal, most importantly a sick or recently dead monkey. No person-to-person transmission has been described. Large animals such as goats, cows, and sheep may become infected with KFD but play a limited role in the transmission of the disease. These animals provide the blood meals for ticks and it is possible for infected animals with viremia to infect other ticks, but transmission of KFDV to humans from these larger animals is extremely rare. Furthermore, there is no evidence of disease transmission via the unpasteurized milk of any of these animals.

Signs and Symptoms: After an incubation period of 3-8 days, the symptoms of KFD begin suddenly with chills, fever, and headache. Severe muscle pain with vomiting, gastrointestinal symptoms and bleeding problems may occur 3-4 days after initial symptom onset. Patients may experience abnormally low blood pressure, and low platelet, red blood cell, and white blood cell counts. After 1-2 weeks of symptoms, some patients recover without complication. However, the illness is biphasic for a subset of patients (10- 20%) who experience a second wave of symptoms at the beginning of the third week. These symptoms include

fever and signs of neurological manifestations, such as severe headache, mental disturbances, tremors, and vision deficits. The estimated case-fatality rate is from 3 to 5% for KFD.

Risk of Exposure: KFD has historically been limited to the western and central districts of Karnataka State, India. However, in November 2012, samples from humans and monkeys tested positive for KFDV in the southernmost district of the State neighbouring Tamil Nadu and Kerala, indicating the possibility of wider distribution of KFDV. The disease has also shown its presence in the states of Maharashtra, Goa, and Gujarat. People with recreational or occupational exposure to rural or outdoor settings (e.g., hunters, herders, forest workers, farmers) within Karnataka State are potentially at risk for infection by contact with infected ticks. Seasonality is another important risk factor as more cases are reported during the dry season, from November through June.

Crimean Congo Haemorrhagic Fever

Crimean-Congo hemorrhagic fever (CCHF) is caused by infection with a tick-borne virus (Nairovirus) in the family Bunyaviridae. The disease was first characterized in the Crimea in 1944 and given the name Crimean hemorrhagic fever. It was then later recognized in 1969 as the cause of illness in the Congo, thus resulting in the current name of the disease. Crimean-Congo hemorrhagic fever is found in Eastern Europe, particularly in the former Soviet Union, throughout the Mediterranean, in northwestern China, central Asia, southern Europe, Africa, the Middle East, and the Indian subcontinent. In India, the confirmed case of CCHF was reported during a nosocomial (infections caught in hospitals) outbreak in Gujarat, Ahmedabad in January 2011. Subsequently outbreaks were reported from different districts of Gujarat every year. During 2012-2015, several outbreaks and cases of CCHF were reported in the states of Gujarat and Rajasthan. Cases were documented from 6 districts of Gujarat (Ahmedabad, Amreli, Patan, Surendranagar, Kutch and Aravalli), 3 districts of Rajasthan (Sirohi, Jodhpur and Jaisalmer) and in state of Uttar Pradesh.

Transmission: Ixodid (hard) ticks, especially those of the genus, *Hyalomma*, are reservoir and vector both for CCHF virus. Numerous wild and domestic animals, such as cattle, goats, sheep and hares, serve as amplifying hosts for the virus. Transmission to humans occurs through contact with infected ticks or animal blood. CCHF can be transmitted from one infected human to another by contact with infectious blood or body fluids. Spread of CCHF has also been reported in hospitals due to improper sterilization of medical equipment, reuse of injection needles, and contamination of medical supplies.

Signs and Symptoms: The onset of CCHF is sudden, with initial signs and symptoms including headache, high fever, back pain, joint pain, stomach pain, and vomiting. Red eyes, a flushed face, a red throat, and petechiae (red spots) on the palate are common. Symptoms may also include jaundice, and in severe cases, changes in mood and sensory perception. As the illness progresses, large areas of severe bruising, severe nosebleeds, and uncontrolled bleeding at injection sites can be seen, beginning on about the fourth day of illness and lasting for about two weeks. In documented outbreaks of CCHF, fatality rates in hospitalized patients have ranged from 9% to as high as 50%. The long-term effects of CCHF infection have not been studied well enough in survivors to determine whether or not specific complications exist. However, recovery is slow.

Risk of Exposure: Animal herders, livestock workers, and slaughterhouse workers in endemic areas are at risk of CCHF. Healthcare workers in endemic areas are at risk of infection through unprotected contact with infectious blood and body fluids. Individuals and international travellers with contact to livestock in endemic regions may also be exposed.

Vectors

Ticks: Ticks are found throughout the world, in tropic and temperate zones. They are divided into two groups: the soft ticks of the family Argasidae (100 species) and the hard ticks of the family Ixodidae (700 species). Hard ticks are capable of transmitting diseases namely, tularemia, Rocky Mountain spotted fever, Lyme disease, Q fever, and tick-borne encephalitis. They can cause a direct injury resulting in a condition known as tick paralysis. The soft tick may transmit spirochetes that cause tick-borne relapsing fever. The tick gets spirochetes when ingesting the blood of an infected animal. The spirochetes then

multiply within the body of the tick, invading the tissues and the body cavity of the tick. Spirochetes are transmitted to the tick's eggs by transovarian transmission, even to the third generation.

a. Hard Ticks (Family Ixodidae): The mouthparts can be seen from above; therefore, the hard tick has a shield on the back and (Fig. 1). The life cycle of the hard-tick is a type of gradual metamorphosis, consisting of four stages: egg, larva (not worm-like), nymph, and adult. The completion of this life-cycle may take from 6 weeks to 2 years. All stages except the egg, feed on the blood of vertebrates, mostly mammals. The female becomes greatly distended while feeding (a period of usually 5 to 10 days). Copulation takes place on the host while the female is feeding. After copulation, the female takes more blood, drops to the ground, finds a sheltered place, and in a few days deposits a gelatinous mass of eggs that may number into the thousands. This oviposition may take several days, after which the female dies. Under favorable conditions, the eggs hatch in about a month; but during cold weather, they may not hatch for several months. Some days after hatching, the six-legged larvae (also called "seed ticks") climb weeds, stems, or twigs or walk over the ground to find a suitable host such as a small mammal. They engorge on the blood of the host, drop to the ground, and molt into the nymphal stage. The nymph then awaits an animal, feeds, drops to the ground, and molts into the adult which then repeats the cycle.



Figure 1. Adult hard tick

b. Soft Ticks (Family Argasidae): The soft ticks are round or oval and lack a shield on the back; their skin is leathery, wrinkled, and tough. In adults, the mouthparts are not visible from above (Fig.2). Their development is similar to hard ticks but they may have two to several larval and nymphal stages (Fig. 3). Soft ticks are secretive in their habits, feeding at night and concealing themselves during the day in crevices or cracks near the nest or roost of the host. The female feeds and lays eggs alternately over a relatively long period. Thus, a single soft tick may feed, on several different hosts during one lifetime, which sharply increases its disease-carrying potential. Many soft ticks feed on birds and reptiles, although others prefer mammals as a host.

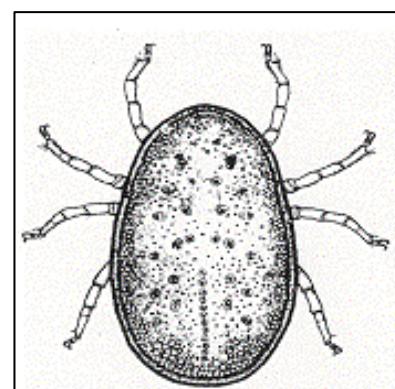


Figure 1. Soft tick

Scrub Typhus

Scrub typhus, also known as bush typhus, is a disease caused by a bacteria called *Orientia tsutsugamushi*. Scrub typhus is spread to people through bites of infected chiggers (larval mites).

History and geographical distribution: Historical and geographical records indicate that the earliest descriptions of scrub typhus symptoms were documented in Chinese literature referred to the vector of scrub typhus as "red insects" commonly found along riverbanks. Following China's documentation, Japan reported cases of scrub typhus in 1899, contributing to an early understanding of the disease.

Global scenario: The disease evolved in the early tsutsugamushi triangle covering the borders of Japan in the northeast, **Pakistan and Afghanistan** in the northwest, **Australia** in the south, and **Islands in the western Pacific Ocean** including Papua New Guinea to the east. Hence the bacteria was named *Orientia tsutsugamushi*. Although the disease was endemic to the tsutsugamushi triangle, now it has spread across the globe with diverse climates and environments that support the chigger mite populations responsible for spreading *Orientia tsutsugamushi*. Recently,

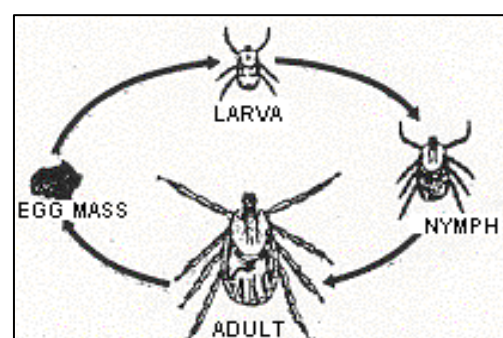


Figure 3. Life cycle of the tick

rickettsioses have been an emerging disease along the Thai-Myanmar border. There are reports of the emergence of scrub typhus in the Maldives and Micronesia.

Indian scenario: In India, during the World War II, the cases of scrub typhus were seen emerging among military personnel's, and in 1965, the disease was noted along the Indo-Burma and Assam borders during the Indo-Pak war (Kelly et al., 2009). Now, the disease has been reported from Rajasthan, Jammu & Kashmir, Vellore, Sikkim, Darjeeling, Nagaland & Manipur, Mizoram, Tamil Nadu, Maharashtra, Kerala, Himachal Pradesh, Meghalaya.

Incubation Period: 1 – 3 weeks.

Clinical picture: A Papule develops at the site of inoculation. The papule ulcerates and eventually heals with development of a black eschar. General symptoms are sudden fever ($>40^{\circ}\text{C}$ [104°F]) with relative bradycardia, severe headache, apathy, myalgia, generalized lymphadenopathy, photophobia and a dry cough. Approximately one week later, a spotted and then maculopapular rash appears first on the trunk and then on the extremities and blanches within a few days. Complications are interstitial pneumonia (30 to 65% of cases), meningoencephalitis and myocarditis. Symptoms generally disappear after two weeks even without treatment. In severe cases with pneumonia and myocarditis, the mortality rate may reach 30%.

Mites

Mites are minute animals. Most species are barely visible to the naked eye. They are found virtually worldwide. There are over 29,000 species of mites, but only a few of them attack humans. They are medically important because certain species transmit scrub typhus (southwest Pacific and the Orient). In attacking a person, various species of mites cause conditions ranging from mild irritation to severe forms of dermatitis.

a. **The Human Itch Mite, *Sarcoptes scabiei*** (Fig.4): This mite causes a disease of the skin known as scabies or the "seven-year itch." The life-cycle consists of egg, larva, nymph, and adult, with the female undergoing two nymphal stages. The mature female burrows into a person's skin, particularly at the hands and wrists, and other areas of the body where the skin creases or folds. As she burrows, she deposits eggs at a rate of about two per day. Egg laying continues for about 4 to 5 weeks, and a single female lays an average of 40 to 50 eggs. Eggs generally hatch in 3 to 4 days, and the larvae migrate from the maternal burrow to form new burrows nearby.

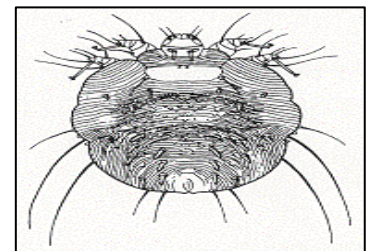


Figure 4. Adult human itch mite

After about 3 days, larvae molt in the burrows to become nymphs. Total development time for females averages 14 to 17 days. After reaching maturity, mating occurs either in the burrows of virgin females or on the surface of the skin after which the fertilized female repeats the process of burrowing and egg laying.

Chiggers (Trombiculid Mites): This is the common chigger or "red bug." Its bite causes an intense itching and infection can result from scratching the bites. In the Asiatic-Pacific area, certain species of this mite are vectors of scrub typhus. The life cycle of the trombiculid mite is very complex. Females lay eggs singly in the soil. In about 2 weeks, the six-legged larva hatches and crawls into nearby vegetation or along the ground in search of a host. Many species feed on a wide variety of vertebrate hosts including reptiles, rodents, large mammals, and humans. The larva feeds at the surface of the host skin, usually requiring 1 to 3 or more days for engorgement. Then the fully fed larva drops off the host, burrows into soil, and molts to an eight-legged nymph. The nymph and later the adult feed on immature stages of other arthropods, frequently the eggs of grasshoppers. Chigger larvae, the only medically important stage, do not disperse far from the point they hatched in their search for a host. Thus, they tend to be much clumped in distribution, forming what are referred to as "chigger islands," in areas of tall grass, weeds, and brushy vegetation. Mites infect man, horse, goat, sheep, cattle, dog etc. They are very tiny, about 0.1 mm, oval in shape and pale to greyish in colour. Adult possess 8 stubby legs while larvae are 6-legged. Eyes are absent. Mouth parts are piercing and sucking type. Some species burrow in skin and make galleries or wounds which causes intense itching. Later, fluid-filled pimples are formed which are covered with scabs. These

contain abundant mites and are the chief sources of infection by contact. Host may lose hairs due to infection.

Mites are very small, ranging from 0.5 to 2.0 mm in length; there are thousands of species, of which many live on animals. Like ticks, they have eight legs and a body with little or no segmentation. The mites undergoes four stages of Egg, larvae, nymph, adults in its life cycle, with only the larval chiggers infected with the bacteria *Orientia tsutsugamushi* act as vectors (Fig. 5)

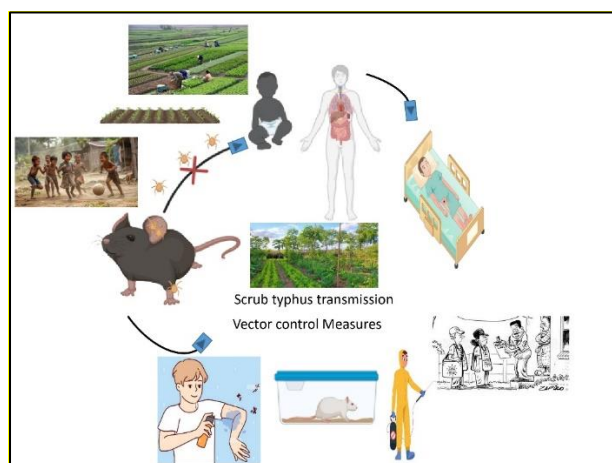


Figure 5. Mites from rodents affecting humans and vector control measures

Distribution: Mites have a very patchy distribution over small areas because of their special requirements. The nymphs and adults need certain soil conditions for their survival and development while the larvae require host animals, such as wild rats, other small rodents and birds. Suitable habitats are found in grassy fields, shrubby areas, forests, abandoned rice fields and cleared forests. The mites are also found in parks, gardens, lawns and moist areas alongside lakes and streams. The larvae wait on leaves or dry grass stems until an animal or human passes by. People usually become infested after walking or standing in mite-infested areas. Bamboo bushes are favoured by the mites in the tropics and subtropics.

Prevention and Control of Tick-borne and Mite borne Diseases

1. Community Awareness

- Awareness should be provided among the community, especially in endemic areas regarding tick and mite borne diseases. A variety of agencies can be contacted to obtain information or speakers for the training of medical, pest management, or other personnel. Additional educational methods include: making brochures, pamphlets and fact sheets available for in-processing personnel; publishing periodic notices in the installation newspaper or plan of the day, particularly warm months and the fall hunting season; and posting warning signs in tick-infested woods or other areas frequented by troops, hunters or hikers.

2. Personal Protection

- Proper clothing should be used to limit access of ticks to the skin, thereby helping to prevent bites.
- Pants should be tucked into the boots or socks, and the shirt should be tucked into the pants.
- Sitting or lying on scrubby areas should be avoided.
- Hanging clothes on scrubs or trees should be avoided.
- Regular disinfesting of pets should be done.
- Long sleeves will help, and a hat will be useful if crouching or crawling in bushes or undergrowth. Light-coloured clothing should be used to make ticks much easier to detect.
- Repellents like Deet 33% (N, N-diethyl-m-toluamide or N,N-diethyl-3-methylbenzamide), permethrin should be used to prevent tick bites. These repellents provide protection for up to 12

hours, depending on environmental conditions. It should be applied in a thin film over exposed skin surfaces, according to label directions.

- Permethrin should be applied to the lower pant legs, crotch, waistband, shirt sleeves, collar, front placket and lower edge of shirt never to skin.
- Permethrin aerosol contains 0.5% permethrin should be sprayed liberally, to the point of dampness, over the entire outside surface of the uniform.

3. Environmental Management

- Where acceptable, clearing of edge habitats by leaf litter removal, mechanical brush control, and mowing or burning vegetation are effective means of tick and mites control in residential areas and certain recreational areas.
- Removal of low-growing vegetation and brush eliminates the structural support that ticks need to contact hosts, thereby reducing the incidence of tick attachment.
- Removing leaf litter and underbrush also eliminates tick habitats and reduces the density of small mammal hosts, like deer mice and meadow voles.
- Mowing lawns and other grassy areas to less than 6 inches (16 cm) greatly reduces the potential for human-tick contact.
- When environmentally acceptable, controlled burning has been shown to reduce tick abundance for six months to one year.
- Another habitat modification technique is to thin early successional shrubs and grasses in early to mid-fall, stressing the overwintering tick population and reducing survivability. This should be done late enough in the season that regrowth does not occur.

4. Chemical control

- Use of an acaricide, formulation (e.g., dust, granule, emulsifiable concentrate) is one of the primary considerations in selecting the type of equipment that will be used to apply an acaricide. This method is not feasible in controlling ticks.
- It is more efficient to allow livestock to collect ticks and then kill the parasites on the host, rather than to apply acaricides to pastures.

Area wide application of acaricides (chemicals that will kill ticks and mites) to control tick habitats (e.g. Leaf litter and brush). Applying acaricides to rodents by using rodent baited tubes, boxes and feeding stations in the areas where these pathogens are endemic. Essentially involve clearing of vegetation allowing the area to dry for a few weeks and then being sprayed with malathion specially when outdoor camping of troops etc. are to be chiggers bites repellents can be used before venturing for activity inside forest areas.

Control measures used to be spraying with malathion dust (now it is not in the approved list of Central Insecticide Board) within a radius of approximately 100 meters around the monkey death spot. Alternate insecticides need to be considered. Spraying should also be done along the forest tracks used by the villagers. if the human dwellings are adjacent to the infested forest spraying should be done in the vicinity of the dwelling also. Wherever possible people should be advised to use tick repellents before going out in the forest. Long term measures include control of *Haemaphysalis* ticks on cattle populations in the area which should be explored and propagated.

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EPIDEMIOLOGY OF LEPTOSPIROSIS: A NEGLECTED PUBLIC HEALTH CONCERN!

Mansi Rajendra Malik¹, Sridharan Subramanian²

¹Tata Institute for Genetics and Society, GKVK Post, Bellary Road, Bengaluru, Karnataka, 560065, India.

²Formerly Directorate of Public Health and Preventive Medicine, Chennai, Tamil Nadu, India.

Email: mansi.malik@tigs.res.in , roadakka@gmail.com

Abstract

Leptospirosis is a perplexing public health concern causing over a million human infections annually. It is a spirochete and is considered an emerging zoonosis due to the transmission of infection from animals to humans and the resulting human encroachment into the forest and wildlife reserves. There are 20 species of *Leptospira* categorised into 24 serogroups with more than 300 serovars. Paddy farming, livestock farming, and exposure to waterlogged conditions are the common modes of exposure to the infection.

Prevalence of Leptospirosis remains underestimated due to lack of awareness in the public and the pathophysiology of the disease. *Leptospira* infection presents acute febrile illnesses that overlap with other viral, parasitic and bacterial illnesses. Co-infection with various other unrelated diseases requires accurate diagnostics and surveillance to estimate the incidence and prevalence of leptospirosis in endemic regions. Misdiagnosis of leptospirosis could lead to Weil's disease, a more severe form of systemic complications. Floods and heavy rain appeared to be among the most important drivers of this infection. Exposure to rodents as well as behaviour and sanitation are often important risk factors indicating potential areas for interventions. Leptospirosis is routinely diagnosed serologically and microscopically, however there is a lack of early and accurate diagnostic solutions that could enable prompt intervention and further mitigation of the disease.

In this chapter, we are trying to understand the pathology of leptospirosis, along with its taxonomy, morphology and transmission dynamics. We have tried to compile the available interventions for disease management, however, emphasizing the need for effective public health surveillance for prevention, and control of Leptospirosis.

Keywords: Leptospirosis, Spirochete, Outer membrane protein, Diagnosis, PCR, Surveillance.

Introduction

Leptospirosis is an important yet neglected zoonotic bacterial disease that occurs in diverse epidemiological settings but imparts its greatest burden on resource-poor populations (Victoriano et al., 2009). Leptospirosis, caused by a spirochete of genus *Leptospira*, is considered the most widespread zoonosis in the world. It has a global distribution with a higher incidence in the tropics and subtropics, ranging from 10 to 100 human cases per 100,000 individuals (Sethi et al., 2010). It is one of the most widespread yet neglected zoonoses, with most reports from South America, the Caribbean, and South Asia. An estimate suggests that a total of 58,900 deaths are reported every year (Bharti et al., 2003). Leptospirosis affects risk groups exposed to animal reservoirs or contaminated environments, sewage workers, military personnel, and individuals partaking in water sports and recreation. However, leptospirosis has a broader health impact as a disease affecting subsistence farmers and, cash croppers, from tropical regions of the world (Barragan, Olivas, Keim, & Pearson, 2017).

The spectrum of clinical presentations of human leptospirosis ranges from asymptomatic to fatal. Most of infections are subclinical or result in mild self-limiting systemic illness. However, the case fatality rate in severe leptospirosis may be as high as 20% (Rajapakse, 2022).

Leptospirosis has a non-peculiar clinical presentation that mimics the signs and symptoms of many other diseases, such as dengue fever, flu and malaria. There is a lack of early, prompt and accurate diagnosis to estimate the incidence and prevalence of leptospirosis in endemic regions.

The spectrum of human disease caused by *Leptospira* spp, ranges from subclinical infection to a severe syndrome of multiorgan infection with high mortality(Rajapakse, 2022). The icteric leptospirosis with renal failure, was first reported over a century ago by Adolf Weil in Heidelberg(Adler, 2015). However, an apparently identical syndrome occurring in sewer workers was described several years earlier. Leptospirosis was certainly recognized as an occupational hazard of rice harvesting in ancient China, and Japan(Victoriano et al., 2009).

In Europe, Australia Leptospirosis was thought to be understood and was recognized amongst febrile illness and particular occupations, giving rise to syndromes such as cane-cutter's disease, swine-herd's disease, and Schlammbieber (mud fever) well before the common aetiology was recognized and identified(Praharee, 2022).

Increase in the incidence of Leptospirosis has been observed after rainfall-related flooding. Where the contaminated animal urine on soil mixes easily with water during floods, making a large population, especially those living in low-lying areas, susceptible to leptospirosis. Most cases of Leptospirosis in India have been reported from the coastal belt (Gujarat, Tamil Nadu, Maharashtra, Kerala, Karnataka, Andaman and Nicobar Islands). Since most of these regions have deltas of large rivers, they are prone to frequent flooding. The population residing in the riverine area are believed to have contributed to these infectious outbreaks. Another reason for the increased incidence in the southern coastal areas of the Indian Sub-continent could be due to the increased survival of *Leptospira* in warm and humid environments(Gupta, Wilson, & Ravindra, 2023a)

Taxonomy

Around late 1980s, genus *Leptospira* was divided into two species, *L. interrogans*, comprising all pathogenic strains, and *L. biflexa*, containing the saprophytic strains isolated from the environment. Both *L. interrogans* and *L. biflexa* are further divided into numerous serovars defined by agglutination after cross-absorption with homologous antigen. Over 60 serovars of *L. biflexa* have been recorded. Within the species *L. interrogans* over 200 serovars are recognized; additional serovars have been isolated but have yet to be validly published. Serovars that are antigenically related have traditionally been grouped into serogroups(Rajapakse, 2022). While serogroups have no taxonomic standing, they have proved useful for epidemiological understanding. The various serogroups of *L. interrogans* are Icterohaemorrhagiae, Canicola, Pomona, Australis, Autumnalis, Pyrogenes, Grippotyphosa, Djasiman, Hebdomadis, Sejroe, Bataviae, Ranarum, Louisiana, Mini, Sarmin(Gupta et al., 2023a).

Morphology

Leptospira is a spirochete, is tightly coiled with hooked ends and displays high motility in the longitudinal axis. They are $\cong 6\text{-}20\text{ }\mu\text{m}$ long and $0.1\text{ }\mu\text{m}$ in diameter. *Leptospira* are fragile and observed only in a phase-contrast microscope or darkfield microscope. They are obligate aerobes that can grow at optimum temperatures of 28°C to 32°C and at a pH range of 6.8 to 7.4. The typical morphology, rotational movement, unique coiled-shaped body, and hooks are the structural characteristics that differentiate *Leptospira* from other organisms(Urbanskas, Karvelienė, & Radzijeuskaja, 2022).

Spirochetes comprise a distinctive double-membrane structure with cytoplasmic membrane and peptidoglycan cell wall, being closely associated and covered by an outer membrane(Gupta et al., 2023a). *Leptospira* are classified based on lipopolysaccharide (LPS), the major antigen for bacteria in the outer membrane, which shows structural heterogeneity based on gene differences. The genome

of *Leptospira* is made up of two circular chromosomes, and it is more prominent when compared with genomes of other spirochetes, such as *Treponema* spp. and *Borrelia* spp., indicating the ability of *Leptospira* species to thrive in different environments(Samrot et al., 2021).

Transmission

Leptospira is transmitted through contaminated water or animal urine that enters humans through open wounds or inhalation of infected samples The bacteria inhabit in the kidney of animals, temporary carriers (cattle), and permanent carriers (rodents)(Fig 1). Endemic or epidemic episodes of *leptospiral* infection occur in regions with high rainfall or natural disasters(Samrot et al., 2021).

The corkscrew-like motility seen in *Leptospira*, allows them to move through more viscous barriers and host connective tissues, contributing to their highly invasive nature. Animals such as rodents, pigs, horses, cattle, dogs and various wild animals such as opossums, deers and pinnipeds are carriers of *Leptospira*(Praharee, 2022).

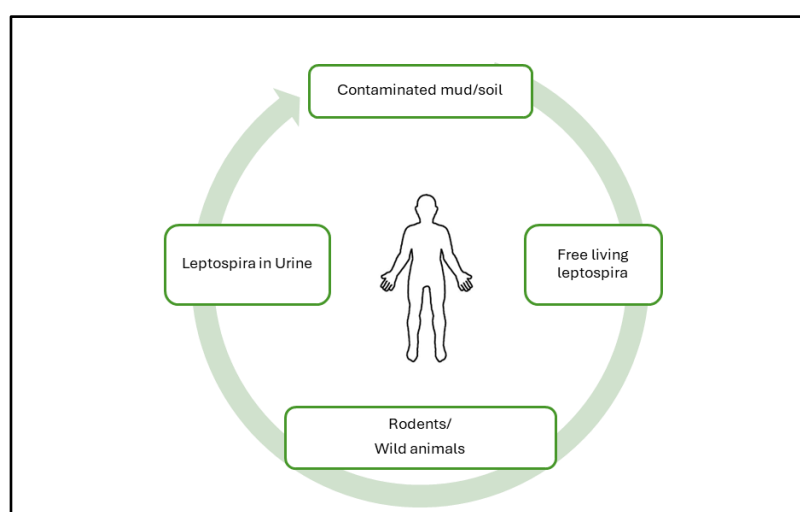


Figure 1. Cyclical representation of transmission of *Leptospirosis*

Humans exposed to such environments are more prone to infection. *Leptospira* can live in a wet environment for several months, depending on suitable environmental conditions, including soils, mud, streams, and rivers, or in organs and tissues of live or dead animals. The majority of mammalian species naturally carry pathogenic leptospires, which are released in the urine of infected animals, including rodents and farm animals, even though they do not show symptoms of infection(Rao, Gupta, Bhalla, & Agarwal, 2003). *Leptospira* has the tendency to inflict chronic renal diseases onto animal carriers. These bacteria tend to accumulate at the proximal convoluted tubules of their host, colonizing and multiplying rapidly from infection and thus are released into the environment via urination(Victoriano et al., 2009).

As a result, leptospirosis is a serious occupational disease that affects a wide range of people, including farmers, slaughterhouse workers, veterinarians, rodent catchers, sewer workers, and others. They can sometimes enter the human body through inhaling urine droplets or drinking water (only when the person who drinks the contaminated water has buccal ulcer thereby creating discontinuous skin inside the mouth) but are rarely passed from one person to another through sexual contact, transplacental from the mother to the baby, or breast milk. Overall, the tendency for each transmission route to occur depends upon the demographical, geographical, agricultural and livestock factors of a particular population(Gupta et al., 2023a).

Climate change and its associated environmental shifts can affect the degree of transmission of leptospirosis. Surveillance for leptospirosis is important for early detection of cases because early treatment is crucial to decrease morbidity and mortality. Surveillance facilitating the assessment of the

incidence, geographic distribution, trends, and risk factors associated with human cases and the identification of outbreaks and potential new animal reservoirs(Bharti et al., 2003).

Symptoms and Diagnosis

Early, rapid and accurate diagnosis of Leptospirosis is challenging for physicians. Mild stages of leptospirosis usually get misdiagnosed as flu or viral fever, however, most of the time, diagnosis is performed only when Weil's disease is set in, marked by pulmonary haemorrhage, Jaundice or renal failure. The initial non-confirmatory symptoms recorded across the literature are fever, cephalalgia, prostration, haemorrhage, Pleural effusion, hepatomegaly and splenomegaly(Gupta, Wilson, & Ravindra, 2023b). This disease can result in multiorgan involvement leading to kidney lesions, interstitial nephritis, uraemia, vascular injury as well as meningitis. Leptospirosis in pregnant women can cause congenital leptospirosis, stillbirth, foetal cardiotocography and foetal death. Post-recovery symptoms are also observed in some patients such as persistent fatigue, mood swings, headache, paresis and depression(Rajapakse, 2022).

The currently established and routinely used diagnostic tools developed for leptospirosis detection include serological methods (Fig 2), such as microscopic agglutination test (MAT), solid phase assay, enzyme-linked immunosorbent assay (ELISA) and indirect haemagglutination assay, direct diagnostic methods (microscopy, particularly phase contrast or dark field microscopy, histochemical staining and immunostaining), culture methods and molecular techniques such as nested polymerase chain reaction (PCR), real-time q PCR, and whole genome sequencing methods(Sykes, Reagan, Nally, Galloway, & Haake, 2022).

Indirect Diagnostic methods

Serological studies are based on detecting specific antibodies against various leptospiral antigens. Usually, serum samples are used for such assays. Serology is preferred over traditional bacterial culture methods, as the latter is time-consuming, given that leptospires have an extended doubling time in culture(Praharee, 2022).

MAT assays have been widely used for detecting leptospirosis through detection of antibodies produced against the antigens of *Leptospira* serovars. This technique utilizes live bacterial cultures and is routinely performed by incubating patient's serum with various serovars of *Leptospira*. A four-fold rise of MAT antibody titre determines *Leptospira* infection. This test is regarded as a gold standard for detecting leptospirosis due to higher sensitivity. However, this is not advisable for higher number of samples and is not cost effective(Samrot et al., 2021).

Other such test is Microsphere immunoassay (MIA) which depends upon live cultures and subjective interpretation of results. It relies heavily on live cultures and subjective interpretation of results. This technique cannot differentiate between classes of anti-leptospiral antibodies. MIA uses the Luminex xMap technology, and can differentiate between IgM and IgG antibodies against *Leptospira*. Similar to MAT, MIA is expensive and requires significant cost reduction for routine implementation(Samrot et al., 2021).

ELISA and Indirect Haemagglutination Assay (IHA) are other popular assays used to determine the IgM and IgG antibodies produced in the patient post leptospirosis infection. The IgM and IgG antibodies, however, are produced only 5-7 days post, *Leptospira* infection and hence these two methods are not ideal for early diagnosis and management of the disease.

One of the most sensitive, yet expensive and technically demanding methods of *Leptospira* detection is flow cytometry, wherein diagnosis of leptospirosis can be done by assessing the light scattering patterns after carrying out the agglutination reaction between the antigen and antibody of a specific serovar(Sethi et al., 2010).

Direct Diagnostic Methods

Direct Diagnosis of *Leptospira* can be done by microscopy wherein *Leptospira*'s can be easily detected under dark field microscopy as thin, coiled, motile organisms in blood and urine samples of patients with

leptospirosis. However, the positivity of dark field microscopy decreases from 100% to 90.9% as the duration of infection increases for more than 8 days (Urbanskas et al., 2022).

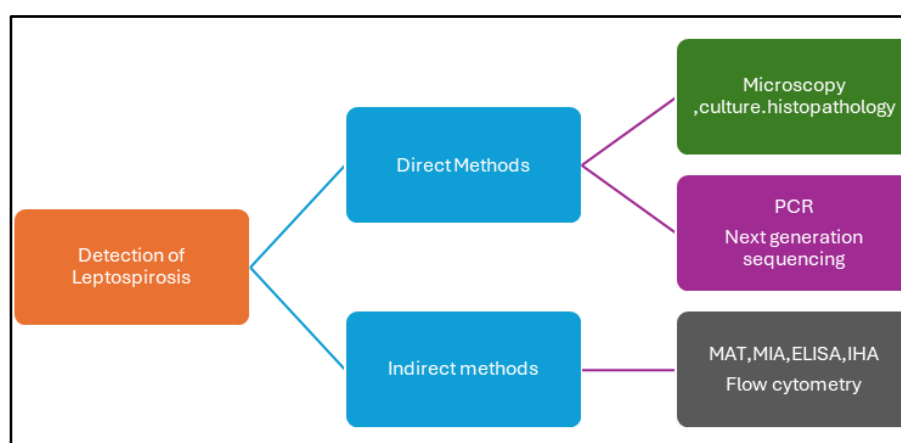


Figure 2. Representation of detection methods for *Leptospira* infection.

Another way to detect the disease is histopathology, wherein formalin fixed, embedded tissue samples can be stained and using microscopy the presence of the bacteria can be reported. The technique revolves around the use of enzymatic or metallic labels on secondary antibodies. (Budihal & Perwez, 2013).

Bacterial culture methods are routine employed by microbiological laboratories. Usually urine and/or blood sample, are inoculated onto a culture flask containing fluid media. Oleic acid-albumin media of EMJH is commonly used in microbiological labs. Antibiotics such as rifampicin, neomycin, actidione are included in the culture media, to enable selective isolation of bacteria from clinical samples. Culturing is time-consuming as the doubling time of leptospirosis is 6-8 hrs and hence this method requires a lot of technical expertise and accuracy to be used as a routine diagnostic assay to detect leptospirosis (Costa et al., 2015).

Realtime Polymerase chain reaction is one of the most sensitive, specific and confirmatory methods employed to detect leptospirosis as early as day 1 of infection from clinical samples.

This can be performed by developing real-time multiplex PCR assays. While the *lipL32* gene is the most common targets used in PCR assays, numerous other gene targets are used to detect clinically relevant *Leptospira*, including *secY*, *flaB*, *rrs*, *lig* genes, *rrl*, and *lipL41* (Gupta et al., 2023a). Multiplex PCR is done by using two sets of primers instead of one in order to increase the specificity of DNA binding and amplification. Nested PCR is one such method which also helps in detecting more specific and sensitive DNA sites using additional sets of primers (Sykes et al., 2022). Finally, whole genome sequencing is an expensive method and requires technical expertise to analyze the data obtained. However this is the most accurate method to identify the serovars of leptospirosis from clinical samples (Guglielmini et al., 2019).

Diagnosis during an Outbreak: During a suspected outbreak of leptospirosis, the field public health staff need a rapid diagnostic test which gives the result immediately and serves as a guide in control of the disease. For this purpose, Lepto Dri Dot card agglutination test developed by the Dutch Royal Tropical Institute in Amsterdam is used. The assay is based on the binding of *Leptospira* specific antibodies in patient's serum to the broadly reactive antigen coated on latex particles leading to a fine agglutination. Results can be obtained within the 60s. The test does not require special storage or sophisticated equipment and can be performed by relatively low-skilled personnel. However, the sensitivity of the test is only about 80%, hence the positive serum may be subject to IgM ELISA or MAT test in the laboratory.

Treatment, Prevention and Management

Most of the clinical cases of Leptospirosis are mild and are often misdiagnosed due to the presentation of acute febrile illnesses that overlap with other vector-borne and infectious diseases. Oral dosage of Doxycycline is the most common antibiotic administered either orally or intravenously to patients.

However other antibiotics such as Amoxicillin and Azithromycin are also used at 500-100mg dosage per day for 5-7 days, depending upon the severity of the infection. Doxycycline is also used as a prophylaxis for travellers and people engaged in farming, livestock and water sport-related activities in the endemic regions(Charan, Saxena, Mulla, & Yadav, 2013)

The use of Probiotics, not just in preventing dysbiosis in patients on antibiotics, but also as a potential therapeutic option for leptospirosis has been explored (Fig 3).

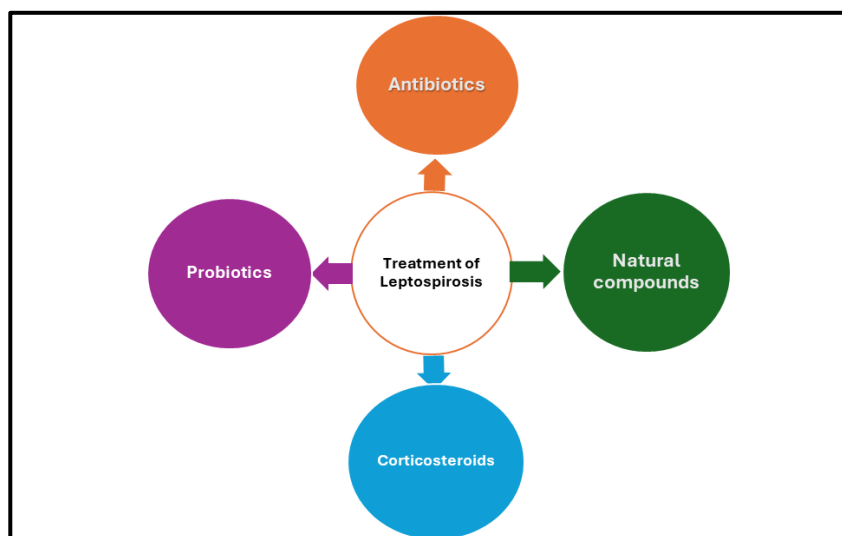


Figure 3. Schematic overview of potential treatment options for *Leptospirosis*.

Various studies have demonstrated the potential of probiotics in mitigating *Leptospira interrogans* pathogenesis, due to immunomodulatory effects. In an experimental model oral administration with live *Lactobacillus plantarum* restored normal body weight, mitigated histopathological signs of disease, and modulated the inflammatory response(Samrot et al., 2021).

There are multiple reports of using corticosteroids and natural plant-based products for alleviation of pain and related symptoms in treating leptospirosis(Samrot et al., 2021).

Vaccination

The development of a universal vaccine against leptospirosis is a challenge due to the wide antigenic diversity of pathogenic *Leptospira* spp. More than 300 pathogenic serovars have been clustered into 24 serogroups throughout the world based on the Lipopolysaccharide (LPS). LPS plays a key role in virulence and varies considerably between different serovars. Alterations in the lipid(O and A) antigens of *Leptospira* have a profound effect on its recognition by the pathogen recognition receptors and consequently the innate immune response(Maia et al., 2022). *Leptospira* have evolved ways to escape the host immune system. Pathogenic *Leptospira*'s can effectively translocate through cell membranes at a greater rate than that of non-pathogenic *Leptospira* for rapid dissemination into the host blood stream and vital organs(Wang, Jin, & Węgrzyn, 2007). Virulent *Leptospira* can rapidly enter kidney fibroblasts and induce apoptosis, making it challenging for immunologists to develop an effective and safe vaccine against the bacteria.

There are numerous studies on leptospirosis vaccines, focused on the bacterial LPS, motility, outer-membrane proteins (OMPs) and lipoproteins. There are a few vaccines that are commercially available against Leptospirosis, comprising of the whole inactivated *Leptospira* (bacterin), and are permitted for human use only in some countries such as China, Japan, France, and Cuba(Xu & Ye, 2017). These vaccines are known to only induce serovar-specific protection and short-term immunity. Also, multiple serious side effects, have been reported for these vaccines, thus questioning the reliability of their long-term use and implementation. Multiple approaches such as DNA vaccines, Live vaccines, multi-epitope vaccines and the use of Toll-Like and Nod-Like Receptor Agonists have been tried for the development of effective vaccines against leptospirosis(Rao et al., 2003).

The advent of the genomics era introduced the world to “second-generation vaccines. Despite all the remarkable advances achieved by various strategies to develop vaccines, only partial response was observed to be elicited against challenge and most of them failed to prevent renal colonization. The subunit vaccines focus not only on the discovery of new antigens, however, parameters such as the use of multicomponent antigens, but use of adjuvants is also ignored, which in turn could direct to humoral and cellular immune response pathways (Samrot et al., 2021). The third-generation DNA and RNA vaccines, courtesy to the advances in the vaccinology research, approaches to aim to vaccinate a patient with genetic material that encodes the target antigen. DNA vaccines failed in the previously reported studies; however, implementation of mRNA vaccines have been considered as a promising new era in vaccinology. The use of exogenous single-stranded mRNA for simulation of the innate immune system is hypothesized to elicit robust B and T cell responses, thus making it an attractive approach for the development of an efficient vaccine against *Leptospira* (Wang et al., 2007).

Public Health Challenges for Prevention and Control

Early diagnosis, treatment and prevention of leptospirosis is a challenge; however, it can help in prompt clinical mitigation of the disease. The most important public health challenge is prompt and accurate surveillance of leptospirosis and the respective serovars from the environment, carrier hosts and suspected clinical samples presenting acute febrile illnesses. Surveillance shall be immensely useful for the early identification of plausible outbreaks, where mass prophylaxis could be enabled in low-resource settings. The burden of leptospirosis has been difficult to estimate due to the lack of reliable epidemiological data from many endemic countries (Barragan et al., 2017). The incidence of leptospirosis mostly remains under-estimated owing to the poor surveillance methods that rely on limitations posed by routinely used diagnostic methods and on the clinicians’ ability to detect and timely treat the disease (Costa et al., 2015).

The involvement of officials from local municipal corporations, sewage boards, water or state irrigation departments, forest departments, veterinarians, clinicians, epidemiologists and scientists is critical to ensure the mapping of the incidence and prevalence of leptospirosis in each geographical niche (Budihal & Perwez, 2013).

To monitor *Leptospira* status, all vulnerable populations, such as sewerage workers, veterinarians, irrigation and forest department personnel, and agricultural workers, during Agri-season may be screened regularly. Treatment may be started immediately in an outbreak based on the results of the Lepto Dry Dot rapid test. All the cattle sheds in the outbreak area/village may be disinfected using a bleaching powder–lime combination in a 1:3 ratio along with the rat runs/street borders. All the public drinking water tanks may be chlorinated to ensure safe water. As a part of BCC activities in the outbreak area, the public may be advised to walk in the streets or open areas only with covered footwear.

Molecular and Genomic surveillance shall further enable understanding of the prevalent serovars of pathogenic, intermediate and non-pathogenic strains of *Leptospira* in the environment. This shall not only enable appropriate policy decisions but also aid in mapping out the epidemiological landscape of leptospirosis in the city, state or country, for effective containment, prevention and control (Guglielmini et al., 2019).

Summary

Leptospirosis is often easily overlooked in the environment as well as in context to the clinical cases, where long-lasting fever is the most common symptom presentation. A One Health approach to the epidemiology, management, surveillance, and control of leptospirosis should be employed, that relies on accessible and accurate diagnostics having human application and along with companion animals and livestock. The environmental route of transmission of leptospirosis is an important aspect of routine surveillance, early diagnosis, and prompt mitigation of the disease in endemic regions of the world. Environmental and clinical surveillance intertwined with public health epidemiology needs to be practised effectively to determine the incidence and prevalence of leptospirosis in vertebrates and as well

as invertebrate hosts. Such measures could help identify vectors and/or host reservoirs responsible for a plausible endemic situation.

In summary, it is crucial to understand the complex interplay of multiple factors that could potentially lead to an outbreak of leptospirosis. It is also important to implement accurate and early diagnostic measures to identify and survey leptospirosis in the environment, in animals, and in humans and work towards prevention and control of this much ignored yet very important public health concern.

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AN INSIGHT INTO CRIMEAN-CONGO HAEMORRHAGIC FEVER: CHALLENGES AND PERSPECTIVES

Sathya Jeevitha B and Jayalakshmi Krishnan*

Vector Biology Research Laboratory, Department of Biotechnology,
Central University of Tamil Nadu, Thiruvavur, India

*Corresponding Author Email: jayalakshmi@cutn.ac.in

Abstract

Crimean-Congo Haemorrhagic Fever (CCHF) is a severe vector-borne viral disease caused by the *Crimean-Congo Haemorrhagic Fever Virus* (CCHFV), a member of the *Nairoviridae* family. The virus is primarily transmitted through *Hyalomma* tick bite. CCHF manifests with nonspecific febrile symptoms such as headache, muscle pain, and gastrointestinal disturbances, which may progress to severe haemorrhagic symptoms, multiorgan failure, and fatality rates of 10% to 40%. Globally, CCHF is endemic in over 30 countries spanning Eastern Europe, Africa, the Middle East, and Asia. India has reported sporadic outbreaks since 2011, primarily in Gujarat and Rajasthan, with mortality rates consistent with global data. The pathogenesis of CCHF involves endothelial dysfunction and cytokine storms, leading to vascular leakage and severe haemorrhagic complications. Early diagnosis is challenging due to similar symptoms with other febrile illnesses, requiring molecular diagnostics like RT-PCR and serological assays. Prevention strategies emphasize tick control, personal protection, and awareness, especially for healthcare workers and individuals in endemic regions. Ribavirin is used occasionally as an antiviral agent, but effective vaccines are still under development. With an estimated 3 billion people globally at risk, coordinated vector control and global cooperation are essential to eliminate its public health burden. This chapter explores the key aspects of CCHF, including its transmission, clinical features, and prevention strategies, highlighting its global significance and the challenges in managing its impact.

Keywords: *Crimean-Congo haemorrhagic fever virus, viral haemorrhagic fever, Hyalomma ticks, zoonotic diseases, public health threats.*

1. CCHF: An Overview and Significance

Crimean-Congo Haemorrhagic Fever (CCHF) is a highly virulent viral disease with a widespread geographic distribution, covering regions such as West China, Southeast Europe, the Middle East, Africa, and Asia. The mode of transmission occurs through the bites of *Hyalomma* tick species, which act as the primary vectors, establishing a strong link to the Crimean-Congo Haemorrhagic Fever Virus (CCHFV). CCHF typically begins with flu-like symptoms, including high fever, headache, myalgia, and profound fatigue. As the disease progresses, gastrointestinal disturbances such as nausea, vomiting, and diarrhea become common. A hallmark of the disease is haemorrhaging, presenting as ecchymosis, petechiae, nosebleeds, bleeding gums, and gastrointestinal haemorrhages, revealing the disease's severity. Severe cases often involve multiorgan failure, particularly affecting the liver and kidneys, leading to a high mortality rate. The case fatality rate ranges between 10% and 30%, depending on factors such as the viral strain and the availability of medical care. Although supportive care has improved, there are currently no specific antiviral treatments for CCHF, highlighting the critical need for preventive strategies for disease elimination (Johnson 2023). This chapter highlights the public health threat imposed by CCHF and delineates the necessity of improved diagnostic tools, vector control measures, and global cooperation, to address its impact effectively.

2. CCHF: From the past

Crimean-Congo Haemorrhagic Fever (CCHF) was first identified during an outbreak in Crimea in 1944–1945, which affected approximately 200 Soviet military personnel and local farmers. Initial investigations pointed to a viral etiology when febrile episodes were induced in patients inoculated with filterable substances from the blood of infected individuals. Further evidence emerged when healthy participants developed less symptoms after being inoculated with filtered suspensions derived from *Hyalomma marginatum* nymphal ticks, confirming the probable tick-borne transmission. In 1967, a team led by a Russian virologist at the Institute of Poliomyelitis and Viral Encephalitides successfully isolated the virus using neonatal white mice, solidifying its identification.

Another pivotal discovery occurred in Congo in 1956, where a virus termed the Congo virus was isolated from the blood of a 13-year-old boy with flu-like symptoms. Subsequent research revealed antigenic similarities between the two viruses, leading to the unified designation as the Crimean-Congo Haemorrhagic Fever Virus (CCHFV). This marked the recognition of CCHF as one of the most severe tick-borne viral infections affecting humans (Whitehouse, 2008). Geographically, CCHFV is distributed across Africa, the Middle East, Europe, and Asia, with cases documented since its discovery in Crimea and the Belgian Congo. By 2000, outbreaks had been reported in numerous countries, including Pakistan, Kosovo, Senegal, Iran, Albania, Kenya, Bulgaria, and Mauritania. Some isolated cases were also identified in Greece, including a laboratory-acquired infection. Limited serological testing suggests the virus may have spread to other regions, such as Hungary, France, Portugal, Benin, and India. Many cases have reported in Bulgaria, Iran, Russia, and Turkey historically.

The spread of CCHFV to previously unaffected regions has been associated with factors such as climate change, population growth, transportation, ecological shifts, and animal migration. After a resurgence in Russia in 2004, outbreaks were noted across South, East, and West Africa, as well as Mauritania. Between 2008 and 2012, the occurrence of CCHF increased significantly, reflecting its epidemic potential. The global concern surrounding CCHF relays from its high fatality rates, risk of nosocomial transmission, challenges in treatment and prevention, and potential for widespread outbreaks (Chinikar et al. 2010; Maltezou et al. 2010; Mertens et al. 2013; Sargianou et al. 2013).

Cultural and Historical Context

CCHF has been recognized for centuries under various names, such as *Khunymuny* (bleeding of the nose), *Karakhalak* (black death), and *Khungribta* (sucking blood). In Central Asia, it has been referred as acute infectious capillarotoxicosis and Uzbekistan haemorrhagic fever. These regional names and historical records emphasize the disease's long-standing global impact and highlight its recognition in diverse cultural and historical contexts (Whitehouse, 2008). This historical overview tells the importance of understanding CCHF's origins and its persistent threat to global public health (Whitehouse 2008).

3. Causative Agent

The Crimean-Congo Haemorrhagic Fever Virus (CCHFV) is a member of the family *Bunyaviridae*, specifically within the genus *Nairovirus*. This family comprises five genera, three of which — *Phlebovirus*, *Nairovirus*, and *Hantavirus* — are associated with haemorrhagic fever in humans. Within the *Nairovirus* genus, there are seven serogroups, with the CCHF serogroup, which includes the *Hazara* virus (HAZV), having CCHFV as its prototype. While CCHFV is recognized as a potent human pathogen causing severe haemorrhagic fever, the *Hazara* virus has not been demonstrated to have similar pathogenic effects in humans (Donets et al., 1977). This distinction highlights the diversity within the *Nairovirus* genus and the unique pathogenic mechanisms of CCHFV that contribute to its high virulence and public health importance (Donets et al. 1977).

Structure of CCHFV

1. **Small (S):** Encodes the nucleocapsid protein, which packages the RNA genome.
2. **Medium (M):** Codes for glycoproteins Gn and Gc, crucial for binding to host cell receptors and viral entry.
3. **Large (L):** Encodes RNA-dependent RNA polymerase and an ovarian tumor protease domain essential for viral replication.

Replication Cycle of CCHFV

- CCHFV exhibits significant genetic diversity due to its segmented genome, which allows reassortment during coinfection. This diversity is further facilitated by the virus's ability to spread even over long distances via migratory birds and livestock transport, contributing to its adaptability and persistence in various ecological niches (Zivcec et al. 2016).

CCHFV infects a broad range of hosts, including livestock and small mammals, often asymptotically (Figure 1). The transmission occurs to humans through:

- ## Role of Ticks

- **Larvae and Nymphs:** Feed on small animals, such as hares and birds.
- **Adults:** Prefer larger hosts, including cattle, sheep, and humans.
- **Vertical Transmission:** Infected ticks transmit the virus transovarially to their offspring, ensuring the virus persists within tick populations.

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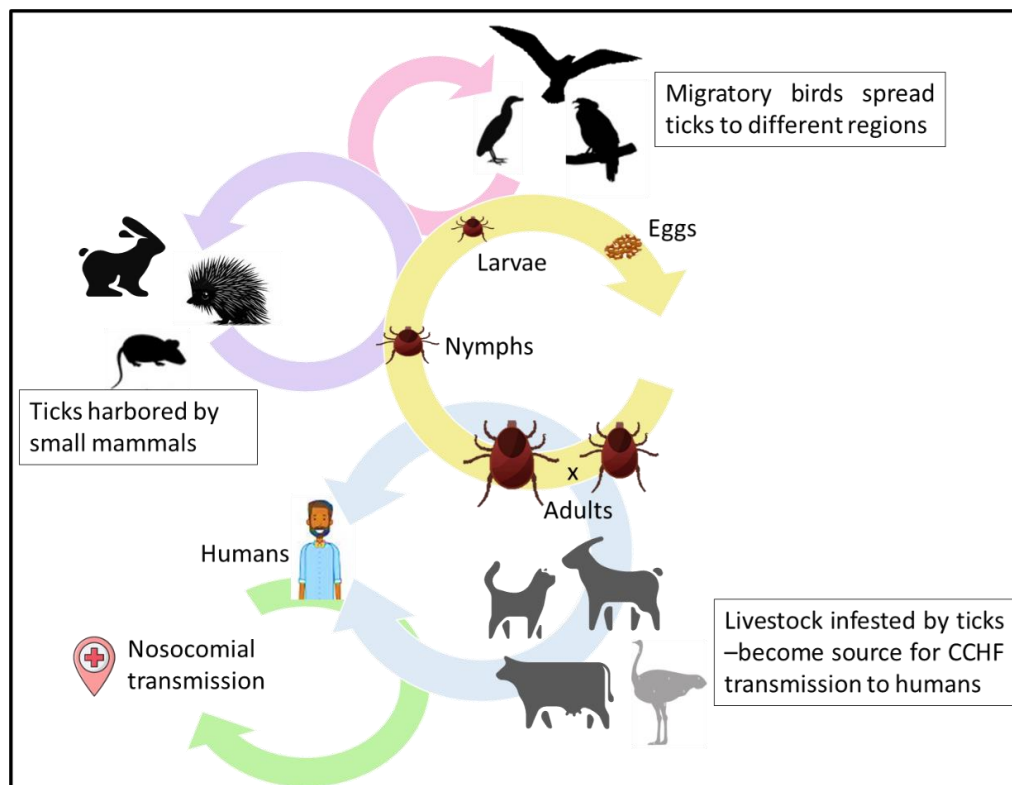


Figure 1. Transmission dynamics of CCHF

Table 1: The tick species of zoonotic importance responsible for causing CCHF include (Behzadi et al. 2021)

Tick Species	Role in CCHF Transmission	Zoonotic Importance
<i>Hyalomma marginatum</i>	Primary vector for CCHFV in Europe and Asia, especially in the Western Palearctic region.	Acts as a critical vector between wild animals, livestock, and humans; infection through tick bites or handling infected animals.
<i>Hyalomma anatolicum</i>	Transmits CCHFV in South Asia and parts of the Middle East.	Common in agricultural settings; poses risk to humans involved in livestock rearing.
<i>Hyalomma lusitanicum</i>	Plays a significant role in maintaining the virus in the environment, particularly in the Iberian Peninsula.	Acts as a bridge for CCHFV transmission to humans, especially in rural areas.
<i>Hyalomma aegyptium</i>	Harbors the virus and spreads it to mammals and reptiles, including tortoises.	Contributes to environmental maintenance of CCHFV and highlights hidden reservoirs in wildlife.
<i>Hyalomma rufipes</i>	Involved in the spread of CCHFV in Africa among wildlife, livestock, and humans.	Feeds on a wide range of animals, creating significant risk for CCHFV transmission to humans in rural areas.

7. Clinical Manifestations of CCHF

The clinical progression of CCHF typically unfolds in four stages: incubation, pre-haemorrhagic, haemorrhagic, and recovery.

1. **Incubation Stage**
 - a. Symptoms appear 1–3 days after a tick bite or 5–6 days post-exposure to infected tissues.
2. **Pre-Haemorrhagic Stage (1–7 Days)**
 - a. Symptoms include fever, muscle pain, headaches, neck and back stiffness, photophobia, nausea, vomiting, diarrhea, and abdominal discomfort.
 - b. Psychological symptoms such as disorientation, drowsiness, and mood changes may also occur.
3. **Haemorrhagic Stage (2–3 Days or Longer)**
 - a. Characterized by petechiae, ecchymoses, and mucosal bleeding. Severe cases may exhibit gastrointestinal haemorrhages, nosebleeds, and bleeding gums.
 - b. Complications include rapid kidney deterioration, liver failure, or pulmonary issues.
4. **Recovery Stage**
 - a. Begins approximately 9–10 days after symptom onset and may extend up to 20 days. Survivors often experience prolonged recovery marked by hypotension, bradycardia, neurological deficits, and hair loss.
 - b. Immunity to CCHFV generally develops following recovery (Bajpai and Nadkar 2011; Shayan et al. 2015; Hawman and Feldmann 2023).

8. Complications and Fatal outcomes

Severe cases of CCHF often result in haemorrhaging, such as gastrointestinal bleeding, haematuria, and widespread ecchymosis. Prolonged prothrombin time (PT) is a critical marker associated with fatal outcomes. The high fatality rate, ranging from 10% to 40%, proves the importance of early diagnosis and supportive care to reduce morbidity and mortality. The long-term complications of CCHF, including neurological and cardiovascular issues, shows the continued research required for delving into the disease's pathophysiology and sequelae (Shayan et al. 2015; Papa et al. 2017).

9. Diagnosis

The diagnosis of Crimean-Congo Haemorrhagic Fever (CCHF) relies on a combination of molecular, serological, and clinical approaches. Molecular methods, particularly RT-PCR, are considered the gold standard for early and accurate detection of viral RNA in blood samples. RT-PCR is highly sensitive and specific, making it indispensable for diagnosing CCHF during the acute phase of the illness. Serological assays, such as enzyme-linked immunosorbent assay (ELISA), are employed to detect IgM antibodies in the acute phase and IgG antibodies in later stages or following prior exposure. However, it may not be reliable to detect antibodies during the early stages of infection through serological tests, emphasizing the importance of molecular diagnostics during this critical period.

Hematological and biochemical tests often reveal abnormalities, including thrombocytopenia, leukopenia, elevated liver enzymes such as aspartate transaminase (AST) and alanine transaminase (ALT), and evidence of coagulopathy, such as prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT). Although virus isolation is definitive, it is rarely performed due to the high infectivity of the virus and the requirement for biosafety level 4 (BSL-4) laboratory facilities. Differential diagnosis should include other haemorrhagic fevers, dengue, malaria, leptospirosis, typhoid fever, and disseminated intravascular coagulation, as some of these overlaps with other clinical features.

Early recognition and laboratory confirmation of CCHF are significant for reducing mortality and preventing nosocomial outbreaks, particularly among healthcare professionals. Strict adherence to biosafety protocols is essential when handling suspected samples to minimize the risk of laboratory-

acquired infections. A robust diagnostic framework is key to ensuring timely detection, effective clinical management, and coordinated public health responses to this highly infectious disease (Raabe 2020).

10. Treatment

The treatment of Crimean-Congo Haemorrhagic Fever (CCHF) is primarily supportive, as there is lack of antiviral therapy. The primary objectives are to manage symptoms, prevent complications, and reduce mortality. Early recognition and timely intervention are critical, especially as the disease can vastly progress to severe haemorrhagic manifestations and multi-organ failure.

a) Supportive Care

Supportive care forms the cornerstone of CCHF management. Hospitalization is often required, particularly in high-dependency or intensive care units, to closely monitor vital signs and organ functions. Fluid and electrolyte balance must be meticulously maintained to prevent dehydration and shock. Intravenous fluids are administered judiciously to avoid fluid overload, particularly in patients with vascular leakage. Blood and blood products, plasma, and platelets, are frequently required to manage coagulopathy and haemorrhagic complications. Regular monitoring of coagulation parameters, liver and kidney function, and complete blood counts is essential to guide treatment strategies.

b) Antiviral Therapy

Ribavirin, a broad-spectrum antiviral, has shown some efficacy against CCHF and is often administered despite limited clinical trial evidence. Early initiation of ribavirin, preferably within the initial few days of symptom onset, is happened to improve outcomes. The typical oral dosage recommended regimen involves a loading dose of 30 mg/kg, followed by 15 mg/kg every four days (6 hours), and then 7.5 mg/kg every eight hours (6 days). Intravenous administration is also an option in severe cases. However, ribavirin treatment needs careful observation for side effects, such as hemolysis and anemia, which may complicate recovery. All therapy must be given by medical persons and clinicians.

c) Intensive Care for Severe Cases

Severe cases of CCHF often needs advanced life-support measures. Complications such as disseminated intravascular coagulation (DIC), acute respiratory distress syndrome (ARDS), and multi-organ failure may require renal replacement therapy, use of vasopressors to manage hemodynamic instability and mechanical ventilation. These interventions aim to stabilize patients while addressing life-threatening complications.

d) Infection Control Measures

Infection control is critical when treating CCHF patients to prevent nosocomial transmission. Patients should be isolated in facilities equipped with appropriate biosafety measures, and healthcare professionals must use personal protective equipment (PPE) when providing care. Waste and fluids from infected patients should be handled and disposed of with extreme caution to prevent secondary infections. Strict adherence to infection prevention protocols is essential to protect healthcare workers and limit the spread of the virus (de la Calle-Prieto et al. 2018).

Pathogenesis

The severity of CCHF is influenced by both host factors including immune status, and the virulence of the virus. The pathogenesis involves a series of interconnected processes that result in systemic inflammation, vascular damage, and multi-organ involvement.

a) Viral Entry and Replication

CCHF is transmitted primarily via the bite of infected *Hyalomma* ticks, accidental contact of infected animal blood or tissues, or exposure to bodily fluids of infected patients. Upon entering the host, the virus targets endothelial cells, hepatocytes, and monocytes. Viral glycoproteins facilitate attachment to cell receptors, followed by entry via endocytosis. Once inside the host cell, the viral RNA genome undergoes replication and transcription in the cytoplasm. The virus hijacks the host's cellular machinery to produce viral proteins and assemble new virions, which are subsequently released to infect neighbouring cells. Rapid replication contributes to high viral loads, which aligns with severe disease manifestations.

b) Immune Evasion and Cytokine Storm

The CCHF virus has evolved mechanisms to evade the host's innate immune defenses by suppressing interferon (IFN) production. It inhibits signaling pathways involving toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I), allowing systemic infection to establish. Concurrently, the infection triggers a cytokine storm characterized by elevated levels of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF- α), interleukins (IL-6, IL-8), and interferon-gamma (IFN- γ). This uncontrolled immune response brings about tissue damage, endothelial activation, and vascular permeability, contributing to disease severity. Elevated levels of cytokines, such as TNF- α and IL-6, are strongly associated with severe CCHF. These cytokines contribute to systemic inflammation and endothelial damage. A delayed IFN- α response enables viral propagation, improvising the disease. Immune dysregulation also activates macrophages, neutrophils, and dendritic cells, which release reactive oxygen species (ROS) and nitric oxide (NO), causing further tissue damage and vascular leakage.

c) Endothelial Dysfunction and Haemorrhagic Manifestations

Endothelial cells are a primary target of CCHFV, and their infection leads to vascular injury and increased permeability. The direct cytopathic effects of the virus, combined with the inflammatory response, compromise vascular integrity, resulting in capillary leakage, edema, and haemorrhage. Platelet dysfunction and consumption, as well as activation of coagulation pathways, further aggravate the haemorrhagic manifestations. Disseminated intravascular coagulation (DIC), characterized by widespread clot formation and depletion of clotting factors, is a common complication, leading to spontaneous bleeding.

d) Hepatic and Multi-Organ Involvement

The liver is a key target organ in CCHF. Viral replication in hepatocytes results in hepatocellular injury, as indicated by elevated levels of liver enzymes (AST and ALT). Liver dysfunction gives rise to coagulopathy by impairing the synthesis of clotting factors. Severe cases often progress to multi-organ failure, involving the kidneys (acute kidney injury), lungs (acute respiratory distress syndrome), and brain (neurological complications). This systemic involvement significantly increases the risk of fatal outcomes.

e) Neurological Complications

Though less common, CCHFV can invade the central nervous system, leading to encephalitis, meningitis, or seizures. Neuroinvasion is thought to occur through blood-brain barrier disruption caused by systemic inflammation and vascular permeability, adding to the disease's complexity.

f) Hemostasis and Coagulopathy

Haemorrhagic diathesis occurs in CCHF, resulting from thrombocytopenia, platelet dysfunction, and coagulation abnormalities. The virus activates both intrinsic and extrinsic coagulation pathways, leading to excessive thrombin generation and fibrin deposition. This hypercoagulable state transitions into consumptive coagulopathy, depleting clotting factors and causing spontaneous bleeding from mucosal surfaces, the gastrointestinal tract, and puncture sites (Bente et al. 2013).

11. Drivers of CCHF Spread

The Crimean-Congo Haemorrhagic Fever (CCHF) spread is influenced by several interconnected factors that facilitate the viral transmission through its primary vector, *Hyalomma* ticks. These drivers include environmental changes, human activities, and globalization, which together enhance the prevalence and geographic spread of the disease.

a) Climate Change

Rising global temperatures, altered precipitation patterns, and habitat modifications have significantly expanded the distribution of *Hyalomma* ticks into new regions. Warmer climates prolong tick activity periods and increase their geographic range, resulting in heightened risks of CCHF outbreaks. These environmental changes also impact livestock and wildlife interactions, creating conditions conducive to tick proliferation.

b) Agricultural Practices

Intensive livestock farming, particularly in endemic regions, has contributed to increased tick infestations. The close interaction between humans and animals in agricultural settings raises the likelihood of zoonotic transmission, making farmers, veterinarians, and slaughterhouse workers particularly vulnerable to CCHF.

c) Other globalization and urbanization practices:

The cross-border movement of livestock and people, coupled with international trade, has facilitated the introduction of *Hyalomma* ticks and the CCHF virus into previously unaffected regions. Livestock transport serves as a primary pathway for ticks to spread, while globalization accelerates the intercontinental transmission of vector populations. Encroachment into forested or rural areas for agriculture, urban development, and infrastructure expansion increases human exposure to ticks and wildlife reservoirs. These activities disturb natural habitats, forcing wildlife and ticks into closer proximity with human populations (Chanda et al. 2023).

12. Global Scenarios

CCHF outbreaks are often unpredictable and associated with significant morbidity and mortality. A few notable outbreaks and their contexts are as follows:

Turkey (2002–2019):

Turkey has reported thousands of CCHF cases from its first outbreak in 2002, making it one of the most affected countries globally. Extensive livestock farming and tick exposure in rural areas contribute to the disease's high prevalence in this region.

Pakistan (2016):

A major outbreak in Pakistan resulted in over 100 confirmed cases. This outbreak underscored the inadequacy of healthcare infrastructure in managing CCHF and highlighted the need for heightened infection control measures.

Spain (2016):

The first indigenous cases of CCHF in Western Europe were reported in Spain, marking the westernmost spread of the disease. These cases emphasize the potential for the virus to provoke in non-endemic regions.

Sudan (2008):

An outbreak in Sudan highlighted the challenges of controlling CCHF in resource-limited settings, where access to diagnostic facilities and vector control measures is limited.

Central Asia (2012):

Large outbreaks in countries like Tajikistan and Uzbekistan showed the endemic nature of CCHF in this region and the need for surveillance and control strategies (Bente et al. 2013).

13. CCHF in India

India has experienced sporadic outbreaks of CCHF, with some regions being more prone due to ecological and anthropogenic factors.

The First CCHF Outbreak in India (2011)

The first confirmed CCHF outbreak occurred in January 2011 in Gujarat. The index case involved a 32-year-old woman from Kolat village in Sanand Taluka, Ahmedabad District, who presented with high-grade fever, nausea, vomiting, and petechial rash. Her condition rapidly deteriorated, leading to haemorrhagic symptoms and death. Investigations revealed contact with livestock, and *Hyalomma* ticks were identified in the vicinity. The outbreak also led to secondary transmission, with two healthcare workers contracting the disease, one of whom succumbed. This incident highlighted the high fatality rate, nosocomial transmission risks, and lack of preparedness in handling such cases. It prompted the Indian government to initiate surveillance programs and enhance diagnostic

a) CCHF in Rajasthan

Rajasthan has reported sporadic cases, particularly in livestock-farming districts. Its proximity to Gujarat increases the risk of spillover. Surveillance studies have detected positive *Hyalomma* ticks for CCHF virus in cattle. While no large outbreaks have occurred, the state remains vigilant, implementing preventive measures such as community awareness and acaricide use.

b) CCHF in Maharashtra

Maharashtra has reported cases linked to livestock trade and travel from Gujarat. Rural districts with high livestock density pose a risk for outbreaks. A suspected case in 2019 revealed the need for heightened surveillance, with efforts focused on livestock screening and human case monitoring in high-risk areas.

c) CCHF in Gujarat

Gujarat is the epicenter of CCHF in India, with recurrent outbreaks since 2011. Districts such as Bhavnagar, Surendranagar, and Kutch are most affected due to extensive livestock farming and the presence of *Hyalomma* ticks. The 2019 outbreak saw multiple cases with a high fatality rate, prompting rigorous surveillance and vector control measures, including treating livestock with acaricides.

Other States with CCHF Prevalence:

- a) Haryana (2020): Reported a confirmed case linked to livestock exposure, highlighting the disease's spread in northern India.
- b) Kerala: Studies on tick surveillance have been conducted, although no human cases have been reported.
- c) Karnataka: The state's significant livestock population places it at risk, prompting vector monitoring programs (Patel et al.).

14. Factors Contributing to CCHF Spread in India

- 1. Livestock Farming: India's large livestock population provides ample hosts for *Hyalomma* ticks, facilitating virus transmission.
- 2. Human-Tick Interaction: Farmers, veterinarians, and slaughterhouse workers frequently interact with ticks, increasing zoonotic risk.
- 3. Climate Change: Rising temperatures and altered rainfall patterns have expanded tick habitats, making more areas vulnerable.
- 4. Healthcare Practices: Nosocomial transmission remains a concern in rural healthcare places where control measures of the infections are inconsistently applied.
- 5. Cross-Border Livestock Trade: The movement of livestock across state and national borders facilitates the spread of *Hyalomma* ticks and CCHFV (Bente et al. 2013; Shayan et al. 2015; Patel et al.)

15. Preventive and Control Measures

Preventing and controlling CCHF requires a multi-sectoral approach, including vector control, public awareness, and healthcare preparedness. Strategies include:

- 1. Vector Control
 - a. Regular application of acaricides to livestock to reduce tick populations.
 - b. Clearing vegetation and maintaining hygienic livestock shelters to minimize tick habitats.
- 2. Personal Protection
 - a. Farmers should wear protective clothing and use tick repellents when handling animals.
- 3. Public Awareness Campaigns
 - a. Educating at-risk populations about CCHF risks and prevention measures.
 - b. Promoting proper handling of livestock and slaughterhouse practices.
- 4. Infection Control in Healthcare
 - a. Use of personal protective equipment (PPE) by healthcare professionals treating suspected cases.
 - b. Adherence to biosafety protocols in handling waste and patient fluids.
- 5. Surveillance
 - a. Monitoring tick populations and testing livestock for CCHFV.
 - b. Tracking febrile cases with tick exposure to ensure timely diagnosis.

Furthermore, CCHFV may be eliminated by heating it to 56°C for 30 minutes and is vulnerable to 1% hypochlorite and 2% glutaraldehyde (Appannanavar and Mishra 2011; Shayan et al. 2015)

16. Conclusion

Awareness is Armor, Prevention is Power – Fight CCHF as it remains an important public health challenge throughout the world, and potential for nosocomial transmission underlining the urgent need for proactive measures. Regular surveillance of tick populations, livestock, and at-risk human populations is essential along with public health campaigns.

Global collaboration is crucial to address the transboundary nature of CCHF. Research into the virus's pathogenesis, immune response mechanisms, and genetic variability should be prioritized to develop effective therapeutics and vaccines. With coordinated efforts across human, animal, and environmental health sectors—adopting a "One Health" approach—CCHF can be better managed, reducing its impact on vulnerable populations.

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ENTOMOLOGICAL SURVEILLANCE

Sridharan Subramanian¹ and P K Srivastava²¹Formerly Directorate of Public Health & Preventive Medicine, Chennai, Tamil Nadu²Formerly National Vector Borne Disease Control Programme (NVBDCP), Delhi

Corresponding Author Email: roadakka@gmail.com

Abstract

Entomological Surveillance is an important component in controlling disease carrying vectors like Mosquitoes, Ticks and Flea is discussed in this article. Entomological Surveillance, especially on mosquitoes, has different methods and techniques. Some of important parameters are dealt in a detailed manner. The parameters used for insecticide resistance, its efficacy, vector incrimination etc., could not be detailed in this chapter due to limited space, however, these are explained and reference can be taken from the guidelines/manuals mentioned in references.

Introduction: Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700,000 deaths annually. They can be caused by either parasites, bacteria or viruses. Malaria is a parasitic infection transmitted by *Anopheles* mosquitoes. It causes an estimated 249 million cases globally, and results in more than 608,000 deaths every year. Most of the deaths occur in children under the age of 5 years. Dengue is the most prevalent viral infection transmitted by *Aedes* mosquitoes. More than 3.9 billion people in over 132 countries are at risk of contracting dengue, with an estimated 96 million symptomatic cases resulting in 40,000 deaths every year. Other viral diseases transmitted by mosquito vectors include chikungunya fever, Zika virus fever, yellow fever, West Nile fever, Japanese encephalitis. Lymphatic Filariasis, commonly known as elephantiasis, is a neglected tropical disease is caused by Nematode worm. This painful and profoundly disfiguring disease is transmitted by *Culex* mosquitoes. In the year 2023, 657 million people in 39 countries were living in the vulnerable areas. Chandipura Virus is transmitted by Phlebotomine Sandfly. Ticks transmit Kyasanur Forest Disease (KFD), Tick borne encephalitis (TBE), Crimean-Congo haemorrhagic fever (CCHF). Bubonic Plague is transmitted by Flea. In order to control/prevent these deadly diseases one must have a thorough knowledge of the vector bionomics so as to select appropriate control measures. This can be achieved only by Entomological Surveillance and monitoring through various means and methods.

Entomological Surveillance – Definition: Entomological surveillance is used to determine changes in the geographical distribution and density of the vector, evaluate control programmes, obtain relative measurements of the vector population over times and facilitate appropriate and timely decisions regarding intervention – World Health Organization. The Surveillance has four major subdivisions *viz*: monitoring, decision-making, assessing effectiveness and identifying trends. Vector wise surveillance is discussed herewith (Table 1).

I. MOSQUITO: These insects are classified under:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Culicidae

Table 1. The following are the diseases transmitted by the Mosquito vectors

Sl. No.	Name of the Disease	Causative Organism	Mosquito genus	Geographical Area
1.	Malaria	Plasmodium - Protozoa	<i>Anopheles</i>	World-wide
2.	Dengue	DEN 1,2,3,4 - flavivirus	<i>Aedes</i>	Tropical and subtropical areas of South America and Africa
3.	Chikungunya	CHIKV - alphavirus		
4.	Yellow Fever	YFV - flavivirus		
5.	Zika	ZKV - flavivirus		Africa, America, India and SE Asia
6.	West Nile virus	WNV - flavivirus	<i>Aedes/ Culex</i>	Europe, Africa, Asia, Australia, and North America
7.	Japanese Encephalitis	JEV - flavivirus	<i>Culex</i>	Asia, Australasia
8.	Lymphatic Filariasis	<i>W. bancrofti</i> - Nematode		Africa, America, India & SE Asia
9.	St. Louise Encephalitis	SLEV - flavivirus		United States
10.	Western Equine Encephalitis	WEEV - alphavirus		
11.	Eastern Equine Encephalitis	EEEV - togavirus	<i>Aedes</i>	North, Central & South America
12.	Venezuelan Equine Encephalitis	VEEV - alphavirus		
13.	Ross River fever	RRV - alphavirus	<i>Culex & Aedes</i>	Tropical Western Australia & Countries in Asia Pacific
14.	Barmah Forest virus	BFV - alphavirus		
15.	La Crosse encephalitis	LACV - bunyavirus	<i>Aedes</i>	United States
16.	Keystone virus	KV - orthobunyavirus		
17.	Rift Valley fever	RFV - bunyavirus	<i>Culex & Aedes</i>	Africa
18.	Buruli Ulcer	<i>Mycobacterium ulcerans</i> - gram positive bacteria	<i>Aedes</i>	Australia

Objective of the Mosquito surveillance is to know its bionomics. The tick mark in the Table (2) below is to be answered to know the bionomics:

Table 2. Activities of adult mosquito

ACTIVITY	WHERE?	WHEN?	WHY?	WHOM?
Resting	✓	✓	✓	NA
Feeding	✓	✓	NA	✓
Breeding	✓	NA	NA	NA

NA - Not Applicable

To answer the above tick marks the following Methods and Techniques are deployed.

1. **COLLECTION - (Adults & Larvae):** There are several methods of adult mosquito collection but the selection and application alone, or in combination of others, as well as the limitations of each method are imposed by:

- The objective of the study for which the collection is made;
- Biology and behaviour of vectors;
- Human behaviour and housing conditions;
- Environmental conditions (natural or modified by man) in which the collections are made like temperature, RH, wind, rain, absence of breeding places, position of breeding places, insecticide spraying, smoke, introduction of animals inside the house, etc. The types of collection and dissection is given hereunder.

- 1.1. Resting Density – Dawn and Dusk
- 1.2. Total Catch (Pyrethrum Spray Sheet Collection)
- 1.3. Catch of Bait – Animals and Humans
- 1.4. Trap Collection
- 1.5. Larvae Collection

2. DISSECTION

- 2.1. Ovary
- 2.2. Stomach
- 2.3. Salivary gland
- 2.4. Spermatheca
- 2.5. Thorax
- 2.6. Head

- 1.1. **ADULT RESTING COLLECTION:** Mosquitoes resting on different surfaces (indoor / outdoor) are collected using a hand aspirator flashlight technique during dawn or dusk (6 am to 9 am or 6 pm to 9 pm). It is expressed in Per Man Hour or 10 Man hour Density. A minimum of 15 minutes per structure is to be spent by a trained insect collector under the direct supervision of an Entomologist. If four such trained insect collectors visit four structures each with two in indoor and two in outdoor, the total time spent on collection shall be four hours with two hours indoor and two outdoor. After collection and counting/segregation of species, the following calculation may be applied to get the Dawn/Dusk/Indoor/ Outdoor Resting density:

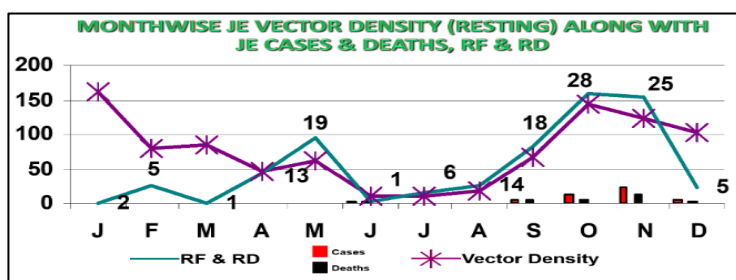
Indoor / Outdoor Resting Density –

$$\text{PMD} = \frac{\text{Number of mosquitoes (male \& female) collected}}{\text{Number of Insect Collector x time spent in search in hour}}$$

Objectives: To study the –

- 1. Density
- 2. Resting Habit
- 3. Distribution on different surfaces
- 4. To calculate the Parity Rate and Physiological age
- 5. Abdominal condition
- 6. Host preference
- 7. Cytotaxonomy
- 8. 24 hr. survival Rate

Materials Required: Suction Tube (Hand Aspirator), Torch light, Test tubes (Labeled), Cotton, Barraud's cage.



Graph showing month-wise vector density (Resting) along with rain fall and rainy days (number) indicates when rainfall increases, the vector density also increases resulting in increased JE cases and deaths in paddy cultivated areas.



Adult resting collection using Suction Tube and Flashlight

1.2. TOTAL CATCH (PYRETHRUM SPRAY SHEET COLLECTION): This method consists of collection of indoor mosquitoes on white cotton sheets after knockdown by space spraying of pyrethrum – kerosene solution in the ration of 1:19. It is expressed in Mean Hut Density. Initially window and door areas along with the eves are covered with a white sheet with the help of wall tacks, then the floor of the structure is fully covered with the white cloth. Once the structure is made and ready for spray, pyrethrum – kerosene solution is sprayed inside the structure by standing in the centre and making a circle resulting in uniform spray. The dosage of spray is 100 to 150ml of solution per 100 cubic feet. Once the spray is done the sprayer leaves the structure and after 15 to 20 minutes the insect collectors enter the structure/hut to collect the knock-down mosquitoes by raising the edges of the white floor cloth and gathering all the mosquitoes in the centre of the cloth and collect each mosquito in a labeled test tube with care using entomological forceps and torch light. A minimum of **five** structures should be sprayed. All the mosquitoes are identified, and the Mean Hut Density is calculated as follows:

$$\text{Mean Hut Density} = \frac{\text{Number of mosquitoes /vectors}}{\text{Number of structures}}$$

OBJECTIVES - To study the:

1. Indoor Resting Density
2. Blood meal
3. Infection & Infectivity
4. Residual Density after Trap collection

TIME OF COLLECTION: Dawn (6.00 am to 9.00 am)

MATERIALS REQUIRED:

1. Pneumatic Hand Sprayer
2. Pyrethrum extract
3. Kerosene
4. Torch light
5. Petri dish
6. Test Tubes (Labeled)
7. Entomological Forceps
8. White cotton sheets
9. Wall tacks

POINTS TO REMEMBER: 2 or 3 sets of white cotton sheets should be in possession so that more than one structure can be prepared for spray. The cotton sheets should be washed thoroughly after every collection. After the knock down of the mosquitoes, the cotton sheet in the floor should not be brought outside the structure for collecting the dead mosquitoes since a gush of wind will spoil all the hard work done.



**Pneumatic Hand Sprayer
(1 litre capacity)**



Collection of dead mosquitoes

1.3. CATCH OFF BAIT - Animals and Humans (Whole Night Biting Adult Mosquito Collection): Mosquitoes are collected while they land on the host to bite or in the process of biting a human* or animal host. Human* and animal baits (Indoor & Outdoor) to be selected and collect the mosquitoes that are landing or in the process of biting. The collection should start at sunset and stop at sunrise (6 pm to 6 am). It is calculated Number of mosquitoes per bait per hour (hour can be specified).

OBJECTIVES

1. To evaluate man - mosquito contact or contact with other animal host.
2. To infer the place and time of biting (Peak biting Time/Endo or Exophagic)
3. To collect live mosquitoes for the study of -
 - Parity Rate
 - Infection & Infectivity Rate
 - Susceptibility Status
 - Longevity

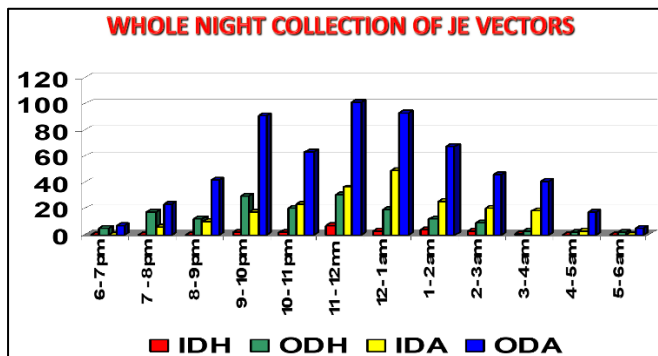
MATERIALS REQUIRED

1. Suction Tube
2. Torch light
3. Test tubes (labeled - minimum of 48 numbers)
4. Cotton
5. Human* & Animal baits



**OD Animal Bait
Collection**

***Human baits cannot be used unless permitted by the ethical committee**



**IDH - INDOOR HUMAN BAIT
ODH - OUTDOOR HUMAN BAIT
IDA - INDOOR ANIMAL BAIT
ODA - OUTDOOR ANIMAL BAIT**

The bar diagram shows the number of mosquitoes collected on various indoor and outdoor, human and animal baits. It can be inferred from the bars that JE vectors are predominantly zoophilic and exophagic.

1.4. TRAP COLLECTION: The method is extensively used for collecting mosquitoes which are flying in search of food, shelter or egg laying site *i.e.*, which are changing from one place to another during their normal movement or due to some artificial influence.

OBJECTIVES: To study the -

1. Relative prevalence of mosquitoes
2. Their Behaviour
3. Effect of residual insecticide or other anti-mosquito measures

CATEGORY OF TRAPS

1. Fixed traps attached to existing structures
2. Portable traps – enclosing baits
3. Mechanical devices for collecting outdoor and indoor mosquitoes

1. **FIXED TRAPS:** There are two types of traps, 1. Exit trap, 2. Entry trap. The Exit traps gives direct information on circulation of mosquitoes from inside to outside and indirect information on outside to inside and the Entry trap is *visevera*.

Degree of exophily / endophily is indicated by:

$$\text{GRAVID} \div \text{FED} = <1 = \text{Exophily} \\ >1 = \text{Endophily}$$



ENTRY TRAP



EXIT TRAP



PORTABLE TRAP ENCLOSING



MECHANICAL DEVICES



INDOOR



OUTDOOR

1.5. LARVAE COLLECTION: Collection of larvae is done in breeding sources (water collections) using a Dipper or Net or Pipette. The measurement of larval density is a vital data for monitoring control programmes against VBD.

OBJECTIVES

1. To establish the breeding habits of different species.
2. To establish the geographical distribution of vectors.
3. To establish the active breeding places.
4. To evaluate the dynamics of development of aquatic stages of mosquitoes.
5. To evaluate the impact of AL measures.
6. To evaluate the impact of adulticide to demonstrate the persistence or absence of a species that cannot be found as adults.
7. To rear adults for taxonomic studies or bio-observations (Bioassay, Susceptibility test or even infection in case of suspected TOT).

SAMPLING PROCEDURES

For purpose of evaluating larval control measures – sampling should be directed to sites where there are larval concentrations. These sites shall serve as Fixed capture stations.

Sampling should be made with a ladle for small water collections or with a net dipper in large collections or a net in wells. The dips should be standardized units of 10 or multiples of 10 per capture station. Positive breeding places with reference to total checked to be recorded. Among positive dip, average no. of IV instar larvae and pupae to be recorded (Average no. of larvae & pupae per positive dip).

POINTS TO REMEMBER: Declaration of positive breeding place depends on the insecticide used and also on day of spray with reference to the day of inspection.



**Larvae
Collection**



Larval Breeding Source

2. DISSECTION: In routine for establishing the Parity, Sporozoite, Oocyst, Infection and Infectivity Rates. In special circumstances – Degree of fertilization (when testing a new larvicide), Identification of chromosomes (to find out sibling species)

OBJECTIVES

1. Ovary Dissection – To establish Parity Rate or Physiological age, Christopher's stages and Chromosomal examination.
2. Stomach Dissection – Oocyst Rate, Tracheolar system and Presence of *mf*.
3. Salivary Gland – To establish Sporozoite Rate.
4. Spermatheca – Presence or Absence of spermatozoa.
5. Thorax – To establish L1 and L2.
6. Head – To establish L3.

PARITY RATE: Parity in mosquitoes is defined as the number of times a female mosquito, has laid eggs. This gives some idea as to the age of a mosquito. Determining the age structure of a population is

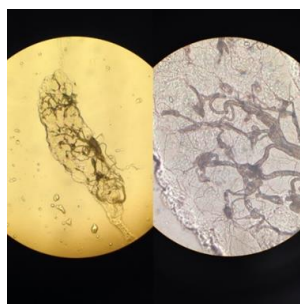
important, as older mosquitoes are more likely to transmit diseases since they have had the opportunity to blood feed on potentially infectious hosts and transmit them to a susceptible host. If the mosquito has not laid eggs (nulliparous), she is likely to be young; if she has laid eggs before (parous), she must have had the opportunity to mate and take a blood meal and is therefore likely to be older. Parity assessment can be performed by the following two techniques:

Detinova Ovarian Tracheation (1962)

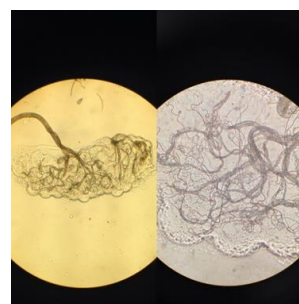
Polovodova technique (1949)

Out of the above two techniques, Detinova OT is simpler to perform but with certain limitations.

DETINOVA OT (1962): Tracheoles are tube-like structures that are part of the respiratory system in insects. In mosquitoes, the tracheoles look like a coiled knot around the forming ovarioles. Due to its resemblance to a ball of yarn, this knot is referred to as a 'SKEIN'. Once the eggs become fully developed and the female oviposits them into the environment, these skeins become loosened or straightened and never become coiled into tight knots again. The presence or absence of tracheole skeins (coiled ends of tracheoles) can be used to classify mosquitoes as either nulliparous or parous. The method is rapid and simple, and a well-developed framework has been established for applying nulliparous/parous classification. However, the inability of this method to distinguish older age classes of mosquitoes and population age structure in natural settings, has prompted the development of additional age grading methods.

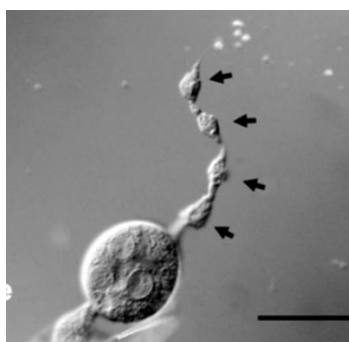


Nulli parous with tracheolar skeins



Parous with loose tracheolar knots

POLOVODOVA TECHNIQUE (1949): The method is based on counting follicular dilatations and estimate the number of times egg laid by mosquitoes. Dissection of ovary for isolation (stretching) of ovarioles so that the stalks may be straightened making possible the appreciation of dilatations, follicular sacs and follicular relics (or degenerated follicles). Careful attempts should be made to isolate ovarioles (at least six) from three parts of the ovary (anterior, middle, and posterior part). The ovarioles which are still attached to the calyx should be carefully stretched and the number of dilatations to be counted. Each dilatation represents one oviposition or egg laying. The physiological age of the mosquito is arrived by expressing as 1d, 2d, 3d and so on or in other words the mosquito has laid eggs once, twice, or thrice and so on. This data can be used to arrive at the calendar age by simply multiplying the number of dilatations with the average number of days of the gonotrophic cycle. Some allowance should be given for 1d since the duration of gonotrophic cycle of nulliparous is more than the parous mosquitoes.



Detached ovariole with four dilatations (arrows)

Parity Rate and Age Grading is an important parameter to be conducted in an indoor residual spray area. The objective of IRS is to reduce the longevity of the vectors such that it does not transmit the disease. Pre – spray and During – spray data are to be compared and regularly monitored. The result of such comparison shall give the impact of spray. The Parity Rate and Age Grading shows the efficacy of the spray and its success in bringing down the longevity of the vectors.

3. AEDES SURVEY: There are several methods for monitoring vectors (mostly *Aedes aegypti*) of arboviral diseases. However, the selection and use of a method requires a clear understanding of the surveillance objectives, the availability of skills and resources, and in some instances the level of infestation. Surveillance may serve to identify areas of high-density infestation or periods of increasing mosquito populations. In areas where the vector is no longer present, entomological surveillance is critical in order to rapidly detect new introductions, before they become widespread and difficult to eliminate. The surveillance has two following types:

- **IMMATURE SURVEY:** The house or premise is basic sampling unit for larval surveys and routinely examined for water-holding potential containers. The existence of mosquito larvae and pupae is checked in these containers. To confirm the species, specimens should be collected and identified with appropriate mosquito larvae identification key. However, the survey may vary as per the infestation of the larvae.

In Moderate to High infested areas:

- Container index
- House index
- Breteau index

In low infested areas:

- Ovitrap
- Larvitrap

- **CONTAINER INDEX:** Percentage of Containers Positive for *Aedes* larval breeding. Significance: Shows the intensity of breeding.

$$\frac{\text{Number of containers positive for } Aedes \text{ larval breeding}}{\text{Number of containers examined}} \times 100$$

>10 – High Risk, 5 to 10 – Moderate Risk, <5 – Low Risk

- **HOUSE INDEX:** Percentage of Houses Positive for *Aedes* larval breeding. Significance: Shows the spatial distribution of *Aedes* larvae.

$$\frac{\text{Number of houses positive for } Aedes \text{ larval breeding}}{\text{Number of houses inspected}} \times 100$$

>10 – High Risk

5 to 10 – Moderate Risk

<5 – Low Risk



Importance of House Index: The areas “a & b” has similar House Index, but in area “b” the positive houses are widespread which makes it more vulnerable for a widespread outbreak.

- **BRETEAU INDEX:** Total No. of containers positive for *Aedes* larval breeding for every 100 Houses inspected. Significance: To assess the vector control operation.

> 50 – High Risk
 5 to 50 – Moderate
 < 5 – Low Risk

NOTE: To calculate a valid Breteau Index, the evaluator should necessarily visit 100 houses and note down the number of positive containers and hence BI is always an absolute number. Since the Index evaluates vector control operation, the evaluator should visit the same house and possibly same container every time for assessment.

- **OVITRAP:** It is a sensitive and economical method to detect the presence of *Aedes* in situations where infestations are low and larval surveys are generally unproductive (e.g., when the Breteau index is < 5).
 - The standard ovitrap is a wide-mouth 0.5 litre glass jar painted black on the outside and equipped with a hardboard or wooden paddle that is clipped vertically to the inside with a roughened side facing inwards. The jar is partially filled with clean water and is appropriately placed in a rain-sheltered site – usually outdoors and close to habitation.
 - Ovitrap are usually serviced weekly, and the paddles are examined for the presence of *Aedes* eggs. The percentage of positive ovitraps provides the simplest index of infestation levels.
- **TYRE SECTION LARVITRAPS** of various designs have been used to monitor oviposition activity, with the simplest of these being a water-filled radial section of a tyre.
 - Tyre larvitrap differ functionally from ovitraps in that water level fluctuations caused by rainfall induce the hatching of eggs, and it is the larvae that are counted rather than the eggs.



Ovitrap



Tyre Section Larvitrap

- **ADULT SURVEY:** Adult mosquito sampling can provide valuable data for studies of seasonal population trends or evaluation of adulticiding measures. However, results are less reproducible than those obtained from sampling immature stages. The following are the types of collection:
 - Resting Collection
 - Sticky Trap collection
 - Adult landing collection
- **Resting Collection:** Resting collection consists of systematic search of *Aedes* mosquitoes resting indoors. The density is recorded either - no. of adults per house or no. of adults per unit of time.
- **Sticky Trap collection:** Various sticky trap devices have been used for sampling adult *Aedes*. They may be designed to be visually attractive, odour-baited, or both, or may simply be located at constricted access points through which adult mosquitoes pass (e.g., points of exit and entry from subterranean habitats such as keyholes in service manhole covers in roads).
- **Adult Landing collection:** Landing or biting collections on humans are a sensitive means of detecting low-level infestations. Rates of capture are usually expressed in terms of

$$\text{Adult landing rate} = \frac{\text{No. of adult mosquitoes captured/no. of baits}}{\text{time spent on collection}}$$

landing/biting per person – hour. The bait usually a male may be asked to remove his shirt or vest and sit in one corner of the room facing the wall. He should be asked to breath profusely for at least 10 minutes. With least disturbance collection should be started as when a mosquito lands on his body. Preferably a mechanical hand aspirator may be used.

II. SANDFLY: Sand flies are tiny insects 1.5–3.5 mm in length, with a hairy appearance, large black eyes and long, stilt-like legs. They are classified under (Table 1):



NOTE: Adult *Aedes* collection should be avoided in endemic locations since the adult could be infected with any ABD virus. However, surveillance tools like various traps may be used to collect the adult mosquitoes.

Kingdom : Animalia
Phylum : Arthropoda
Class : Insecta
Order : Diptera
Family : Psychodidae

Table 1. Diseases transmitted by Sandfly

SL. NO	DISEASE	CAUSATIVE ORGANISM	TYPE	RESERV OIR	DISTRIBUTION
1.	Chandipura	<i>Chandipura virus</i>	<i>Rhabdoviridae</i>	--	Maharashtra and Gujarat State
2.	Pappataci Fever	<i>Naples virus</i> , <i>Sicilian virus</i> , <i>Toscana virus</i>	3 Serotypes of <i>Phlebovirus</i>	--	S. Europe, N. Africa, The Balkans, E. Mediterranean, Iran, Iraq, Pakistan, Afghanistan, India
3.	Carrion's disease	<i>Bartonella bacilliformis</i>	<i>Gram negative Bacteria</i>	Human	Peru, Ecuador, Southern Columbia
4.	Vesicular Stomatitis	VSV	<i>Rhabdoviridae</i>	--	Central & SW United States, Canada, Mexico, Central & South America
5.	Visceral Leishmaniasis	<i>L. donovani</i>	<i>Protozoa</i>	--	Bangladesh, Brazil, Ethiopia, India, Sudan, Kenya, Somalia
6.	Cutaneous Leishmaniasis	<i>L. donovani</i>	<i>Protozoa</i>	Human (PKDL)	All Tropical and Subtropical regions of the world
		<i>L. tropica</i>		Dog & Rodent	

SAMPLING METHODS OF ADULT SAND FLIES: Vector surveillance aims to detect the presence of a vector in a given population or to determine vector abundance for estimating the risk of disease transmission, and hence the sampling methods required will depend upon the questions to be addressed. Sampling methods exist for both the adult and the immature stages of sand flies. The most used trapping methods are light traps for host-seeking females and sticky traps or aspirator collection for the resting population.

LIGHT TRAP: Light traps are used extensively in field studies on sand flies. The most widely used trap is a battery-operated light-suction trap, with the main advantage being that it can be left overnight to collect sand flies.

STICKY TRAP: Sticky traps constitute a method of sampling sand flies (Table 2) by interception rather than attraction. Standardized pieces of white un-waxed paper or cards are soaked in castor oil and placed in sand fly resting places overnight (at least two or three consecutive days). To obtain quantitative results, either one or both sides of the paper must be completely exposed. The number of papers used at each sampling event is normally constant. These traps are generally inexpensive and easy to manufacture in large numbers.



LIGHT TRAP



STICKY

Table 2. Collection Method of sampling sand flies

STAGE	TARGET POPULATION	COLLECTION METHOD	ADVANTAGES	DISADVANTAGES
Adult	Resting population both males and females Feeding population from animals	Hand aspirator collection	1. Collection of live sand flies 2. Reveal where sandflies rest during the day, to aid control measure. 3. Resting collection tend to target males which can permit identification to species where females are morphologically indistinguishable. 4. Can be used to measure population changes	1. Labour intensive, difficult to standardize. 2. Specimens(sandflies) easily gets damaged.
	Resting population both males and females	Sticky Trap	1. Inexpensive and easy to make 2. Large numbers can be used. 3. May be used for diurnal collection. 4. Rows of traps at floor to ceiling can be used to sample intra-domiciliary adult activity.	1. Only dead specimens can be collected. 2. Ineffective in areas with high relative humidity.
	Host-seeking females (but others like gravid and males are also attracted)	Light Trap	1. Standardization is possible. 2. Overnight night collection is achieved.	1. Attracts only specific species. 2. Distance/Range of attraction is limited. 3. Specimens often damaged and dead.

Immature	Immature Stages	Emergence Trap	Live sandflies can be collected	Exact breeding sites are difficult to locate.
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III. TICK: Ticks are small Arachnids, typically 3 to 5 mm long. They are classified under (Table 3):

Kingdom: Animalia

Phylum: Arthropoda

Class: Arachnida

Order: Acarina

Family: Ixodidae (Hard Tick), Argasidae (Soft Tick)

Table 3. Diseases transmitted by Ticks

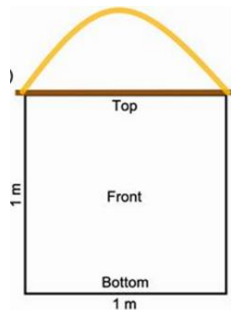
Sl. No.	DISEASE	VECTOR	CAUSATIVE ORGANISM	TYPE	DISTRIBUTION
1.	Kyasanur Forest Disease (KFD)	<i>Haemaphysalis spinigera</i>	KFD V	<i>Flaviviridae</i>	Karnataka State, Shivamogga District
2.	Crimean Congo Haemorrhagic Fever (CCHF)	<i>Hyalomma</i>	CCHF V	<i>Bunyaviridae</i>	Asia, E. Europe, C,W&S Africa, Madagascar
3.	Rocky Mountain Spotted Fever (CCHF)	<i>Dermacentor Rhipicephalus</i>	<i>R. Rickettsii</i>	Gram-ve Coccobacillus	Western Hemisphere
4.	Tick Borne Relapsing Fever	<i>Ornithodoros (Soft Tick)</i>	<i>Borrelia recurrentis</i>	<i>Spirochete</i>	Western US, British Columbia, C&S America
5.	Q fever	<i>Rhipicephalus, Dermacentor, Amblyomma</i>	<i>Coxiella burnetii</i>	Gram-ve Bacteria	Sporadic throughout world
6.	Lyme Disease	<i>Ixodes scapularis</i>	<i>Borrelia burgdorferi</i>	<i>Spirochete</i>	N. America & Europe
7.	Ehrlichiosis	<i>Amblyomma americanum</i>	<i>Ehrlichia chaffeensis</i>	Gram-ve Bacteria	S. Atlantic & S & C America
8.	Babesiosis	<i>Ixodes scapularis</i>	<i>Babesia microti</i>	Protozoa	N. America, Europe & Asia
9.	Powassan viral Disease	<i>Ixodidae & Dermacentor</i>	POS V	<i>Flaviviridae</i>	N. America & Far east Russia
10.	Tick Borne Encephalitis	<i>Ixodes ricinus</i> <i>Ix.persulcatus</i>	TBE V-Subtypes: 1.European 2. Far eastern 3. Siberian	<i>Flaviviridae</i>	C & E Europe, North Asia - Regions of Russia, China & Japan
11.	Colorado Tick Fever	<i>Dermacentor</i>	CTF V	<i>Reoviridae</i>	US & Canada (Mountain areas)
12.	Omsk Haemorrhagic Fever	<i>Dermacentor</i>	OHF V	<i>Flaviviridae</i>	Siberia (Russia)
13.	Russian Spring Summer Encephalitis	<i>Ixodidae & Haemaphysalis</i>	RSSE V	<i>Flaviviridae</i>	Russian, Central Europe & SE Asia

TICK COLLECTION: Tick surveillance is intended to monitor changes in the distribution and abundance of ticks and the presence and prevalence of tickborne pathogens in order to provide actionable, evidence-based information to clinicians, the public and public health policy makers. Key questions address when and where humans are at risk for exposure to ticks and tickborne pathogens. Two of the most commonly used sampling techniques for host-seeking hard ticks are dragging and flagging. These methods are used for the active collection of ticks, and although they remove ticks from the environment, they are not recognized to have an effect on diminishing or controlling tick populations.

DRAGGING: In dragging method a cloth is dragged across the top of the vegetation or forest floor and regularly checked for the presence of ticks. The size of the cloth (usually 1 x 1 sq.mt.) allows for determination of the area sampled; the distance of metres dragged is multiplied by the size of the cloth in order to determine the total area dragged, which is needed to determine ticks per unit area. To drag, sample a predetermined area of at least 750 sq.mt., a grid or linear transects can be established. Drag sampling along the same grid or transect can be done repeatedly for longitudinal studies to define host-seeking phenology or compare year-to-year density. Corduroy is also commonly used in drag cloths. The fabric ridges increase surface area such that the sampling cloth size may be greater than 1 sq.mt. which may be a consideration in density estimates. A thick white flannel cloth material can also be used, as it is easy to wash, durable, less expensive, and easier to find at a local fabric store. When soiled, the drag cloth can be laundered with non-fragrance laundry soap in a regular washing machine.

The Ticks are collected from the cloth and examined and species wise segregated. To determine the presence of ticks per unit area the following calculations may be made:

If the Total distance dragged is 1000 mts. multiply the same with the dimensions of the cloth used to get the presence of ticks per unit area. The per unit area is equal to the dimensions of the cloth used.



Drag Cloth Design



Tick Drag and Collection by the Zonal Entomological Team, Coimbatore

FLAGGING: The flagging technique uses a similar device as the previously described drag cloth, but more closely resembles a flag attached to a pole. The collector uses this adapted flagging device by waving



Tick collection using Flagging Method: Demonstration by Institute of Vector Control & Zoonoses, Hosur, TN

it over vegetation, representing the motion of waving a flag. The benefit of flagging is that the collector has more control over the cloth and dowel so the technique can be used in a more targeted manner to collect a certain species or life stage of tick, especially adult ticks, and usually aims to collect a minimum sample size of ticks. It is useful in collecting the tick from vertical vegetation like shrubs, tall grass, etc. Flagging is generally used to determine the presence or lack of detection of ticks and tick density is not typically evaluated using this method. Further, flagging is also more difficult to standardize between collectors, as sampling effort is not standardized, adding challenges to comparisons of results of different flagging studies.

IV. FLEA: The adult fleas are small wingless insects, usually less than 5mm long, with a heavily sclerotized and distinctive laterally flattened body. They are classified under (Table 4):

Kingdom: Animalia
 Phylum: Arthropoda
 Class: Insecta
 Order: Siphonaptera
 Family: Pulicidae – 200 genera with 3000 described species

Table 4. Diseases Transmitted by Flea

Sl. No.	DISEASE	ORGANISM	TYPE	GEOGRAPHICAL DISTRIBUTION
1.	Bubonic Plague	<i>Yersinia pestis</i>	Gram negative <i>Coccobacillus</i>	Worldwide
2.	Murine Typhus	<i>Rickettsia typhi</i>	Gram negative Bacteria (<i>Rickettsia</i> of <i>Typhus</i> group)	Worldwide
3.	Tungiasis	<i>Tunga penetrans</i>	Flea(Jigger Flea)	Africa, The Caribbean, Central & South America, India
4.	Tularemia	<i>Francisella tularensis</i>	Gram negative <i>Coccobacillus</i>	United States, Canada & Mexico

FLEA COLLECTION FROM RODENTS: Wild rodent should be collected from the rat burrows in the forest area and placed on a white plastic basin for collection of fleas immediately in the field. The rodents' fur to be thoroughly brushed with a toothbrush to collect the flea by hand or with forceps or sometimes even with a rubber bulb attached glass tube for suction and stored individually in sterile cryovials with 95% ethanol. Later, in the laboratory, the carcasses of the rodent should be checked to verify that all fleas had been collected. For each rodent, the total number of fleas extracted should be recorded. In domestic areas, rat traps may be used. More than 100 to 200 rat traps should be placed in domestic and peri domestic areas in the night and collected in the morning. Rats which has been trapped should be anesthetized or euthanized by cervical dislocation and the same procedure of collecting the fleas should be followed. Flea Index may be calculated as follows:

$$\text{Absolute Flea Index (all flea index)} = \frac{\text{Total no. of Fleas collected}}{\text{Total Rats examined}}$$

$$\text{Cheopis Index} = \frac{\text{Total no. of } X. \text{ cheopis} \text{ collected}}{\text{Total Rats examined}}$$

CRITICAL VALUE OF FLEA INDEX:

Absolute Flea Index = >3

Cheopis Index = >1



Rat Dissection for Spleen smear

After ensuring that all fleas are collected from the rats, the carcasses should be dissected for Spleen smear, stained with polychromatic stains such as Wayson, Wright or Giemsa. The plague bacilli can be appreciated in oil immersion lens like a safety pin because of its bipolar appearance.



Wild rat collection in the forest area by IVC&Z, Hosur as a part of Plague Surveillance

CONCLUSION: The above entomological parameters are only crucial ones out of more than 50 parameters to be studied by entomologists and suggest vector control experts/ personnel engaged in vector control operations. This emphasizes the significance of entomological monitoring and need of entomologists which not just to check the breeding, therefore it may be suggested that the success of prevention, control and elimination of vector borne diseases will not be possible without skilled entomologists/ medical entomologists or public health entomologists.

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Anopheles stephensi - THE EMERGING CHALLENGE IN MALARIA ELIMINATION

R S Sharma¹, P K Srivastava² and Kalpana Baruah³

¹Former HOD & Additional Director, Centre for Medical Entomology & Vector Management National
Centre for Disease Control Delhi

²Former Joint Director & Head, Entomology & Vector Control Division, NCVBDC Delhi

³Former Additional Director, & Head, Entomology & Vector Control Division, NCVBDC Delhi

Abstract

Asian urban malaria vector and has been one of the major drivers for malaria transmission in cities across India, Iran and Pakistan, as well as the Arabian Peninsula. The species is currently expanding its geographical range in Africa. The rapid spread of *An. stephensi* across Africa presents a significant challenge to malaria elimination. It was detected in Africa for the first time in 2012, in the city of Djibouti and subsequently reported from Ethiopia, Sudan, Somalia, Somaliland, Nigeria, Kenya, Ghana and Eritrea.. There are increasing reports of the Asian malaria mosquito, *An. stephensi* invading and spreading in Eastern Africa. The spread of *An. stephensi* is one of the multiple biological threats for achieving malaria elimination in the affected countries. The increasing invasion and influence of this vector is indicative of weaknesses in vector surveillance and control programs. Regular and extensive investigations are needed in both urban and rural areas, especially in countries serviced by the Indian Ocean trade routes, to establish the full extent and future trajectories of the problem. More importantly, instead of tackling this vector species as a stand-alone threat, affected countries should adopt more integrated and multi-sectorial initiatives that can be sustained and malaria elimination in urban areas and urbanised rural areas are achieved. In India *An. stephensi* transmitted urban malaria programme was started long back in 1971 and initially it covered 23 towns. It was extended to 5 more towns in 1972 for anti-larval measures which was later expanded to 131 towns but studies on changing bionomics of *An. stephensi* is not studied to generate scientific evidence on variations in different ecogeographic situations.

Introduction

The emergence of *An. stephensi* in different countries poses a critical challenge to the on-going initiatives for malaria elimination. *An. stephensi* is a mosquito species that is capable of transmitting both *Plasmodium falciparum* and *P. vivax* malaria parasites. Unlike the other main mosquito vectors of malaria, it thrives in urban and man-made environments. Originally native to parts of South Asia and the Arabian Peninsula, *An. stephensi* has been detected, to date, in 7 countries in the African continent.

An. stephensi was reported to transmit malaria in urban areas and port cities like Bombay (now Mumbai) as the breeding of this species was associated with fresh water collection, construction sites etc. The vector control therefore was managed largely by source reduction and biological control. In addition to port cities, anthropogenic activities like digging of wells, piped water supply, introduction of canal irrigation etc., supported *An. stephensi* establishment in other cities e.g. Delhi, Lucknow, and Hyderabad. It is established that unplanned urbanization and deficient water supply prompts water storage practices, favouring creation of more mosquito-genic conditions which result in increased transmission of malaria by *An. stephensi*.

National Malaria Eradication Programme (NMEP) launched in 1958 focused mainly on use of DDT-insecticide for indoor residual spray in rural areas where problem of *An. stephensi* did not exist. The

programme which achieved significant success in 1965 had faced massive resurgence in 1976 due to various administrative and technical reasons. The infrastructures were not adequate and mature enough to take up surveillance and vigilance. This produced a large number of secondary cases due to the re-introduction and relapse of malaria. Added to this was the problem of urban malaria transmitted mainly by *An. stephensi* of which the management or control was the responsibility of local bodies. Malaria cases increased in towns and started diffusing to the rural areas, due to inadequate staff and the shortages of mosquito larvicidal oil as described in 'Malaria resurgence in India: a critical study' by Sharma and Mehrotra 1986.

Climate and environmental factors, including temperature preferences, play a pivotal role in emergence and establishment of *An. stephensi*. Its ability to adapt to urbanization and exploit human-built water storage facilitates its spread to newer areas. Studies suggest that *An. stephensi* could increase annual *P. falciparum* malaria cases by 50% if strategic interventions are not implemented (Todele and Dejene 2023). Mapping thermal transmission suitability using satellite imagery becomes crucial for predicting its spread and informing targeted interventions. During one of the surveillance studies in December 2016, evidence for the presence of *An. stephensi* in Sri Lanka was first reported from two sites in the Mannar Island (Dharmasiri et al., 2017). Current vector control strategies, recommended by WHO may need augmentation to combat *An. stephensi* effectively (WHO, 2023).

Another serious threat to the global health effort in Africa is rapidly developing due to the emergence and spread of the invasive malaria vector, *An. stephensi*. The presence of *An. stephensi* has been reported in Djibouti, Ethiopia, Somalia, and Sudan (WHO 2020). The existence of *An. stephensi* detected accidentally indicated the insufficient capacity of surveillance systems in the area to find or identify the vector (WHO 2020). However, after extensive training in 2021, the vector surveillance system in Sudan confirmed the distribution of *An. stephensi* near areas of high movement of humans, animals, and goods across international borders. These findings suggest a high risk of potential for non-detected spread of *An. stephensi* in countries at confirmed, high, and potential risk of malaria.

Now *An. stephensi* has adopted in African region and transmitting malaria outbreaks. Earlier malaria vector found across parts of South Asia and the Middle East. It is best known as the primary vector of urban malaria in India and is also an important vector in Iran, Iraq, Afghanistan, and Pakistan, where it transmits both *Plasmodium falciparum* and *Plasmodium vivax*. In the past two decades, *An. stephensi* has begun invading Africa. It was detected for the first time in 2012 in Djibouti and has since been reported in Ethiopia, the Republic of Sudan Somalia and Somalia, Nigeria, Kenya, Ghana, and Eritrea (Fig 1,2).

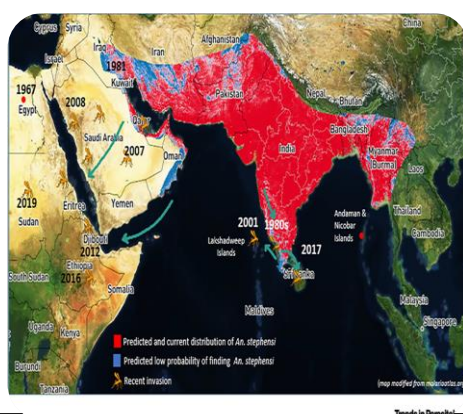


Figure 1. The spatio-temporal pattern of range expansion of *An. stephensi*

(Source (<https://apps.who.int/malaria/maps/threats>

Distribution: India, Pakistan, South China, Iran, Burma Iraq, Thailand, Bahrain
Invaded region: Djibouti(2012), Ethiopia (2016), Somalia (2019),
 Nigeria (2020),Yemen (2021) Ghana (2020)

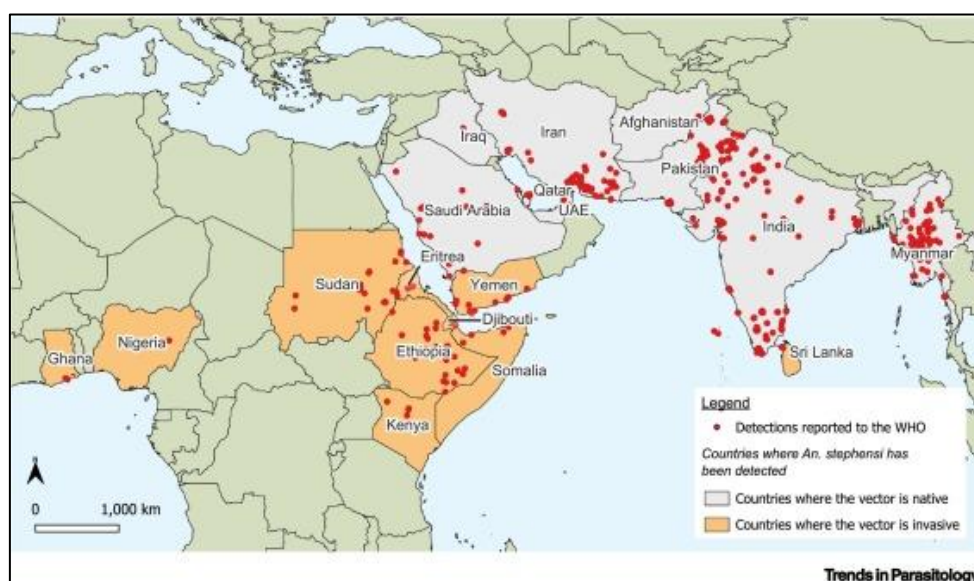


Figure 2. A map depicting the known range of *An. stephensi*.

(Source: <https://apps.who.int/malaria/maps/threats/>)

Now this mosquito species posing a potential threat for outbreaks of urban malaria, as well as complicating control and elimination of malaria in **Africa** ((Russell TL, et al 2020 and RBM 2021). WHO initiative aimed at stopping the further spread of *An. stephensi* in Africa. To support an effective regional response, WHO is recommending a 5-pronged approach

- Increasing collaboration
- Strengthening surveillance
- Improving information exchange
- Developing guidance
- Prioritizing research

Understanding *An. stephensi*

There is a wealth of data regarding the malaria vector *An. stephensi* bionomics in Asia which is applicable to understanding its invasion across sub-Saharan Africa. *An. stephensi* bionomics are highly variable between populations across Asia (Sharma 1986), but there are several important characteristics seen widely that set it apart from the native vectors in Africa. Its ability to exploit both animal and human hosts, and rest both indoors and outdoors, is likely to reduce the impact of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) which are core interventions for malaria elimination. The emergence of *An. stephensi* in Africa therefore poses a multifaceted challenge to malaria elimination efforts. Collaborative efforts are essential for tackling the *An. stephensi* invasion. Understanding its profile, characteristics, and the factors contributing to its spread coupled with on-going research are crucial for devising targeted control strategies and ensure continued progress in the fight against malaria in endemic countries.

Understanding the ecology and behaviour of *An. stephensi* will critically inform control measures and provide prerequisite knowledge for explaining new larval and control tools to contain its spread. Three races in this species, namely *An. stephensi* (type form), *An. stephensi* variety *mysorensis*, and *An. stephensi* (intermediate form), have earlier been reported by several investigators. These different forms have different distributions and roles in malaria transmission (Roz et al. 2024). These forms can be distinguished among each other based on egg morphology (Sweet and Rao 1937, Rutledge et al. 1970,

Subbarao et al. 1987). The number of ridges among the eggs are usually 16–19 in type form, 13–16 in intermediate form and 10–14 in *mysorensis* form. The type and intermediate forms tend to be efficient vectors for malaria, while the *mysorensis* form tends to be zoophilic (prefer animal blood to human blood), which has limited role as a vector of malaria. In urban settings, *An. stephensi* (type) tends to be anthropophilic (prefer to ingest blood meals from humans), endophagic (prefer to feed indoors), endophilic (prefer to inhabit/rest indoors), and their juvenile stages can be found in artificial containers (e.g., water barrels, wells, fountains, cisterns). In rural settings, *An. stephensi* (*mysorensis*) is considered to be zoophilic, exophilic (prefer to inhabit/rest outdoors), and their juvenile stages can be found in irrigation canals, stream pools, and stream margins.

Larval ecology: *An. stephensi* breeds in a number of different water-bodies and man-made breeding places in urban areas, but predominantly in artificial containers, curing tanks, overhead tanks, and ground level water tanks (Fig 3, 3a).



Figure 3. Breeding places for *An. stephensi* type



Figure 3a. *An. stephensi mysorensis* breeding places in rural area

Bentley (1911) described the breeding of *An. stephensi* in wells, cisterns and fountains in Mumbai city in 1904 (Dhir, 1969) detected the breeding of *Anopheles stephensi* in man-made breeding places like construction sites in Delhi. In rural areas, the larvae of *An. stephensi mysorensis* may exist in many aquatic habitats, such as streams, tanks, ponds, underground tankas in desert areas, swamps, marshes, and other sources of standing water. They may also occupy smaller environments, such as tree holes, leaf axils, and

man-made containers. The larvae of *An. stephensi mysorensis* exclusively prefer to occupy stone pots and earthenware containers (Sinka et al., 2011, Fig 3a). This species is also able to endure high levels of salinity, and have been found to breed readily in water where the salinity is equal to or even surpassing that of sea water (Manouchehri et al., 2017). **Adults Behaviour:** They rest in human dwellings and cattle sheds. Inside human dwellings they may rest on hanging objects, behind curtains etc. Outdoor resting has been observed in wells and underground cement tanks. Their biting is throughout the night but peak biting is from 19.00 to 04.00 hrs. Feeding habits indicate that these are mainly zoophilic, but may feed indoor and outdoor (Endophagic and Exophagic) indiscriminately on human and cattle particularly at high density level. The flight range is about 1-3 kms.

Regardless that it may feed outdoors during the summer, when weather is warmer and humans and animals are more likely to sleep outside in the open air. Although indoor feeding habits have shown no variation between seasons, adult females tend to feed more often at night during the summer rather than during the day in winter. *An. stephensi* shows a greater preference for humans over animals in urban areas, where they can be found year-round.

Invasive effect of *An. stephensi* - it was observed that *An. stephensi* (type form), the vector of urban malaria, had shown a southward geographical spread on the Indian mainland due to rapid urbanization and water storage practices. During the 1970s, the species invaded Goa on the western coast and Kanyakumari on the tip of the southern coast in the 1980s. The species finally entered Kerala and contributed to the malaria outbreak during 1996 at Valiathura near Thiruvananthapuram airport, where over 100 cases of malaria were recorded. This was followed by another outbreak in Kasargod during 1998 when 405 cases and three deaths were reported. In both these outbreaks *Anopheles stephensi* was responsible. During March 2000, a brief survey of two islands, viz. Agatti and Kavaratti, revealed the presence of *Anopheles stephensi*, a known vector of urban malaria in the country. Earlier on the mainland, the southward peninsular spread of *An. stephensi*, including in Kerala, a non-malarious state, was recorded and the species was reported to be involved in two major outbreaks of malaria. It appears that over the years *An. stephensi* has invaded the Lakshadweep islands and gained a permanent foothold on these islands due to the availability of a large number of community hand rain-harvesting cement storage tanks. These developments also threaten the northern islands of Maldives which are about 90 km south of Lakshadweep, where water storage practices are identical.

Genesis of *An. stephensi* transmitted urban malaria:

An. stephensi consists of three major biotypes: type, intermediate and *mysorensis* forms. These biotypes differ in their morphology, ecology, behaviour and mating characteristics. In India, the *An. stephensi* type form is considered the most important vector of malaria in urban areas. The intermediate form is a more common vector in rural areas. The *mysorensis* form is generally considered to be a poor vector of human malaria due to its preference for feeding on animals; however, it has been confirmed as a human malaria vector in some locations, such as in the Islamic Republic of Iran.

An. stephensi transmitted urban malaria, as a specific problem in India, was first recognized in 1969, after an in-depth review of the situation of malaria in India by Madhok Committee (Madhok, 1970), though it was first recognized in the early part of the 20th century as malaria vector in urban areas. Many of the local bodies carrying out anti-larval operations earlier failed to continue due to a paucity of funds and quick containment of malaria outbreaks in limited cities viz., Bombay, Delhi, Lucknow, etc., which did not precipitate in a perception that malaria was a major problem. The large-scale migration of population, creation of slum clusters, construction activities, rapid urbanization and water storage practices have additionally contributed to the establishment of malaria foci. *An. stephensi* control is therefore based on source reduction, larviciding, minor engineering interventions, legislative measures, building bye-laws and human factors.

Strategies against invasive *Anopheles stephensi*: Generally, in responding to invasive species, three sequential approaches of feasibility, efficacy and impact of control interventions can be considered so that an evidence-based strategy can be built for control, containment and elimination.

Control is accepting that the species has invaded certain areas, and controlling it is to mitigate a potential increase in the area.

Containment is allowing the species to remain in a certain area but conducting surveillance and intensive control, particularly at the fringes, to prevent further spread.

Elimination is to ensure that the species from the invaded area is eliminated by building on efforts made under control and containment.

Tracking the spread of *An. stephensi* is also very important in view of its invasion to newer areas and WHO Malaria Threats Map has a dedicated section on invasive vectors, including *An. stephensi*. All confirmed reports of the presence of *An. stephensi* therefore, need to be reported to WHO to enable an open sharing of data and an up-to-date understanding of the vector's distribution and spread.

An. stephensi Control: Anopheles transmitted malaria control guidelines provide the control of vectors by source reduction, filling and levelling, channelizing, de-silting, de-weeding, periodical cleaning of drains, solid waste disposal, sanitation, empty water container once in a week, recurrent anti-larval measures like Temephos, *Bacillus thuringiensis*, insect growth regulators (IGRs) and biological control agents by application of larvivorous fish viz, *Gambusia affinis* and *Pocelia reticulata*. The control of these vectors lies primarily in the implementation of urban bylaws to prevent mosquito breeding in domestic and peri-domestic areas, residential blocks and government/commercial buildings, construction sites etc. Use of larvivorous fish in the water bodies such as slow-moving streams, lakes, ornamental ponds, etc. is also recommended. Larvicides are used for water bodies, which are unsuitable for use of larvivorous fish. Awareness campaigns are also undertaken by Municipal Bodies/Urban area authorities. Guidelines for legislative control by the National Centre for Vector Borne Diseases Control (NCVBDC) is also shared for its adoption and effective implementation. Municipal Corporation of Greater Mumbai, Navi Mumbai Municipal Corporation, NCT Delhi, Chennai Corporation, Bengaluru corporation, Mangalore, Chandigarh, Bhopal, Agartala, Thane and Goa etc., have enacted on Building Bye-laws with provision to prevent mosquitogenic conditions on the exterior of the buildings and clauses in the contract to keep curing tanks free of mosquito breeding during the construction phase and dismantling of the same before issuance of occupancy certificate like Mumbai and Navi Mumbai Corporation.

Challenges: Challenges include limited surveillance, human behaviour influencing transmission, and the need for tailored control measures in different communities. Resistance to conventional treatments and climate change further complicate control efforts. Since the malaria problem in towns/cities is not perceived as a major threat, no structured health care delivery system like the primary health care system as in rural areas has been established. The provision of meeting operational costs for malaria elimination activities is to be ensured by Urban Local Bodies.

The associated challenges are the area limitations for anti-larval measures in certain pockets of the city and not the entire towns/city limits. The source reduction drives in domestic areas are usually hampered by denial of entry to public health personnel for security reasons. The other challenges include limited community mobilization, multi-sectoral collaboration, and the absence of appropriate civic legislations. The entomological surveillance by skilled entomologists and integrated vector management (IVM) with proper resource allocation are the core challenges for timely tackling the problems. Most disturbing issues to be resolved are urbanization and population Growth which pose several innate issues of resource availability, insecurity.

Spatial Spread: Urban towns are expanding under population pressure spatially. There is growth of sub-cities, for example, Gurgaon sub-city, Greater Noida, Dwarka in National Capital Territory of Delhi, Navi Mumbai and Greater Mumbai, etc. These projects lack infrastructure, water supply, solid waste removal in comparison to the actual need resulting in heavy vector breeding potential.

Vertical spread: Mega towns are now expanding vertically creating new avenues of breeding. For example, firefighting exigencies require the building of two storage tanks one at the ground and the other at the top with the high breeding potential of *An. stephensi*.

Discussions and conclusion: It is established that *An. stephensi* has the potential to spread quickly hence strengthening entomological surveillance is of utmost importance which can determine the extent of the spread of *An. stephensi* and its role in transmission. It is essential to formulate the target specific control measures and assess its impact. Human malaria case surveillance should be used to investigate the potential impact of the vector's presence on malaria, particularly in urban areas. Such surveillance might

provide an indication of the presence of *An. stephensi* in areas where it has not yet been detected. Increased collaboration among National malaria programmes, researchers, funders, and other stakeholders conducting surveillance, research and control of *An. stephensi* is needed to ensure that knowledge is shared, resources are used optimally, and key activities are prioritized. Cross border collaboration for which countries should work together to ensure an effective regional approach. Improving information exchange Information on the presence of *An. stephensi*, as well as on successes and failures in attempts to control the vector, needs to be documented and shared widely and rapidly at both national and international levels to determine best practices and inform the response across invaded areas. It will be important to evaluate the impact of vector control interventions, and particularly new tools, against *An. stephensi*.

There is a need of national responses to *An. stephensi* which should be part of a comprehensive response to malaria vectors as guided by the WHO Global technical strategy for malaria 2016–2030. The WHO Global Vector Control Response 2017–2030 provides a framework for investigating and implementing integrated activities across vector-borne diseases. In urban areas, there is a need to target the most vulnerable sections of society who suffer a double burden of insufficient protection from *An. stephensi* malaria transmission due to inadequate housing and living conditions, and limited financial resources. Existing health and governance structures in urban environments need to invest in programs to manage *An. stephensi* population effectively using established methods and tools for mosquito control.

In India National Rural Health Mission (NRHM) and National Urban Health Mission (NUHM) highlighted financial and technical support to achieve the objectives of malaria elimination and preventing mortality and morbidity due to malaria with improved surveillance and source reduction. NUHM should be operationalized with technical and financial support for *An. stephensi* control for urban Malaria elimination in the country.

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DENGUE VECTOR BREEDING AND TRANSMISSION PERTAINING TO NEW CONSTRUCTION SITES AT CHENNAI, TAMILNADU, INDIA

S SELVAKUMAR

Chief Vector Control Officer
Greater Chennai Corporation, Chennai

Vectors are living organisms that can transmit infectious pathogens from animals or insects to humans. One such vector is Arthropods, which are well known carriers of pathogens that are harmful to the ever increasing world population of both animals and humans alike. They carry deadly pathogens and parasites which may affect human beings. This happens on a larger scale and leads to epidemics and sometimes more drastically pandemics (Mehlhorn, 2008; Mehlhorn et al., 2012).

Mosquitoes are a group of exopterygota insects belonging to the order Diptera. There are around 3600 species of mosquitoes that are known to exist around the world. Indian Mosquito fauna is known to comprise around 393 species belonging to 49 genera. In Tamil Nadu there are 18 species that belong to 7 genera (Prakash et al., 2014). Mosquitoes exhibit complete metamorphosis with a life cycle consisting of egg, larva, pupa and adults. Only female mosquitoes bite animals and people in order to get a blood meal which is required to produce eggs. These mosquitoes are regarded as the greatest menace and WHO has aptly termed them “Public Enemy Number One” (WHO, 1996) The three major mosquito’s genera viz., Aedes, Anopheles and Culex, are a threat to millions of people around the world. They act as vectors for pathogens that are devastating for mankind. They also carry parasites which are capable of transmitting diseases, viz., malaria, filaria, yellow fever, dengue, Chikungunya and Zika virus fever (WHO, 2014, 2016). Amongst the major vector-borne diseases, dengue is a fast-spreading endemic disease in more than one hundred countries. Dengue which is transmitted by Aedes species was identified by WHO as a neglected disease remains the same (WHO, 2010).

The virus responsible for dengue the disease is called the Dengue Virus also denoted as DENV. It spawns a very wide range of sickness in humans. This ranges from inappropriate infection to classic DF and in more serious cases it can also lead to fatal Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) (Gubler, 2010). “DENV is a single stranded RNA virus which belongs to the family Flaviviridae of genus Flavivirus, having four serotypes (DENV1, 2, 3 and 4)”.

Since there are no known medicines or any viable vaccines available at the moment, Prevention of dengue through vector control is the only viable solution that is available to us to prevent outbreaks. Studies on vectors are very much necessary. The Vectors of dengue are able to thrive when there is an abundance of water. Therefore, with foresight and decisive action a healthy Chennai can be created for our posterity. It is a world-wide known phenomenon that “malaria is incidental to building construction” wherein the conditions at construction sites promote vector breeding and the tropical aggregation of labor (who are all carriers of the malaria parasite) results in focal outbreak. If not detected the foci spreads to neighborhoods and gets established in due course of time. This was what happened in Chennai. But recently, dengue has joined the race to add more problems to public health. There is a long history of construction sites acting as breeding grounds for vector mosquitoes throughout the world.

The difference between domestic breeding sites and new construction sites is that the later is able to provide an undisturbed aquatic environment for these deadly vectors to breed and spread like wildfire. The present study aims at conducting a comprehensive analysis of the role of new construction sites in the breeding of vector mosquitoes. Any developmental activity that leaves a trail of public health issues should be considered as bad engineering and all our efforts should be taken for sustainable development.



Figure 1. *Aedes aegypti*

Aedes aegypti which is commonly known as yellow fever mosquito can be recognized by its distinct white and black markings on its legs. A distinct feature of this mosquito is a lyre shaped structure on its thorax. They exhibit sexual dimorphism where the females are larger than males. When viewed under the microscope females are found to possess small palps tipped with silver or white scales, and antennae have sparse short hairs, however those of males are feathery.



Figure 2. Female (center and right) and Male (left) *Ae. aegypti* E.A. Goeldi, 1905

Aedes aegypti mosquitoes can be found in abundance during monsoon and post monsoon season. They need clean water to survive and reproduce. *Aedes aegypti* are able to survive in urban and semi urban environments even when there is scarcity of water because of water storage practices. The mosquito has adapted itself well to tropical climates and therefore they are able to thrive in Chennai.

Breeding and Life Cycle

The primary vector for dengue virus is *Aedes aegypti*. It thrives in urban and semi urban environments. They prefer to breed in non-natural containers like containers used to store water (plastic buckets, drums, water tanks) along with items like bottles, tyres, and discarded coconut shells. In the context of new construction sites cement tanks, mixers, overhead tanks. In unfavorable conditions like extreme dry environments their eggs are able to remain viable for more than a year in a dry state. These dry eggs are able to hatch out when water is available in abundance again. If the female who laid the eggs was carrying dengue virus, then the virus is also able to remain in the eggs for the same period in dry state. The mosquitoes that emerge when water becomes available again will be ready carriers of this deadly virus. Eggs are laid in moisture laden surfaces just above the water line and the eggs hatch out once the water level goes above the eggs. They exhibit complete metamorphosis with all four stages that include egg, larva, pupa and adult.

Resting Habits, Flight range, Feeding habits and Biting time

This mosquito is found to prefer resting in dark areas of rooms, in the corners of houses. They will also be found resting in dark clothing and darkly coloured furniture and also on curtains and beds.

The average flight range of *Aedes aegypti* is 100 to 300 meters depending on several factors like geographical location and land and sea breeze. When these factors are taken into consideration, they are able to traverse distances as far as 400 meters.

Males feed on fruit but females require a blood meal for eggs to develop. They were generally considered as day biters and are found to be more active during the daytime. They are sensitive biters and will be found biting several times and several people to complete its meal.



Figure 3. *Aedes albopictus*

Aedes albopictus commonly known as Asian tiger mosquito can be recognized by its distinct white stripes that begin on the dorsal surface of the head and that runs along the thorax. They also exhibit sexual dimorphism where the females are larger than males. The antennae in males are found to be bushier when compared to females. A very distinct characteristic feature to identify the Asian tiger mosquito is the presence of a single silvery-white line of tight scales. This line begins between the eyes which continues down the dorsal side of the thorax. This marking is the easiest and surest way to identify the mosquito.

Aedes albopictus are a feral species of mosquitoes that are known to exist in areas beyond the semi urban domain. However due to the expansion of the city of Chennai they have become the secondary vector for dengue. They can be found in areas like gardens and parks. They are known to co-breed in garbage areas.

Breeding and Life Cycle

Aedes albopictus is the secondary vector for dengue virus in non-domestic environments. They prefer to breed in natural habitats like tree trunk holes, latex collection cups found in rubber plantations and coconut shells

Resting Habits, Flight range, Feeding habits and Biting time

This mosquito is found to prefer resting in outside habitats like shrubs, bushes, and grass. They are mostly found in non-domestic settings however of late they are also being found in domestic areas as well. The average flight range of *Aedes albopictus* is 400 meters. They are also able to spread their eggs through passive transportation. It is also a day-biter, and they prefer to feed on vertebrate hosts that include humans. They are aggressive biters and will not relent till blood meal is procured from its chosen host.

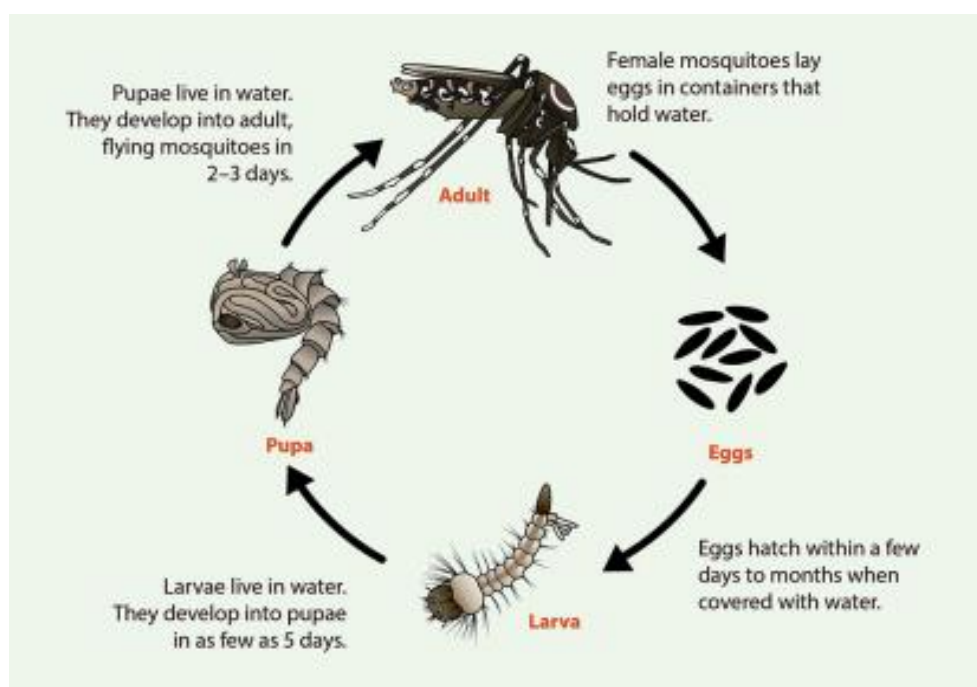


Figure 4. Life cycle of *Aedes* species

CENSUS OF DENGUE CASES IN CHENNAI

Chennai city is located on the east coast of the Bay of Bengal. The Greater Chennai Corporation is the civic body responsible for the administration of the city. The public health and hygiene department is responsible for the health and sanitation of the state. The vector control officer holds the responsibility of controlling the vector and disease spread in each zone. In Chennai, as per the 2011 Indian census, the population is 4,646,732. However, its urban/metropolitan population is 8,653,521. Of the four southern Indian States Tamil Nadu is affected the most and further the number of dengue cases is the highest in the city of Chennai which has all for DENV serotypes. Of the four southern Indian States Tamil Nadu is affected the most and further the number of dengue cases is the highest in the city of Chennai which has all for DENV serotypes. Therefore our study focussed on the breeding sites of the Mosquito vector that transmits Dengue.

Identifying and categorizing Dengue Vector breeding sites pertaining to New Construction sites in Chennai, Tamil Nadu, India

Chennai is stratified into three regions, viz., North, Central and South regions. These three regions are divided into Fifteen Zones which are further split into 200 divisions. The North region consists of Zone 1-5 and is divided into Divisions 1 to 63, the Central region Consists of Zone 6-10 and is divided into divisions 64 to 142 and the South region consists of Zone 11-15 and is divided into divisions 143 to 200. Zone 1 starts at Tiruvottiyur and Zone 15 ends in Sholinganallur.

Categorization of breeding Sites

The categorization and characteristics of different types of construction projects like the mega constructions, Commercial and Apartment complexes, Individual houses are one of the silent breeding sites of mosquitoes that transmit dengue. New construction sites exhibited many *Aedes* breeding in areas with water stagnation. New construction sites (Fig 5) with the sole purpose of understanding the correlation between the breeding of Dengue vectors i.e., *Aedes aegypti* and *Aedes albopictus* and transmission of Dengue to the neighboring vicinities.



Figure 5. *Construction sites*

From the study it has become evident that these temporary aquatic habitats found in these new construction sites seem to provide a greater breeding potential to these life-threatening vectors. New Construction sites are classified into 3 types Viz, Mega projects, Commercial and residential (apartments) and Individual houses. Sites from each Region i.e., North, Central and South Chennai have been documented.

Influence and significance of breeding habitats for dengue transmission

Entomological Survey

Standard entomological techniques were utilized for the survey. Qualitative larval sampling was conducted in all temporary aquatic habitats. Live larvae were collected and reared in the laboratory until adults emerged and they were identified. The breeding prevalence of *Aedes* Species was detected in diverse breeding areas of all the categorized sites. Every accessible larval breeding habitat like sunken portions, metal and plastic drums, Cement mixers and cement tanks, plastic buckets, lift pits were inspected.

Disease Data

RT-PCR results along with data pertaining to Dengue cases of Chennai divided into Regional, Zonal and Divisional wide reports from January 2020 to December 2021 was obtained from the Greater Chennai Corporation. Standard statistical tools were employed to analyze the Data.

Detection of Dengue Virus (DENV) from Vectors

All mosquito pool samples collected from the field will undergo molecular analysis. The sample processing will take place in a Type II Biosafety Cabinet with HEPA filters. RNA will be extracted using a Kingfisher Flex Nucleic Acid Extractor according to the manufacturer's guidelines. The purified RNA will be analyzed for Dengue Virus (DENV) using RT-PCR with a commercial kit. DENV-positive RNA samples will then undergo serotyping by Real-Time PCR using a Multiplex RT-PCR Kit, which detects and differentiates specific DENV RNA and its serotypes.

Control measures to control Dengue Vector breeding in New Construction Sites

The key concern here is the role that construction sites play in **dengue vector proliferation**. The open nature of these sites, combined with the presence of water and debris, creates opportunities for

mosquitoes to breed undisturbed. Whether the project is large (mega construction), medium (commercial construction), or small (individual houses), all of these sites can be vulnerable to the spread of dengue if proper precautions (e.g., eliminating standing water, covering containers, and ensuring the construction site is regularly cleaned) are not taken. It may be essential to include vector control measures in construction planning and to regularly monitor these sites to mitigate the risk of dengue transmission.

Total number of dengue cases region wise

For the year 2020 Zones 1 to 15 recorded a total of 139 dengue cases (Fig 6). The North region (Zone 1-5) showed a total of 48 cases. The Central region (Zone 6 -10) showed a total of 56 cases and the South region (Zone 11 – 15) showed a total of 35 cases. For the year 2021 Zones 1 to 15 recorded a total of 839 cases. The North region (Zone 1-5) showed a total of 189 cases. Central region (Zone 6 -10) showed a total of 374 and the South region (Zone 11 – 15) showed a total of 276 cases. January showed a total of 18 cases, September was seen to record 15 cases, October being the second highest for the year showing a total of 25 cases, November showed 14 and December recorded the highest at 29 cases. 2021 saw a sharp rise in cases from the month of August showing a total of 102 cases, 153 cases for September, 173 for October being the highest for the year, 111 in November and 93 in December respectively. The sharp rise in cases could be attributed to the decline in Covid-19 infections, relaxation of lockdowns and increase in the number of construction sites resuming activities and also because of newer constructions (Fig 7).

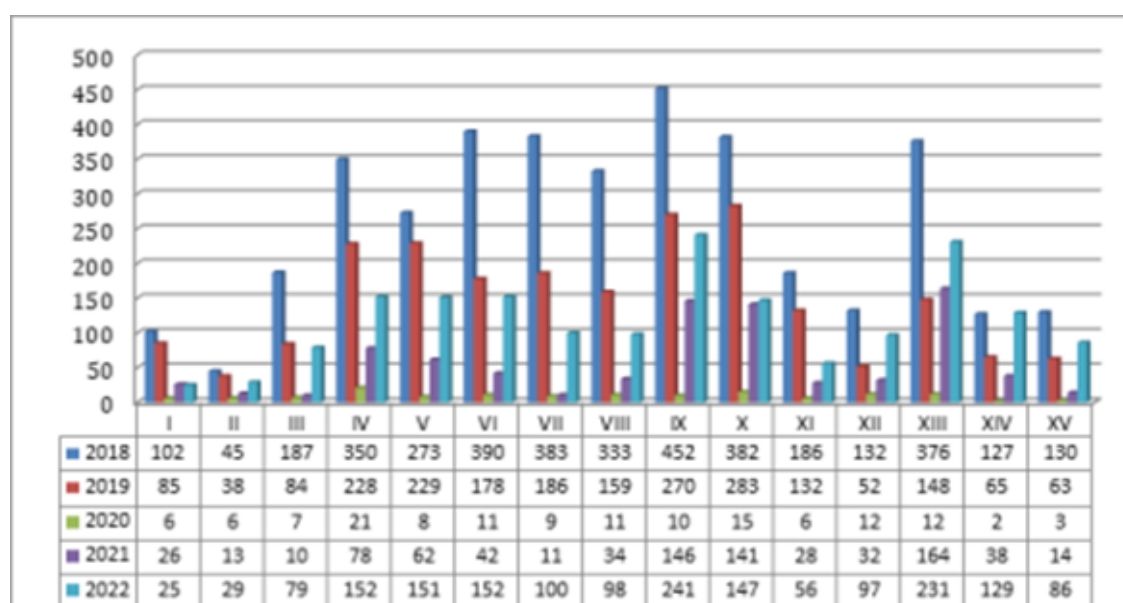


Figure 6. Zone and year wise dengue cases reported in Chennai city-2018 to 10.12.2022

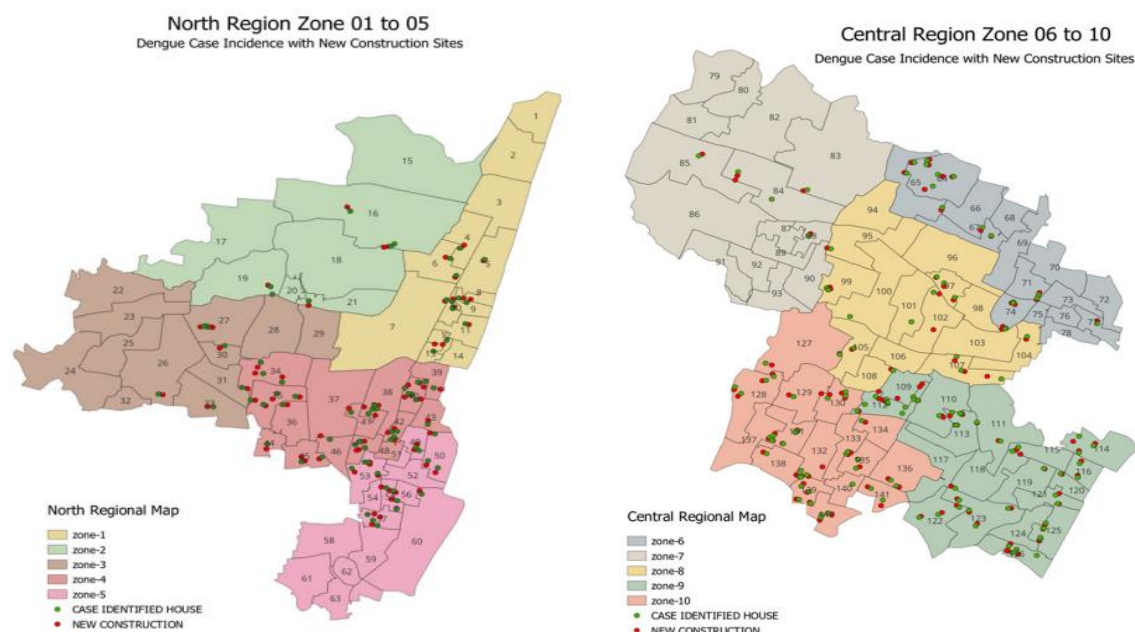


Figure 7. Zonal Distribution Map- Dengue cases Chennai

Detection of Dengue Virus (DENV) from Vectors

All the Mosquito Pools collected from the field are to be subjected to Molecular Analysis. Blood samples are sent from GCC to DPH who conduct the RT-PCR tests and send back the results to GCC. The sample processing to be carried out in a Type II Biosafety Cabinet with HEPA filters. RNA extraction will be done using King Fisher Flex Nucleic Acid Extractor as per manufacturer's instructions. The purified and extracted RNA will be subjected to DENV RT-PCR analysis using a commercial kit. DENV RT-PCR Positive RNA Extracts to be subjected to Dengue virus (DENV) serotyping by Real Time PCR using Multiplex RT-PCR Kit for the detection and differentiation of Dengue Virus specific RNA and its serotypes.

Every hospital will need to report to GCC about dengue patient information. Once this data reaches GCC field entomologists will be deployed to the patient's home address to ascertain if other members in the patient's family and neighboring families have also been affected by dengue.

Control measures for dengue vector breeding in new construction sites

Source reduction, elimination of container habitats that are favorable oviposition sites and which permit the development of the aquatic stages; by tightly fitting lids or covers on containers or by killing larval and pupal stages using insecticides. Agro-industrial modifications of habitats of mosquitoes along with climatic variations influence outbreak of these diseases by mosquito species as vectors in the study area. Mosquitoes utilize a great variety of water sources for breeding. These include ground pools, water in artificial containers, water holding tree holes and leaf axils. Depending upon the species involved, the distance of dispersal from breeding areas varies from a few meters to many kilometers.

Saline water

Aedes mosquitoes are able to breed even when temperatures go as high as 39.8 °C. *Ae.aegypti* breeds in alkaline water ranged between 7.5 and 8.5 ph. increasing the salinity of the water by adding rock salt will discourage the breeding of *Aedes* and will thereby bring about a reduction in the transmission of the disease.

Larval and adult control

Regular anti-larval work most preferably on a weekly basis is needed to control the immature stages of mosquitoes. Anti-adult control measures also need to be implemented on a weekly basis or on a fortnightly basis at the least by spraying and fogging.

Surveillance of workers

Weekly to fortnightly surveillance of construction that includes temperature checks and collection of blood samples and also rendering the necessary treatment to workers is recommended. Increased surveillance is very much needed during monsoon seasons.

More than half of the world's population are affected by Dengue. Southeast Asia is one of the hotspots. Temperate climates with strong levels of relative humidity not only aid the breeding of mosquitoes but enable them to breed to a greater extent. Chennai has seen dengue cases increase over the past decade. Rainfall definitely plays a vital role in the spread of this deadly disease.



Chennai is located at 13° 04N and 80° 17E on the southeast coast of India. It is found in the northeast corner of Tamil Nadu abutting the Bay of Bengal. Chennai experiences temperatures between 15.8 °C and 44 °C and RH ranges between 61% and 80%. Chennai receives an average of 1300mm rainfall every year with the highest mounts being during monsoons.

Factors that are known to influence *Aedes* breeding like maximum daylight time have not been taken into consideration in the present study as the lighting environments in new construction sites are completely different when compared with usual breeding sites. New construction sites provide completely dark environments for these vectors to breed. Domestic breeding sites will always have some form of disruption to breeding as water will never be stored for extremely long periods of time. Stored water will either be discarded, or it will remain in constant use causing some form of turbulent activity preventing mosquito breeding. In domestic breeding sites we have to take into consideration the intervention of the local governing bodies

that will usually provide some form of control measures in the form of fogging, misting, spraying of larvicides and also to control adults. New construction sites in China and they have concluded that new construction sites satisfy all the necessary criteria for the breeding of *Aedes*. Construction sites are more than capable of being super breeding sites and they pose a significant health risk to neighboring communities.

Conclusion

The significant role of new construction sites in aiding the breeding of *Aedes* mosquitoes, particularly during the monsoon seasons. The transportation of infected ovaries through equipment like cement mixers and the flight distance of dengue vectors contribute to the spread of dengue from one area to another. The favorable conditions in new construction sites, such as undisturbed environments, temporary aquatic habitats, and micro-climates, provide ideal breeding grounds for these vectors.

Control measures for dengue vector breeding in new construction sites include source reduction, elimination of container habitats, and the use of insecticides. Increasing the salinity of water and regular anti-larval and anti-adult control measures are also effective strategies. Surveillance of workers and construction sites, especially during monsoon seasons, is crucial for early detection and prevention of dengue outbreaks.

This emphasizes the need for enhanced public awareness, interdepartmental coordination, and robust control measures to mitigate the impact of dengue in Chennai. By addressing these challenges, Chennai

can develop effective strategies to reduce the burden of vector-borne diseases and improve public health outcomes.

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TOOLS AND TECHNOLOGY FOR VECTOR CONTROL

Sridharan Subramanian¹ and P K Srivastava²

¹Former Chief Entomologist, Directorate of Public Health & Preventive Medicine, Tamil Nadu

²Former Joint Director, Head, Entomology & Vector Control Division, National Vector Borne Disease Control Programme (NVBDCP), Delhi- 110054

Corresponding Author Email: roadakka@gmail.com

Abstract

Mosquitoes, flies, bugs and other vectors transmit viruses, parasites and bacteria that infect millions of people globally. They cause many diseases, including malaria, dengue, leishmaniasis, Chagas disease, Zika virus and Japanese encephalitis. Control of these vectors is absolutely essential to bring down the case and death due to vector borne diseases mentioned above. Vector control methods differ according to the bionomics of the vectors.

Defenition: Vector control is any method to limit or eradicate the mammals, birds, insects or other arthropods (here collectively called "vectors") which transmit disease pathogens. The most frequent type of vector control is mosquito control using a variety of strategies. Several of the "neglected tropical diseases" like Lymphatic Filariasis are spread by such vectors.

Introduction: Rapid unplanned urbanization, changing land use patterns and increased international travel and trade bring humans into more frequent contact with vectors, while climate and other environmental changes fuel their spread worldwide. In recent years, vector-borne diseases have moved into new territory, many diseases once limited to tropical and subtropical zones are now increasingly seen in temperate areas. 80% of the world's population is at risk of one or more vector-borne disease. 17% of the global burden of communicable diseases is due to vector-borne diseases. Over 700,000 deaths are caused by vector-borne diseases annually. Certain vector borne viral diseases like Zika and Dengue do not have specific curative medicines or presently preventive vaccine (Dengvaxia and Qdenga vaccines are available in United States, in India the Dengue vaccine has undergone first phase of clinical trials and the vaccine is expected to be commercially available only by mid-2026). In this scenario the vector control against these diseases gain paramount importance. Other diseases like Malaria where specific drugs and vaccines are available, JE where specific vaccine is available, the vector control plays a major role in control of these diseases. Lymphatic filariasis, a neglected tropical disease has neither a direct curative medicine nor preventive vaccine, vector control is the only choice for the reduction of incidence. Various methods of vector control using various tools and insecticides are dealt in this article.

THE GLOBAL VECTOR CONTROL RESPONSE 2017-2030

Reducing the burden and threat of vector-borne diseases that affect humans, which kill mites and ticks, are not strictly insecticides, but are usually classified together with insecticides.

CHALLENGES	MILESTONES 2020	MILESTONES 2025	TARGETS 2030
Reduce mortality due to vector-borne diseases globally relative to 2016	By at least 30%	By at least 50%	By at least 75%
Reduce case incidence due to vector borne diseases globally relative to 2016	By at least 25%	By at least 45%	By at least 60%
Prevent epidemics of vector-borne diseases*	--	In all countries without transmission in 2016	In all countries

**Rapid detection and curtailment of outbreaks to prevent spread beyond the country.*

Based on their chemical nature they are classified as:

- Organic insecticides
- Inorganic insecticides
- Synthetic insecticides

Based on toxicity, it is classified into four types:

- Extremely toxic – Colour: red, symbol: skull and poison, oral LD50: 1-50
- Moderately toxic – Colour: blue, symbol: danger, oral LD50: 501 – 5000
- Highly toxic – Colour: yellow, symbol: poison, oral LD50: 51 – 500
- Less toxic – Colour: green, symbol: caution, oral LD50: >5000

Based on the mode of action, it is classified as physical poisons, nerve poisons, respiratory poisons, protoplasmic poisons. To accomplish the task of controlling the vectors we need the following:

1. Killing agent (Insecticides)
2. Tools or Equipments to disperse the killing agent into the appropriate environment.
3. Methodology of doing the above.
4. Techniques of operating such equipments.

1. KILLING AGENT

INSECTICIDES: Any toxic substance that is used to kill insects. Such substances are used primarily to control pests that infest cultivated plants or to eliminate disease-carrying insects in specific areas. Insecticides can be classified in several ways, on the basis of their chemistry, their toxicological action, or their mode of penetration. In the latter scheme, they are classified according to whether they take effect upon ingestion (stomach poisons), inhalation (fumigants), or upon penetration of the body covering (contact poisons). Acaricides, general poisons, and chitin inhibitors. Based on the mode of entry in the insects, it is classified as contact poisons, fumigants poisons, stomach poisons, and systemic poisons. Insecticides have been a cornerstone in human health, livestock, modern agriculture and pest control, playing a vital role in safeguarding crops. However, their use comes with a complex mix of advantages and disadvantages.

Advantages of Insecticides

- **Disease Control:** Insecticides play a critical role in controlling vector-borne diseases such as malaria, dengue fever, and Zika virus by targeting disease-carrying insects like mosquitoes.
- **Livestock Protection:** Insecticides help protect livestock from pests that can cause diseases and discomfort, thus improving animal health and productivity.
- **Increased Agricultural productivity:** Insecticides protect crops from pests, ensuring higher yields and reducing the risk of crop failure. This is crucial for maintaining food security and stabilizing the supply chain. Insecticides can also help maintain the quality of produce, reducing blemishes and damage caused by the pests that can affect marketability and consumer preference.
- **Convenience and Efficiency:** Insecticides are relatively easy to apply and can cover large areas quickly, making them a convenient option for large-scale public health and agricultural operations.

Disadvantages of Insecticides

- **Human Health:** Exposure to insecticides can pose health risks to humans, including acute poisoning and long-term effects such as cancer and neurological disorders. This is a significant concern for public health and farm workers and communities near treated areas.
- **Environmental Impact:** Insecticides can harm beneficial insects such as bees, butterflies, and other pollinators, disrupting ecosystems and reducing biodiversity. Runoff from fields can lead to contamination of soil and water bodies, affecting aquatic life and potentially entering the human food chain.

- **Insect Resistance:** Over time, insects can develop resistance to insecticides, rendering them less effective and necessitating the development of new, often more potent chemicals. This cycle can lead to increased costs and potential environmental harm.

Integrated Vector Management (IVM) strategies, which combine chemical, biological and physical methods, can help mitigate some of these disadvantages by promoting sustainable and environmentally friendly insect control practices. The challenge lies in balancing the immediate benefits of insecticides with long-term ecological and health considerations, ensuring that we protect human health, our crops and our planet.

In India, The National Centre for Vector Borne Diseases Control has approved an array of insecticides for public health use to be chosen as per the needs and situations.

ADULTICIDE	LARVICIDE
INDOOR RESIDUAL SPRAY:	OIL
1. DDT 50% WP	1. MLO 100% Petroleum product
2. Malathion 25% WP	CHEMICAL
3. Deltamethrin 2.5% WP	1. Temephos 50% EC
4. Cyfluthrin 10% WP	BIOLARVICIDE
5. Lambdacyhalothrin 10% WP	1. Bacillus thuringiensis var Israelensis – Strain 164, Serotype H-14 5% WP
6. Alphacypermethrin 5% WP	2. Bacillus thuringiensis var Israelensis – Strain ABIL, Serotype H-14 5% WP & AS
7. Bifenthrin 10% WP	3. Bacillus thuringiensis var Israelensis 12 Aqueous Suspension (12AS)
INDOOR SPACE SPRAY	INSECT GROWTH REGULATOR
1. Pyrethrum 2% Extract	1. Diflubenzuron 25% WP
2. Cyphenothrin 5% EC	2. Pyriproxyfen GR 0.5% Granules
OUTDOOR SPACE SPRAY	
1. Malathion Technical Grade	
2. Cyphenothrin 5% EC	

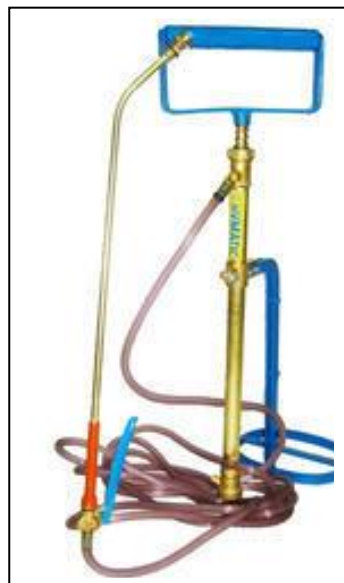
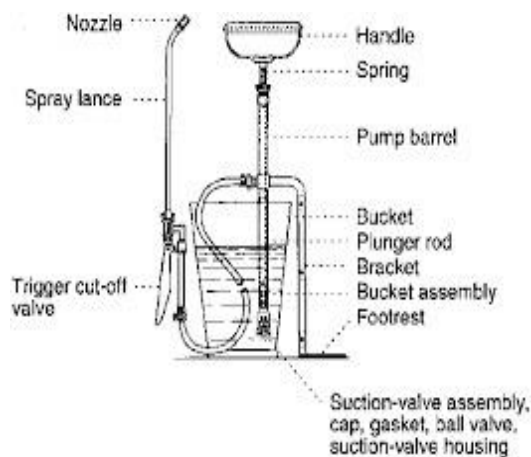
** Details like Dilution and Dosage please refer NCVBDC web site*

2. TOOLS OR EQUIPMENTS TO DISPERSE THE KILLING AGENT INTO THE APPROPRIATE ENVIRONMENT

- **STIRRUP PUMP:** The pump is mounted on a footrest or stirrup, is inserted in the spray liquid in a bucket. A hose attached to the pump leads to the spray lance. It is used for Indoor Residual Spray against adult mosquitoes. Two persons are needed, one to pump and one to direct the spray. The pressure varies with the speed of pumping, and so it is difficult to make uniform spray

applications. The Trigger valve, Foot valve, Washers and Nozzle should be cleaned regularly. Plastic Hose-to-Body joints should be tightly and perfectly fitted to avoid leakage.

STIRRUP PUMP



➤ METHODOLOGY OF IRS

Definition

IRS is the application of a long-lasting, residual insecticide to potential malaria vector resting surfaces such as internal walls, eaves and ceilings of all houses or structures (including domestic animal shelters) where such vectors might come into contact with the insecticide. When carried out correctly, IRS is a powerful intervention to rapidly reduce adult mosquito vector density and longevity and, therefore, to reduce malaria transmission. When a vector comes into contact with a sprayed surface, it absorbs a lethal dose of insecticide, thereby reducing its lifespan. . This results in a progressive decline in vector density and longevity, especially among older female mosquitoes, and a reduction in overall vectorial capacity, thereby contributing to a reduction in malaria transmission.

Objectives of IRS: The objectives of IRS are to reduce, and ultimately interrupt, disease transmission by reducing vector survivorship, density, and human-vector contact, in a manner that is safe for human health and the environment.

Outcomes of IRS: When applied properly, IRS is a powerful malaria vector control intervention, rapidly reducing vector-transmission capacity and malaria incidence. IRS provides maximum mass effect on the vector populations when it is applied at high coverage levels.

Applying insecticide: The insecticide suspension has to be sprayed evenly and at the recommended application rate over all sprayable surfaces. The amount of insecticide that is sprayed on a surface is determined by a number of different factors.

Factors that are influenced by the spray operator during the spraying:

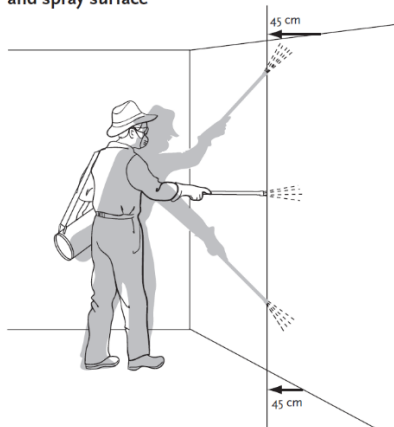
- Distance from the nozzle tip to the surface being sprayed (should be kept at 45cm).
- Speed of movement of the nozzle over the surface.
- When using a Hand Compression sprayer - air pressure in the sprayer (should be maintained at 172–380kPa (25–55psi) or (1.7–3.8 bar) for a sprayer without a 1.5 bar CFV.

Factors that are not directly influenced by the spray operator, but that require attention during preparation for actual spraying are:

- The concentration of insecticide in the suspension.
- The nozzle tip aperture size.

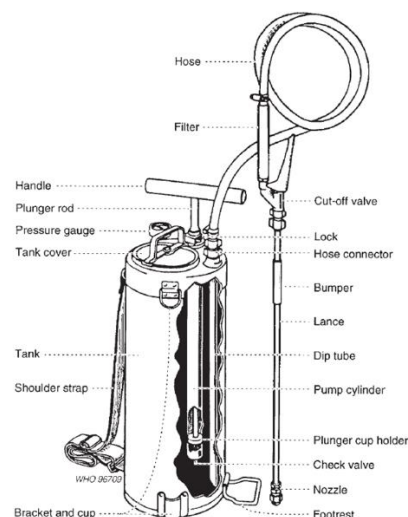
Insecticide spray procedure: The first swath is from top to bottom. After the first swath, the spray operator should take a step sideways to get to the middle of the next swath and cover the second swath from bottom to top. The correct footwork should be maintained together with the hand spray speed to generate the correct rhythm. The correct timing for spraying a 2 m swath is 5 seconds (i.e. each linear metre covered should take 2.2 seconds). Timing may be aided by mentally counting “one thousand and one, one thousand and two, one thousand and three”, etc. Adjust the mental counting procedure according to the local language.

Maintaining the distance between spray nozzle and spray surface



- **HAND COMPRESSION SPRAYER:** Consists of a tank for holding a liquid insecticide formulation, which can be pressurized by means of a hand pump attached to it. The compressed air forces the liquid from the tank via a hose with a cut-off valve, a lance and a nozzle. The sprayer can be used for Residual and Larval spray and only one person is required for the operation of this sprayer. The sprayer should withstand the pressure and comply to relevant Country Standards (BIS).

HAND COMPRESSION SPRAYER



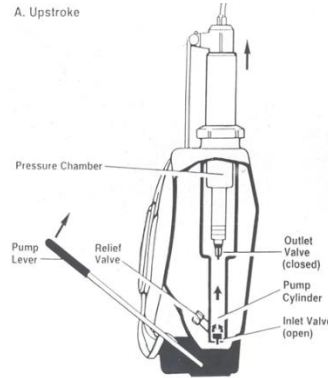
- **KNAPSACK SPRAYER:** The Sprayer is carried on the back. A frame or shield prevents contact between the tank and the back. It is a continuous type of sprayer with a fairly constant discharge rate. The person maintains pressure in the tank by pumping air by a lever with one hand and

directs the spray lance with the other. The knapsack sprayer can be used for spraying breeding sites with larvicides but should not be used for residual wall-spraying.

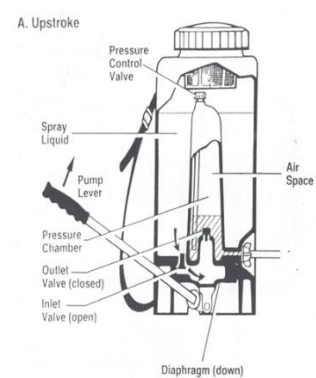
KNAPSACK SPRAYER



PISTON TYPE

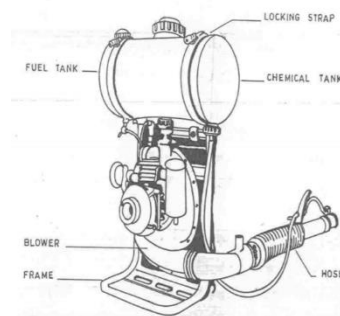


DIAPHRAM TYPE

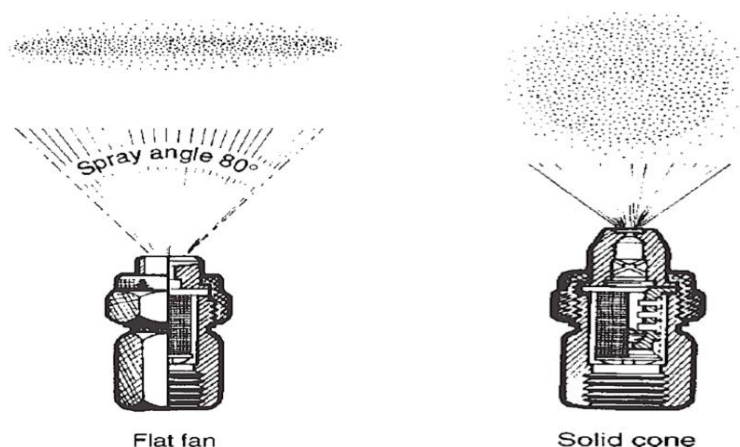


- **POWER SPRAYER:** It is a simple two-stroke engine driven sprayer used in Public Health and Agriculture. It is carried on the back of the operator. It is used for spraying against endophilic mosquitoes in case of an outbreak of Dengue and mites for the control of Scrub typhus. It consists of a 1.2 - 3.0 hp high speed petrol engine, a blower, a 12 lit chemical tank, delivery hose, fleeted air hose, flow regulator knob and a plastic atomizer grate. The tank is filled with the required chemical solution. When the engine is started, the blower generates a high velocity air to which the chemical solution is fed. The chemical mixed air stream is broken into fine droplets at the atomizer grate and sprayed. The particle size is about 50 μ m.

POWER SPRAYER



NOZZLES USED FOR SPRAY: Flat fan Nozzle is used for Residual Spray, while Solid cone is used for Larval Spray. It is vital to clean and maintain these nozzles.



- **Control Flow Valve:** Newer pumps with Control flow valve (CFV) are recommended by WHO to have uniform spray. These pumps have completely replaced Stirrup pumps used for IRS in Kala azar control. Advantages of CFV are, during IRS, the flow rate needs to be constant. If there is no CFV, the pressure decreases gradually, and it has to be pumped again. To avoid a decrease in flow rate, and to ensure an even discharge as tank pressure drops, it is recommended that flow regulators (*i.e.*, 1.5 bar CFV) are fitted in the nozzle tip. By using a CFV, the operator (spray-person) gets constant pressure at the nozzle that ensures the output remains the same during the application and also ensures that the droplet spectrum does not change. When pressure in the tank falls below the amount set by the valve, the spray immediately shuts off.

Control Flow Valve

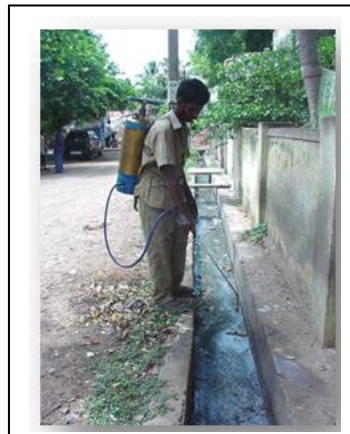


WHO recommended 8002E flat fan nozzle: 80° swath + liquid output of 0.2 US gallons (750ml) per min at 3 bar pressures = 550 ml/min at 1.5 bar pressure and uniform spray.

LARVICIDING: Larviciding is the application of biological or chemical insecticides that kill the immature stages of mosquitoes and is one of the approaches of larval source management (LSM), along with habitat modification, habitat manipulation and biological control. Larviciding to control immature stages of mosquitoes is usually limited to peri-domestic and or domestic-use containers that cannot be destroyed, eliminated or otherwise managed. It is also implemented in and around houses, drains, wells etc. Larviciding has to be done weekly/fortnightly to avoid emergence of adults. It is difficult and expensive to apply chemical larvicides on a long-term basis. Therefore, chemical larvicides are best used in situations where it is feasible and other core interventions like IRS is not acceptable. Establishing the precise timing and location are essential for maximum effectiveness.



Temephos Application



Bti Application

THERMAL FOG OPERATION: Adulticide by thermal fogging is accomplished by providing a specific dose of small air borne (or aerosol) hundreds of millions of tiny drops into which the mosquito or other vectors will fly and impinge drops on wings, legs and body and provide a lethal contact dose. Thermal fogs are produced by special equipment through which the insecticide (usually dissolved in oil with a suitable flash point) is vaporized by being injected into a high-velocity stream of hot gas. It is used for any pest control task where active substances should be uniformly distributed, even in inaccessible places, without leaving undesirable residues. This fogging method is the solution for treating large areas and spaces in short duration especially during an outbreak of vector borne disease and with minimum quantity of pesticide solution, less operational work and with little harm to the environment (less residues, no penetration into the ground). Space treatments should be timed to coincide with the period of flight activity of the mosquitoes, *e.g.*, after dusk and before dawn for *Anopheles spp.*, and late morning (after sunrise) and early evening (just before sunset) for dengue vectors. The success of the application depends on the relation between the droplet size and the intrinsic toxicity of the insecticide on the target insects. The optimal droplet size of the insecticide aerosols ranges from 10-20 μ m VMD for mosquitos and for larger flies it is 30 μ m VMD. An advantage of thermal fogging from the public relation standpoint is the dramatic visibility of this operation, the local inhabitants thus having clear evidence that authorities are indeed taking positive action against their mosquito problem.

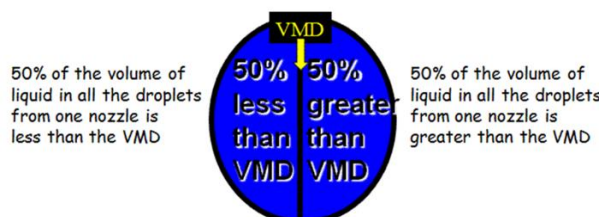
- **VEHICLE-MOUNTED THERMAL AEROSOL FOG GENERATOR:** Used in big cities and municipal corporations. The cost of operation is very high, but a huge area can be covered in a short time. The fogging operation should be done only in motorable roads with the vehicle speed of about 6 to 8 kms/hour and against the wind direction. The fogging operation may cause disruption in traffic regulation, especially in the evenings. For one hour of operation, the fog machine may consume 100 litres of HSD, 5 litres of Technical Malathion/Pyrethrum and 10 litres of Petrol plus vehicle running cost. The VMD of the particle size should be less than 25 μ m such that the particles are airborne for a long time and impinge in the body of the flying insects resulting in their death. This is a big and heavy fog machine. The maintenance of this fog generator should be done by a trained mechanic. For regular servicing and maintenance, the company of purchase may be addressed. However, checking the electrolyte level (acid level) in the battery, its terminals, fanbelt, filter inside the formulation tank, fog injection assembly, spark plug in the engine and the heating chamber, oil level, fog ejection nozzle may be inspected and attended-to regularly.



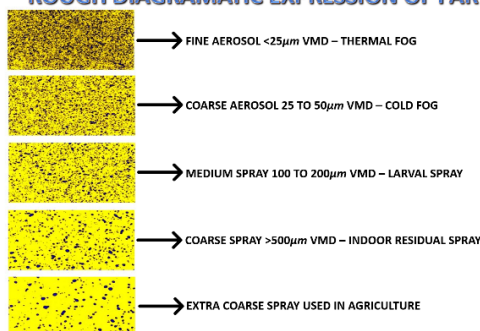
- **HAND OPERATED THERMAL FOG GENERATOR:** Widely used in Public Health for the control of vectors especially endophilic. Very simple to operate, needs minimum manpower and fairly low cost of operation. Since the weight of the machine is around 10 kgs, it can be taken even to high-rise buildings. The output particle size should be less than $25\mu\text{m}$ VMD. Mixing of insecticide and the diesel should be done in a separate container and mixed well before transferring to the formulation tank. If this procedure is done inside the formulation tank the insecticide at the bottom of the tank will clog the filter at the end of the formulation in-take hose. When the fogging season is over or if there is going to be a gap of 1 to 2 months, clean all the parts, clean the formulation tank thoroughly with soap. Reassemble all the parts and start the fog operation with soap water in the formulation tank, this will clear all the lines and the valves en route.



DROPLET/PARTICLE SIZE: The Particle size is expressed in volume median diameter (VMD) which refers to the midpoint droplet size, where half the volume of the spray contains droplets with a diameter less than that of the VMD; droplets in the other half by volume have a diameter larger than that of the VMD. The diameter of a droplet whose volume, if multiplied by the total number of droplets, will equal the total volume of the formulation. The particle size should be measured at least once in every 6 months or before the start of fog operation of the season.



ROUGH DIAGRAMATIC EXPRESSION OF PARTICLE SIZE



MOSQUITO NET: Mosquito nets have been in use since very early times to protect people against blood sucking insects at night, they also help to protect against other creatures such as spider, cockroach, beetle, lizard, snake, and rat. They also provide privacy when made thicker and opaquer. Sleeping under a bed net reduces the risk of man-vector contact as mosquitoes bite at night and is thus an effective preventive measure. But ordinary mosquito nets provide limited physical barrier between mosquito and man and protection as they may still bite through the net or get inside the net following improper use. The Insecticide Treated bed nets (ITNs) or Long Lasting Impregnated Bed nets (LLINs) provide better and effective protection by keeping away mosquitoes as well as killing them. ITNs and LLINs also kills or keeps away other nuisance insects - cockroaches, bedbugs, houseflies, fleas, etc.

- **INSECTICIDE TREATED BED NETS (ITNS):** Insecticide treatment is recommended for synthetic nets (nylon, polyester), as treatment of cotton nets is not cost-effective, and effect of insecticide is not long lasting. Insecticides used for mosquito nets are not harmful to people, if used correctly. These nets should be treated with correct amount of insecticide mixed with correct amount of water. The nets should be fully soaked in the insecticide-water mixture and after allowing the excess liquid to drip by gently squeezing, let the net dry flat in a plastic sheet in a shade and later it may be hung to finish up drying. Avoid frequent washing of the net as it will remove the insecticide from the net.
- **LONG LASTING INSECTICIDAL NETS (LLINS):** LLINs are mosquito nets which have the insecticide incorporated in their fibre, so that it is not removed by as many as 20 washes. Because these nets have an even and quality controlled insecticide application, they are generally more effective than conventional ITNs. Furthermore, the LLIN is more cost-effective (as it can be used for 3-5 years) than distribution of conventional bed nets and treating them with insecticide once or twice a year. Generally, for a targeted village, the required number of LLINs should be distributed in one single operation. In addition to distribution to targeted high-risk villages aiming at complete population coverage, LLINs should be given to pregnant women in high risk areas and to special groups such as children in tribal schools and hostels. Number of LLINs = Number of households x 2.4.

CONCLUSION: Vector control operations should be conducted systematically and correctly following all the instructions given by the State Health Directorate and National Centre for Vector borne Diseases, Delhi. The operations are costly and should be supervised thoroughly by supervisors of different levels (Block, District and State). The operations whether Indoor Residual Spray, Larval Source Management or Thermal Fog, if conducted systematically shall protect the public from these deadly and dreadful diseases there by desired results of bringing down the case incidence can be achieved.

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AI FOR VECTOR DISEASE CONTROL

Srishti Srivastava, Pradeep K. Srivastava

University of Southern Indiana, USA, and Absolute Human Care Foundation, India

Correspondence Email(s): fsrishti@usi.edu, pradeepksrivastava@gmail.com**Abstract**

Vector-borne diseases (VBDs) remain a significant global health challenge, causing millions of infections and deaths annually. Traditional control methods, while effective, often face limitations such as environmental impact and resistance development. Artificial Intelligence (AI) has emerged as a promising tool to revolutionize VBD control strategies. This chapter explores the potential applications of AI in various aspects of VBD management. By leveraging AI algorithms, we can enhance surveillance, prediction, and intervention efforts. AI-powered early warning systems can predict outbreaks in advance, allowing for timely and targeted interventions. Advanced image analysis techniques can be used to monitor vector populations and identify potential hotspots. Additionally, AI can optimize resource allocation and improve the effectiveness of control measures. However, the successful implementation of AI in VBD control requires addressing several challenges, including data quality and availability, model interpretability, and ethical considerations. By overcoming these challenges and fostering interdisciplinary collaboration, we can harness the power of AI to significantly reduce the burden of VBDs and improve global health outcomes.

Keywords: Artificial Intelligence, Vector-Borne Diseases, Public Health, Machine Learning, Disease Control

Vector-borne diseases (VBD), such as malaria, dengue fever, and Zika virus, have posed significant threats to global health for centuries (DJ., 1998). These diseases are transmitted to humans through vectors, primarily mosquitoes, ticks, and flies, and have caused numerous epidemics and pandemics throughout history. Traditional methods of controlling VBD have included chemical control (insecticides), environmental management, and public health campaigns aimed at reducing vector populations and preventing human exposure (Wilson, 2020). The advent of artificial intelligence (AI) has brought new and promising tools to the field of disease control. Figure 1 illustrates that the AI healthcare market is poised for explosive growth. By 2025, it's projected to surpass \$2 billion for the first time. This momentum will continue, with a 146% increase between 2025 and 2028. By 2030, the market is expected to exceed \$10 billion and further soar to \$21.74 billion by 2032. AI's ability to analyze vast amounts of data, identify patterns, and predict outbreaks has the potential to revolutionize the control and prevention of VBDs. By leveraging machine learning algorithms, AI can enhance the precision and efficiency of existing control measures, making it possible to respond more effectively to emerging threats (Bohr, 2020).

This chapter explores the application of AI in controlling VBDs, highlighting the methods, successes, and challenges associated with integrating AI into public health strategies. The discussion will encompass various AI techniques, including machine learning, neural networks, and predictive modeling, and their real-world applications in monitoring, predicting, and controlling disease outbreaks. The goal is to provide a comprehensive understanding of how AI can be harnessed to combat vector-borne diseases and improve global health outcomes.

Background and Related Work

Vector-borne diseases (VBDs) pose a significant global health threat, causing millions of infections and deaths annually. These diseases, transmitted by biological vectors like mosquitoes, ticks, and fleas, have historically been challenging to control. Traditional methods, such as insecticide and larvicide spraying, often face limitations like environmental impact, resistance development, and operational inefficiencies. Recent advancements in artificial intelligence (AI) offer a promising avenue to revolutionize VBD control strategies. By leveraging AI's capabilities in data analysis, pattern recognition, and predictive modeling,

researchers and public health officials can gain deeper insights into the epidemiology of VBDs and develop more effective interventions. Some of the key areas where AI is being applied to VBD control include the following.

- (i) **Surveillance and Early Warning Systems:** AI-powered systems can analyze data from various sources, including satellite imagery, weather data, and social media, to identify areas at risk of outbreaks.
- (ii) **Vector Control:** Smart spraying: AI-powered drones and robotic systems can optimize insecticide application, reducing chemical usage and minimizing environmental impact and Larval source mapping: AI algorithms can analyze aerial and satellite imagery to identify and prioritize breeding sites for targeted larvicide spraying.
- (iii) **Disease Prediction and Forecasting:** Predictive modeling: AI models can forecast the spread of VBDs based on factors like climate conditions, vector behavior, and human population density. And Risk assessment: By analyzing historical data and real-time information, AI can assess the risk of outbreaks in specific regions.
- (iv) **Community Engagement and Behavior Change:** personalized messaging: AI-powered tools can deliver targeted health messages to individuals based on their specific needs and preferences. And Gamification: AI can be used to create interactive and engaging games to educate people about VBD prevention and control.

The authors in (Pley, Lowe, Montgomery, & Yacoub, 2021) explore the integration of AI and digital technologies in the surveillance and management of vector-borne diseases, particularly in the context of climate change. It emphasizes the sensitivity of these diseases to climatic variations and highlights how innovative surveillance systems can incorporate climatic data to enhance outbreak prediction and response. The authors discuss the importance of community involvement in the design of these technologies to ensure their effectiveness and sustainability. Additionally, the paper outlines the potential of mobile health (mHealth) applications and satellite data in improving disease monitoring, advocating for a comprehensive approach that considers social, economic, and environmental factors influencing disease dynamics. The paper, (Tom & Febin Daya, 2024), explores the application of machine learning (ML) algorithms to enhance the vector control of permanent magnet synchronous motors (PMSMs). Traditional control methods, such as Proportional-Integral (PI) controllers, are often sensitive to parameter variations, which can affect performance. The authors investigate various ML regression techniques, including linear regression, support vector machine regression, and feedforward neural networks, to improve speed control in PMSM drives. Simulation results demonstrate that the ML-based controllers outperform conventional PI controllers, achieving better speed tracking and reduced transient responses. This study highlights the potential of ML to optimize motor control systems, particularly in dynamic operating conditions. The paper (Mshani, Siria, Mwanga, Sow, & et al., 2023) reviews the applications of infrared (IR) spectroscopy and ML techniques in malaria surveillance and diagnostics. It highlights the potential of these technologies to enhance the detection of malaria parasites and improve vector control strategies. The authors discuss how combining IR spectroscopy with ML can accurately predict key epidemiological parameters, such as mosquito age and infection status, thereby facilitating more effective malaria management. They identify significant research gaps and propose a target product profile (TPP) to guide the development of these technologies for use in low-income settings. The review emphasizes the need for further validation and standardization to ensure the successful integration of IR-ML systems into routine malaria control programs. The authors in the book chapter, (Motta, Badaró, Santos, & Kirchner, 2023), discuss the transformative potential of AI in enhancing the identification and management of mosquito species that transmit diseases like malaria. It highlights how traditional methods of mosquito identification, which rely on human expertise, are time-consuming and require extensive training. The authors explore various AI techniques, including machine learning and deep learning, that can automate the classification process, improving efficiency and accuracy. By leveraging these technologies, the study suggests that AI can significantly contribute to vector control strategies, ultimately aiding public health efforts in combating vector-borne diseases.

AI-Driven Strategies for Vector-Borne Disease Control: Technology Deep Dive

AI offers the potential to revolutionize VBD control by providing more accurate, efficient, and targeted interventions. Here are some key AI-driven strategies:

Predictive Mapping and Risk Assessment Technologies:

1. *Convolutional Neural Networks (CNNs) in Vector Habitat Prediction*

Technical Architecture: Convolutional Neural Networks are specially designed deep learning models that excel at processing spatial data, making them ideal for analyzing satellite imagery and geospatial information.

Practical Application Scenario: a CNN could analyze satellite imagery of a region and identify potential water-logged areas, agricultural landscapes with irrigation systems, urban zones with poor drainage, and seasonal landscape changes affecting vector habitats

2. *Random Forest Algorithms for Risk Probability Calculations*

Comprehensive Data Integration Approach: Random Forest algorithms excel at combining multiple data sources to create probabilistic models with high predictive accuracy.

Data Sources Integrated: satellite imagery, climate records, historical disease transmission data, population density information, healthcare infrastructure data, environmental monitoring records

Integrated Implementation Example: for Malaria Risk Prediction: a hypothetical deep learning model for malaria transmission risk would collect multi-source geospatial data, process satellite imagery using CNNs, apply Random Forest algorithms for risk calculation, and generate high-resolution risk probability maps. The potential performance metrics for evaluation of the efficacy of the AI-driven approach could be, prediction accuracy, spatial resolution, or neighborhood-level granularity

Advanced Vector Surveillance Technologies:

1. *Computer Vision with Transfer Learning for Vector Identification*

Technical Foundation of Transfer Learning: transfer learning represents a breakthrough in AI image recognition, allowing models to leverage knowledge from pre-trained datasets to excel in specialized tasks like mosquito species identification.

2. *Generative Adversarial Networks (GANs) for Vector Population Modeling*

Core Conceptual Framework: GANs represent a unique machine learning paradigm that mimics a competitive game between two neural networks: the generator network and the discriminator network. The generator network creates highly realistic synthetic data indistinguishable from real-world observations. The discriminator network distinguishes between real and synthetic data with maximum accuracy.

Example Integrated Implementation: an AI-powered drone system using object detection algorithms could autonomously survey and map vector populations in remote areas, transmitting real-time data to health monitoring centers.

Personalized Health Recommendations:

1. *Transformer-Based Natural Language Processing*

Architectural Foundation of Transformer Models:

- Attention mechanism enables nuanced context understanding, processes complex linguistic relationships, and allows dynamic information prioritization.
- Key processing provides contextual language comprehension, personalized information extraction, and multi-modal data interpretation

2. *Federated Learning*

Fundamental Architectural Approach: federated learning uses a decentralized learning methodology via local model training. This approach processes data on individual devices, maintains complete data privacy, and minimizes centralized data exposure. Moreover, the aggregated learning mechanism only shares model updates, and not raw data, thus preserving individual privacy.

Example Implementation: a mobile health application using federated learning could provide individualized disease prevention recommendations based on personal health data and local transmission risks.

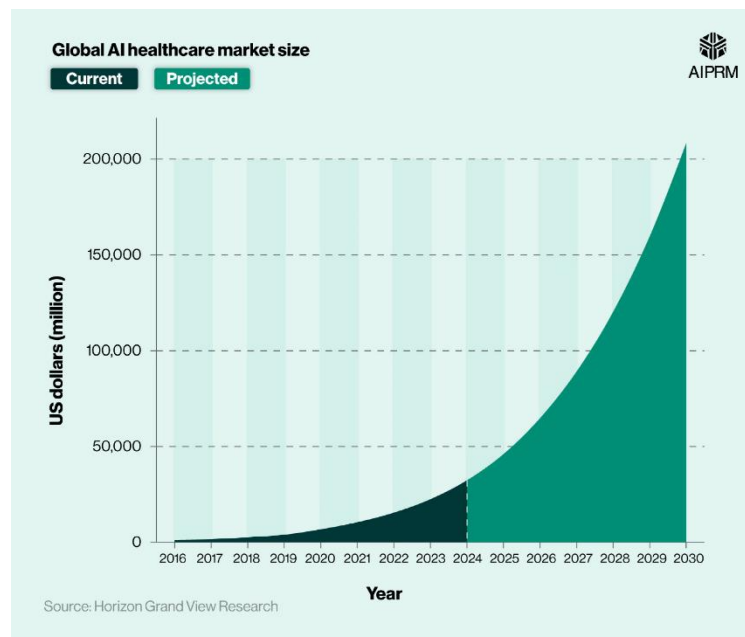


Figure 1. A breakdown of the global AI market size in healthcare. (Report, 2024)

Conclusions and Future Directions

While promising, AI-driven vector control strategies must continue addressing challenges such as, ensuring data privacy, maintaining algorithmic accuracy, and developing adaptable models for rapidly evolving disease ecosystems. The convergence of AI, epidemiology, and environmental science represents a transformative approach to combating vector-borne diseases, offering hope for more effective, proactive global health management. Possible future research directions in this area could be to develop more robust, context-aware AI models, create adaptable frameworks for emerging vector-borne threats, and enhance interdisciplinary collaboration between AI, epidemiology, and public health experts.

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CLIMATE CHANGE IMPACTS ON VECTOR-BORNE DISEASES IN INDIA: A NARRATIVE

Ramesh C Dhiman

Former Scientist G, ICMR
National Institute of Malaria Research, DELHI-110075;
Email: r.c.dhiman@gmail.com

Climate affects every walk of life including health. The major components of climate are temperature, rainfall, Relative Humidity, wind velocity and radiation. Though we are well versed with the word 'climate' but in recent years the word 'climate change' has emerged. In fact climate change can be termed as the changes in climatic conditions over a span of three decades or more. Let us make it clear that year to year changes in climatic conditions are called as climate variability, while daily changes in climatic conditions denote weather. The intergovernmental Panel of climate change (IPCC) which is a panel of 195 countries, publishes its assessment reports on the changing climate, its projected impacts on different facets of life in the form of IPCC reports and summary for policymakers. The sixth Assessment Report (AR6) of IPCC projects rise in temperature to the tune of around 5° C by the year 2100 (Fig.1).

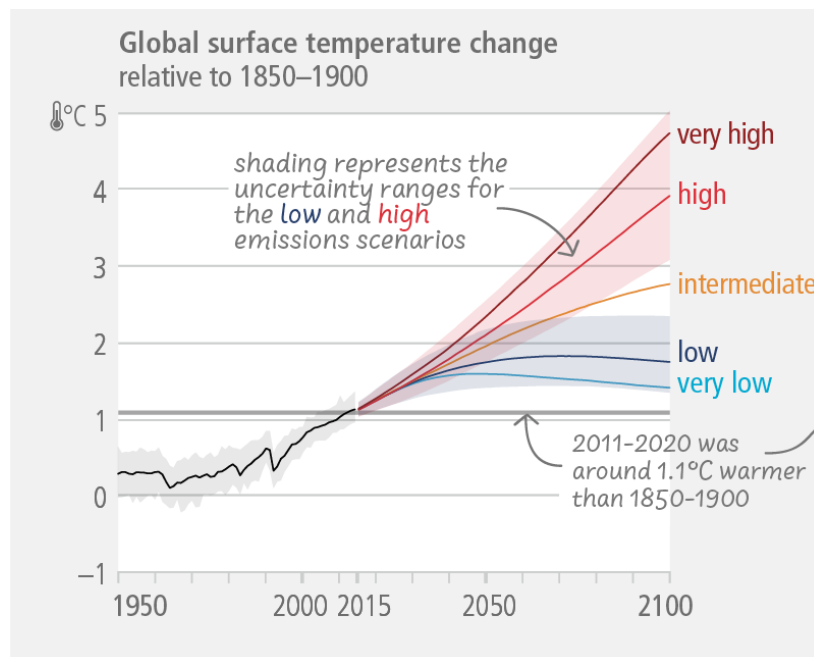


Figure1. Global surface temperature changes in °C relative to 1850–1900 (Source: Climate change 2023 synthesis Report. Summary for Policymakers)

The vector Borne Diseases (VBD) are the diseases transmitted by the vectors which are generally insects like mosquitoes, sand flies, and arthropods like ticks and mites. In India, the major VBDs are malaria, dengue, chikungunya, lymphatic filariasis, leishmaniasis and Japanese encephalitis which are covered under national programme, i.e. National center for Vector Borne Diseases (NCVBDC). In addition to these six VBDs, scrub typhus, Kyasanur Forest Disease, Zika virus, Crimean Congo Hemorrhagic fever and Chandipura virus are also emerging.

As the arthropod vectors are cold blooded creatures, their body is affected by external temperature conditions. In severe hot, there is mortality while at lower temperatures, the development is either arrested or takes quite longer time. Transmission of any VBD takes place only within a suitable range of

temperature and/or Relative Humidity. The thresholds of some important VBDs are given by Dhiman et al. 2024. The life of vectors particularly mosquitoes are affected by three sites of temperature, i.e. temperature of water bodies where mosquitoes breed, temperature of indoor resting habitats where they rest and the temperature of outdoor where mosquitoes visit for egg laying and search of host for bloodmeal. Even there is difference in the indoor and outdoor temperatures at a particular site (Fig 2). The temperature we usually use for analysis in studies is the air temperature. If we take into consideration the differences in indoor versus outdoor temperatures, its impact on extrinsic incubation period of pathogen varies. In a study undertaken in Delhi, the impact of indoor versus outdoor temperature has been depicted on EIP of dengue (Fig 3).

The T and RH not only affects the developmental cycle of vectors species, but the development of parasite/virus also depends. The presence/absence of a particular disease in a geographic area depends on the climate suitability. Even high or low endemicity is also related with most suitable, moderate or least suitability. The climatic, ecological factors as risk factors of malaria are elucidated by Sarkar et al – (2019). Based on the spatio-ecological model the country was classified into five zones characterized by various levels of malaria transmission risk (very high; high; moderate; low; and very low. It was found that about 13% of the country is under very high malaria risk, which includes the malaria- endemic districts of the states of Chhattisgarh, Odisha, Jharkhand, Tripura, Assam, Meghalaya and Manipur. The study also showed that the transmission risk suitability for *P. vivax* is higher than that for *P. falciparum* in the Himalayan region. The field study also highlighted that the low to moderate risk zones are prone to outbreak of malaria when the suitable T and RH are met in the area. In areas with extremes of weather, the vector species do not survive till completion of development of pathogen while at colder temperature, it takes such a longer time in completion of developmental cycle of pathogens, by that time the vector does not survive. The reason of high malaria endemicity in Jharkhand, Chhattisgarh, Odisha and northeastern states may be attributed to most suitable conditions. In western part of India like Rajasthan, parts of Haryana, Punjab and Gujarat, malaria endemicity is low as the climate suitability is for a limited period. i.e. 4-6 months.

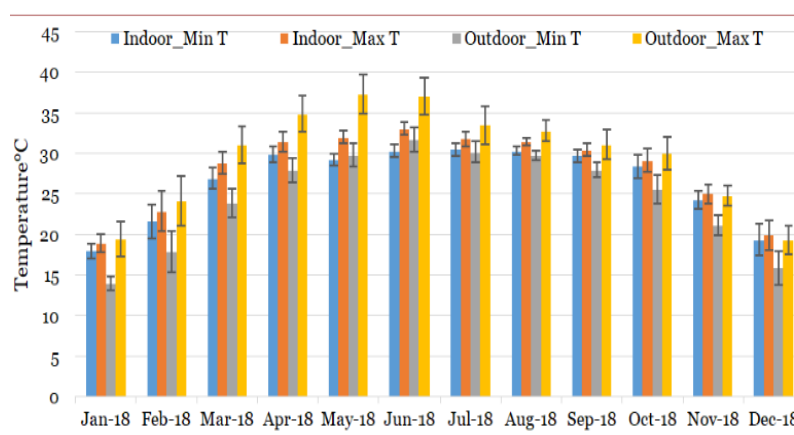


Figure 2. Monthly Recorded Indoor (a) and Outdoor (b) Temperature from January to December 2018 in Okhla, Delhi (Source: Singh et al 2022 J Com Dis 54 (1): 60-66)

The climate change has already affected the emergence of new foci of malaria and dengue transmission in Himalayan region of India. The states of Uttarakhand and Himachal Pradesh are reporting cases of malaria from hitherto malaria free areas and witnessing outbreaks of dengue.

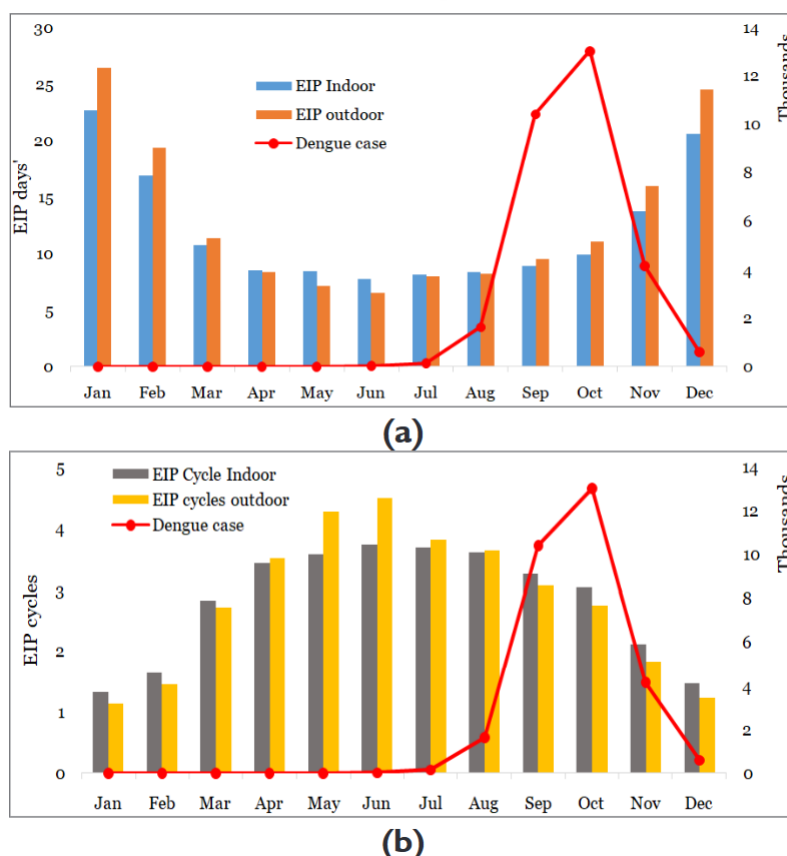


Figure 3. Number of Days required to Complete One EIP Cycle (b) Number of Monthly Possible Infectious Cycle of Dengue Virus in Mosquito Vector in Outdoor and Indoor Temperature Conditions (Source: Singh et al 2022 J Com Dis 54 (1): 60-66)

As regards the spatial and temporal distribution of dengue in the country, it is expanding rapidly. The annual cases of dengue have surpassed the cases of malaria in the India (289235 dengue Verses 227564 malaria cases in 2023). The reason of spatial distribution is due to several factors like scarcity of water leading to water storage, introduction of piped water supply in rural areas, opening of foci of transmission in new areas particularly in Himalayan region and overall intense transmission due to increased temperatures in etc.

The role of climatic factors in KFD has also been elucidated recently. The importance of requirement of rainfall, forest with foliage during non-transmission while the transmission occurs from November to May when there is no rainfall (Singh et al 2019). Based on 14 bioclimatic factors like mean temperature of wettest quarter, mean temperature of warmest quarter, annual precipitation, precipitation of wettest and driest quarter etc. projected scenario of distribution of *Hemaphysalis spinigera* ticks has been generated which reveals that the areas along the western ghats is most suitable for ticks prevalence(Pramanik et al 2021).

Scrub typhus is a disease of concern now a days as its distribution has expanded to Himalayan region, southern India as well as deserts of Rajasthan. With changes in climatic conditions, the intensity of transmission is likely to increase in northeastern states as well as in Himalayan states. D Cruz et al (2024) based on retrospective data of 15 years in Vellore (Tamil Nadu) found that for an increase of 1 °C in mean temperature, the monthly ST cases reduced by 18.8% while for 1% increase in mean relative humidity (RH), there is an increase of 7.6% of monthly ST cases. Similarly, an increase of 1 mm of rainfall contributed to 0.5 to 0.7% of monthly ST cases (after 2 months) depending on the variables included in the analysis. Rise in temperature has a negative impact on scrub typhus during the same month. As the

mites prefer to lay eggs in a temperature range of 20°C-30°C, high temperature areas are not suitable for transmission. That is why the transmission months vary from area to area eg. In Rajasthan the transmission occurs during monsoon and post monsoon months.

Impact on Vectors: As far as the impact of climate change on vectors is concerned, the changes in temperature are bound to affect the developmental cycle of vector species resulting in shorter duration of life cycle at increased temperature. The impact of this phenomenon will be more in Himalayan region which has low temperatures. In a study undertaken in Uttarakhand, it was found that the man hour density of *An. culicifacies* in hilly areas of Nainital district was up to a maximum of --- and the vectors were available only for 3-4 months. In 2013, after a span of about 25 years, the temporal distribution as well density of vectors has increased tremendously. It indicates that the months of transmission of malaria have increased setting the suitability for indigenous transmission. The increased temporal distribution and high density of vectors has been corroborated by the emergence of cases of malaria from the foci which were hitherto free from malaria (Dhiman et al., 2019).

In an interesting study (Dhiman et al., 2013) to find out the role of altitude vis-a-vis density of malaria vectors, the study provided the evidences of decrease in temperature with increase in altitude, even within a district resulting in variation in temporal distribution of malaria vector. With the increase of 67 m altitude between plains and foothill village, there was a reduction in temperature to the tune of 1.1°C and with further increase in altitude of 416 m between foothill and hilly villages, the temperature

decreased by 0.27°C. The difference in temperature at three altitudes affects the Transmission windows (TWs) of both *Plasmodium vivax* (Pv) and *P. falciparum* (Pf), and opening of TWs are inversely proportional to altitude. In the plains, the TW for Pv and Pf were open for 11 and 10 months respectively, while 10 and 9 months in the foothills and 9 and 8 months, respectively for both the parasites at hilly altitude. Overall, the availability of *Anopheles culicifacies* and *An. fluviatilis* decreased with increase in altitude altitude from foothills to hilly areas.

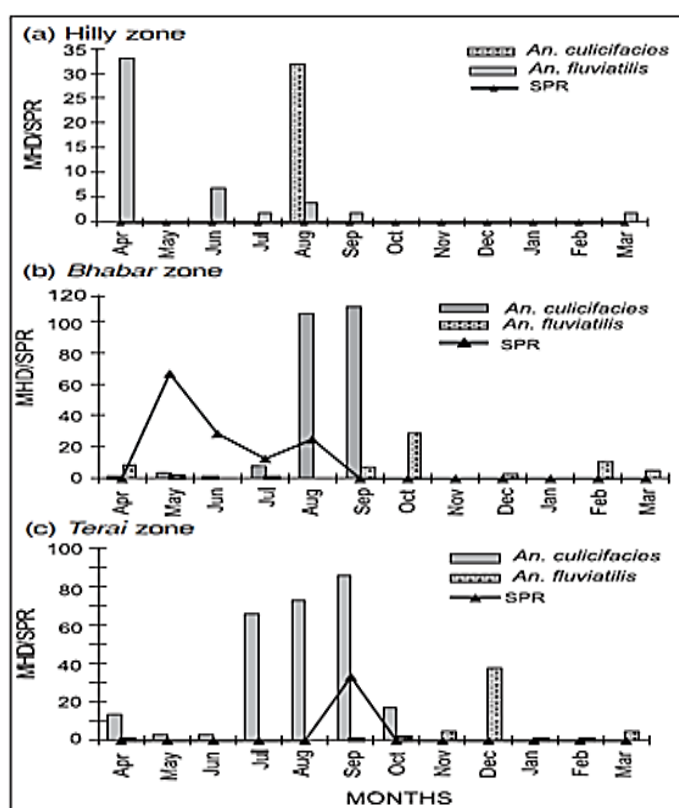


Fig. 1: Vector density and SPR in three physiographic zones of Kumaon region, Uttarakhand state

a. Shukla et. al, 2007

b. Man Hour Density of vectors in Bhorsa village(Nainital)

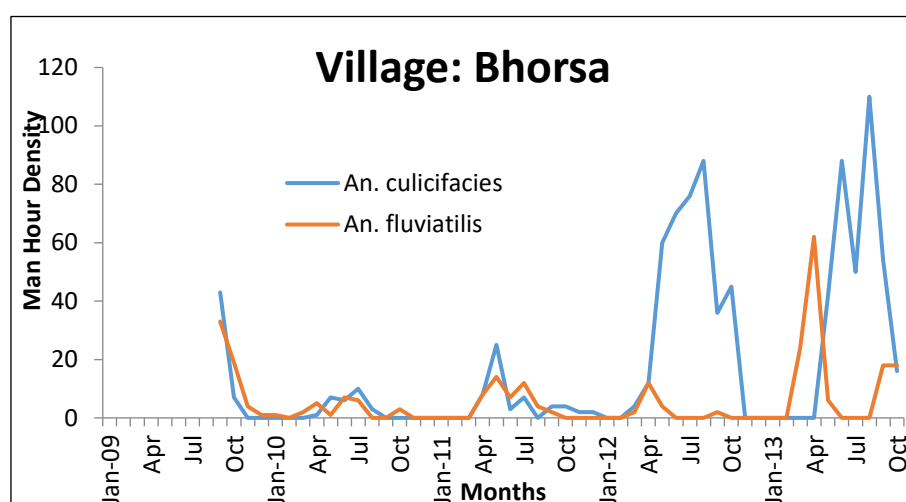


Figure 4. Density of malaria vectors (*An. culicifacies* and *An. fluviatilis*) in 1998-99 Versus 2012-2013 in district Nainital (Uttarakhand)

Way forward

There is ample sensitization at the level of policymakers and researchers. Awareness amongst vulnerable communities is need of the hour. Vulnerable maps of diseases like Scrub typhus and CCHF should be generated by researchers and health program should undertake periodic surveillance for detection of cases in hitherto disease free areas. The responsible nodal agency like NPCCHH should implement health adaptation plans so that calendars of disease- specific activities are meticulously adopted and implemented. The district nodal officers should have disease specific guidelines and action plans for addressing the adverse impacts of climate change.

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ABOUT THE AUTHOURS/EDITORS



Dr M. Raja, has 14 years of experience in the field of Entomology(Biocontrol) and Teaching. He obtained M.Sc. degree in Zoology and then Ph.D. from the Madras University. He was worked as a JRF at Sun Agro Biotech Research Centre, Porur, Chennai from 2008 to 2010 and Research associate at Nutricon Private Ltd, Marimalainagar, Kanchipuram District. He has guided 04 research scholar for Ph.D. degree (Guiding 4) and guided 09 for M.Phil and 30 students for M.Sc. He has to his credit 32 research papers from National and International peer reviewed journals and authored/co-authored/edited number of books notably Fauna of Loyola and Water an Elixir of Life: An Akashic Record with recognition of International Standard Book Numbers. He has completed 5 minor projects and sequenced 16 fungal species and 4 bacterial species and DNA sequence was submitted in the Gene bank. Dr. M. Raja is Fellow of The Royal Entomological Society London, the Entomological Society of India, the Entomology Academy of India and members of various societies. He was served as Joint secretary (Loyola College Teaching Association), Club advisor, ERP Coordinator in Loyola College. Recipient of Awards and Honours from various esteemed institutions on Best Paper Presentation and Outstanding Faculty Award. Presently he is Associate Professor and Director to Centre for Insect Molecular Studies and Environmental Research, Department of Advanced Zoology and Biotechnology, Loyola College (Autonomous), Chennai-600 034 601301, Tamil Nadu, India.



Dr Pradeep Kumar Srivastava was Head of Vector Control & Entomology division of National Vector Borne Disease Control Programme (NVBDCP), Govt. of India. He has been Country's nodal officer for elimination of Lymphatic Filariasis, headed the Planning and Coordination Division of NVBDCP, Delhi. He was In-Charge of Urban Malaria and National Filaria Control Programme in India. He served in different states as the Regional Entomologist and as Scientist in Department of Biochemistry, AIIMS, New Delhi and Malaria Research Centre now known as NIMR. He was Elected as Annual fellow member of Royal College of Entomological Society London in 1993. He has worked as Temporary Adviser to WHO on many occasions in Member countries of SEARO and WHO Headquarter Geneva. He has chaired and co-chaired scientific sessions on Lymphatic Filariasis in USA and France. He has been a part of Indian delegation on Conference of Parties (COPs) 2017 for DDT reduction in vector control. He has framed state specific Malaria elimination Plan in Jharkhand state with WHO support in 2019. He has been Co-chair of Vector Control Working Group under Asia Pacific Malaria Elimination Network (APMEN). He has more than 60 scientific publications and has authored many guidelines including 'Operational Manual for Integrated Vector Management in India', and chapters in various books. He has been conferred Vestergaard Frandsen Award 2019 by National Academy of Vector Borne Diseases (NAVBD), India. He has supported in Vector Control Need Assessment of India and Timor-Leste and drafted the "National Integrated Vector-Borne Diseases strategic plan Timor-Leste (2021-2030)" funded by WHO. He has been as one of Core expert team members of WHO in Malaria Programme Review in India as well as in drafting National strategic Plan for Malaria Elimination in India (2023-2027). Besides, he is working as Director, Absolute Human Care Foundation -a non-profit organization and devoting himself in capacity building of entomologists and vector control experts.



Dr. S. Selvakumar, working as a field Entomologist and having an experience of more than three decades. He obtained M.Sc. degree in Zoology and then Ph.D. from the Madras University. His credentials, the introduction of fiber boats to control the vectors in inaccessible areas in Chennai waterways (248 km length, for spraying of larvicides). Creation of Web-based Real Time Vector Control Monitoring System, during December 2018 in Greater Chennai Corporation. Organized a training Programme for noted Entomologists from Sri Lanka during 2007 and in-turn received a Certificate of "Malaria Free country" from WHO. Introduced Drones for mosquito control activities for the First time in India, during 2021, currently using 6 drones for spraying larvicides in all the waterways in Chennai. Published more than 15 research papers in International journals and a paper in Dengue Bulletin (WHO), Volume 41, 2020 is noteworthy, which is applicable for all Dengue reporting countries to find out the Dengue outbreak years. Has been invited as a Guest Lecturer in various institutions, including IIT Chennai, to create awareness on mosquito control among students' community. Specialized in introducing new strategies and innovative logistics, for vector control activities. He is currently working as a Chief Vector Control Officer, Greater Chennai Corporation, Chennai.

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